

Synchronization of motor unit firings: an epiphenomenon of firing rate characteristics not common inputs

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Submitted 8 May 2015; accepted in final form 16 October 2015

Kline JC, De Luca CJ. Synchronization of motor unit firings: an epiphenomenon of firing rate characteristics not common inputs. *J Neurophysiol* 115: 178–192, 2016. First published October 21, 2015; doi:10.1152/jn.00452.2015.—Synchronous motor unit firing instances have been attributed to anatomical inputs shared by motoneurons. Yet, there is a lack of empirical evidence confirming the notion that common inputs elicit synchronization under voluntary conditions. We tested this notion by measuring synchronization between motor unit action potential trains (MUAPTs) as their firing rates progressed within a contraction from a relatively low force level to a higher one. On average, the degree of synchronization decreased as the force increased. The common input notion provides no empirically supported explanation for the observed synchronization behavior. Therefore, we investigated a more probable explanation for synchronization. Our data set of 17,546 paired MUAPTs revealed that the degree of synchronization varies as a function of two characteristics of the motor unit firing rate: the similarity and the slope as a function of force. Both are measures of the excitation of the motoneurons. As the force generated by the muscle increases, the firing rate slope decreases, and the synchronization correspondingly decreases. Different muscles have motor units with different firing rate characteristics and display different amounts of synchronization. Although this association is not proof of causality, it consistently explains our observations and strongly suggests further investigation. So viewed, synchronization is likely an epiphenomenon, subject to countless unknown neural interactions. As such, synchronous firing instances may not be the product of a specific design and may not serve a specific physiological purpose. Our explanation for synchronization has the advantage of being supported by empirical evidence, whereas the common input does not.

motor unit; motoneuron; firing instances; common input; synchronization

FOR NEARLY ONE CENTURY, the occasional synchronization of motor unit firing instances has been explained by various speculative notions. None of these notions has ever been substantiated with convincing empirical evidence. Adrian and Bronk (1928) first postulated that relatively few central inputs projected to relatively large numbers of motoneurons, causing pairs of motoneurons with common inputs to fire synchronously. Buchthal and Marsden (1950) agreed that the central spread of excitation was one possible explanation for synchronization. However, they also proposed that synchronous motor unit firing instances could just as likely be the result of firing characteristics intrinsic to the motoneurons. Later, Person and Kudina (1968) further suggested that synchronization could be a consequence of synchronizing and desynchronizing inputs

converging on the motoneurons. None of these studies could prove the notions they put forth, and all posited that the actual physiological factors responsible for synchronization remained to be determined by additional empirical evidence. Nonetheless, without any new evidence, Sears and Stagg (1976) put forth the notion that measurements of synchronization could be used to approximate the synaptic connections shared by motoneurons.

Subsequent work by Nordstrom et al. (1992) popularized the notion of the “common input” by proposing that the number and strength of physical connections shared by human motoneurons could be determined based on the quantity of synchronous motor unit firing instances that they produce. Dozens of reports have since adopted this notion, including the following, among others: 1) within the same muscle (Garland and Miles 1997; Kamen and Roy 2000; Keen et al. 2012; Laine and Bailey 2011; Mellor and Hodges 2005; Nordstrom et al. 1990; Schmied et al. 1993); 2) across synergistic muscles (Barry et al. 2009; Bremner et al. 1991a, b; Carr et al. 1994; Gibbs et al. 1997; Keen and Fuglevand 2004; McIsaac and Fuglevand 2007; Powers et al. 1989; Wings and Santello 2004); 3) during tremor (Halliday et al. 1999; Logigian et al. 1988; Semmler and Nordstrom 1995); 4) in various neuromuscular pathologies (Baker et al. 1992; Farmer et al. 1990, 1993; Kirkwood et al. 1984; Mottram et al. 2010; Schmied et al. 1999); 5) during various muscle contraction paradigms, such as precision grip tasks (Hockensmith et al. 2005; Huesler et al. 2000; Kilner et al. 2002; Santello and Fuglevand 2004; Wings et al. 2006); 6) in exercise training (Boonstra et al. 2009; Dartnall et al. 2008, 2011; Griffin et al. 2009; Mochizuki et al. 2005; Schmied and Descarreaux 2010); and 7) during muscle fatigue (Boonstra et al. 2008; Grönlund et al. 2009; Holtermann et al. 2009; Semmler and Nordstrom 1998).

Yet, despite its apparent wide acceptance, the notion that under voluntary conditions, common inputs cause synchronization remains an unverified hypothesis. Moore et al. (1970) were among the first to caution that synchronization could be caused by a variety of anatomical configurations of inputs to motoneurons. They reasoned that proof of any one configuration, such as common inputs, required additional evidence beyond the occasional synchronization of the firing instances. However, their warning was not carefully considered until the work of De Luca et al. (1993). In an extensive study of the amplitude and time-varying properties of synchronization, they found no evidence to suggest that common inputs were responsible for synchronization. Instead, they proposed that synchronous firing instances were more likely an epiphenomenon of the firing characteristics of the motor units.

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Findings from other studies have also brought into question the validity of the common input notion. For example, Semmler et al. (2000) reported that synchronization was similar between young (~25 yr old) and elderly (~75 yr old) subjects. Yet, it is well documented in these studies, among others, that the quantity of cortical neurons, the number of cortico-spinal projections, and the excitability of inputs to motoneurons are all reduced with increasing age (Eisen et al. 1996; Hallett 2000; Henderson et al. 1980; Oliviero et al. 2006; Rossini et al. 1992). If common inputs actually caused motor units to fire synchronously, one would expect that deterioration of synaptic inputs would include, at least in part, deterioration of common inputs that would ultimately result in changes to the degree of synchronization. The fact that synchronization appears to remain unchanged with increasing age decreases the cogency of the common input notion.

In our recent work, De Luca and Kline (2014) provided a rigorous analysis of the different synchronization detection methods that have been used to infer common inputs between motoneurons. We found that all previously used methods were subject to a relatively high degree of false detections and incorrect estimation of synchronization. In fact, some methods, such as the common input assumption approach, even led to the ascription of common inputs to motoneurons whose firing instances manifested no statistical dependence.

In this report, we describe two experiments intended to shed light on our understanding of the circumstances that lead to the incidence of synchronous motor unit firing instances. However, before progressing, we highlight some methodological details that we implemented to avoid the shortcomings of many previously reported studies.

1) Our analysis of synchronization is based on data obtained from natural, voluntary isometric contractions, maintained at constant force levels in human subjects. Early studies that advanced the common input notion by Kirkwood and Sears (1978) and Sears and Stagg (1976) were based on experiments of respiratory muscles in anesthetized cats. Later studies, claiming that common inputs caused synchronization, used human subjects but were limited to contraction paradigms, in which subjects manipulated the force to maintain fixed firing rates of the monitored motor unit action potential trains (MUAPTs).

2) The constant force isometric contractions in our protocol ranged from 5 to 50% of the subjects' maximal voluntary contraction (MVC). The majority of previous publications did not report the force levels during which synchronization was calculated but instead, reported that contractions typically were limited to <5% MVC [see, for example, among others, the work of Keen et al. (2012); McIsaac and Fuglevand (2007); and Nordstrom et al. (1992)].

3) Our surface electromyography (sEMG) decomposition technology yielded a data set more than one order of magnitude greater than any previously studied. Past studies of synchronization were typically limited to fewer than five MUAPTs per individual contraction.

The experiments performed in this study had two intended aims: 1) to test the veracity of the common input notion and 2) to investigate an alternative, empirically based explanation for the synchronization of motor unit firing instances.

For the first aim, subjects were required to track a two-force level paradigm that was maintained at a relatively low force

level and then increased to a relatively higher force level. We compared the degree of synchronization measured between MUAPTs as their firing rates progressed throughout the increasing force contraction. On average, across all subjects, muscles, and force levels tested, synchronization decreased as the force level increased. We could not find any empirically supported explanation consistent with the common input notion that could account for the decreased amount of synchronization.

For the second aim, we investigated associations between the behavior of synchronization and the firing characteristics of the motor units. We found that synchronization varies as a function of two characteristics of the motor unit firing rate: the similarity and the slope as a function of force. De Luca and Contessa (2012) and De Luca and Hostage (2010) have shown that the firing rates are arranged in an inverse hierarchical order, according to motor unit recruitment thresholds. Concurring findings have been reported in these studies, among others (Holobar et al. 2009; McGill et al. 2005; Person and Kudina 1972). We refer to this construct as the Onion Skin property.

The results of the experiments in this study and those of our previous works by De Luca and Kline (2014) and by De Luca et al. (1993) indicate that synchronization is likely an epiphenomenon, subject to countless unknown neural interactions.

METHODS

Experimental Design and Protocol

We conducted experiments on four male and two female subjects who volunteered for the study. The subjects were between 21 and 23 yr of age and had no known history of neuromuscular disorders. Before participating, all subjects read, indicated they understood, and signed a consent form, approved by the Institutional Review Board at Boston University. Two muscles were studied from each subject during two types of voluntary isometric contractions: index finger abduction of the first dorsal interosseous (FDI) muscle of the hand and leg extension of the vastus lateralis (VL) muscle of the lower limb. Isometric contraction force was measured via load cells and was displayed on a computer monitor to provide visual feedback for the subject.

sEMG signals were recorded using a five-pin decomposition EMG (dEMG) sensor, previously described in De Luca et al. (2006). The sensor was placed on the skin over the center of the muscle belly. The sEMG signals from the four pairs of electrodes in the sensor were differentially amplified, filtered with a bandwidth of 20–450 Hz, sampled at 20 kHz, and stored in computer memory for offline data analysis (for an example, see the four sEMG signals in Fig. 1). Before recording data, we measured the MVC force by 3 brief maximal contractions. Each MVC was 3 s in duration, followed by a rest period of 3 min. The MVC of greatest value was used to normalize the isometric force for later comparison across subjects.

For the experimental protocol, subjects proceeded to track a series of target isometric contractions displayed on the computer screen using the output of the force sensor. Each subject performed two contraction paradigms that were designed to fulfill two specific purposes.

Testing the common input notion. We implemented a protocol of three isometric trapezoidal trajectories, each with two constant force regions, to evaluate the behavior of synchronization across changes in contraction force level. We did so for the FDI muscle and separately for the VL muscle. For the FDI, the first trajectory started at 0% MVC, increased to a first constant force level at a rate of 10% MVC/s, was sustained at 5% MVC for 25 s, then again increased to a second constant force level at a rate of 10% MVC/s, and was sustained at 15%

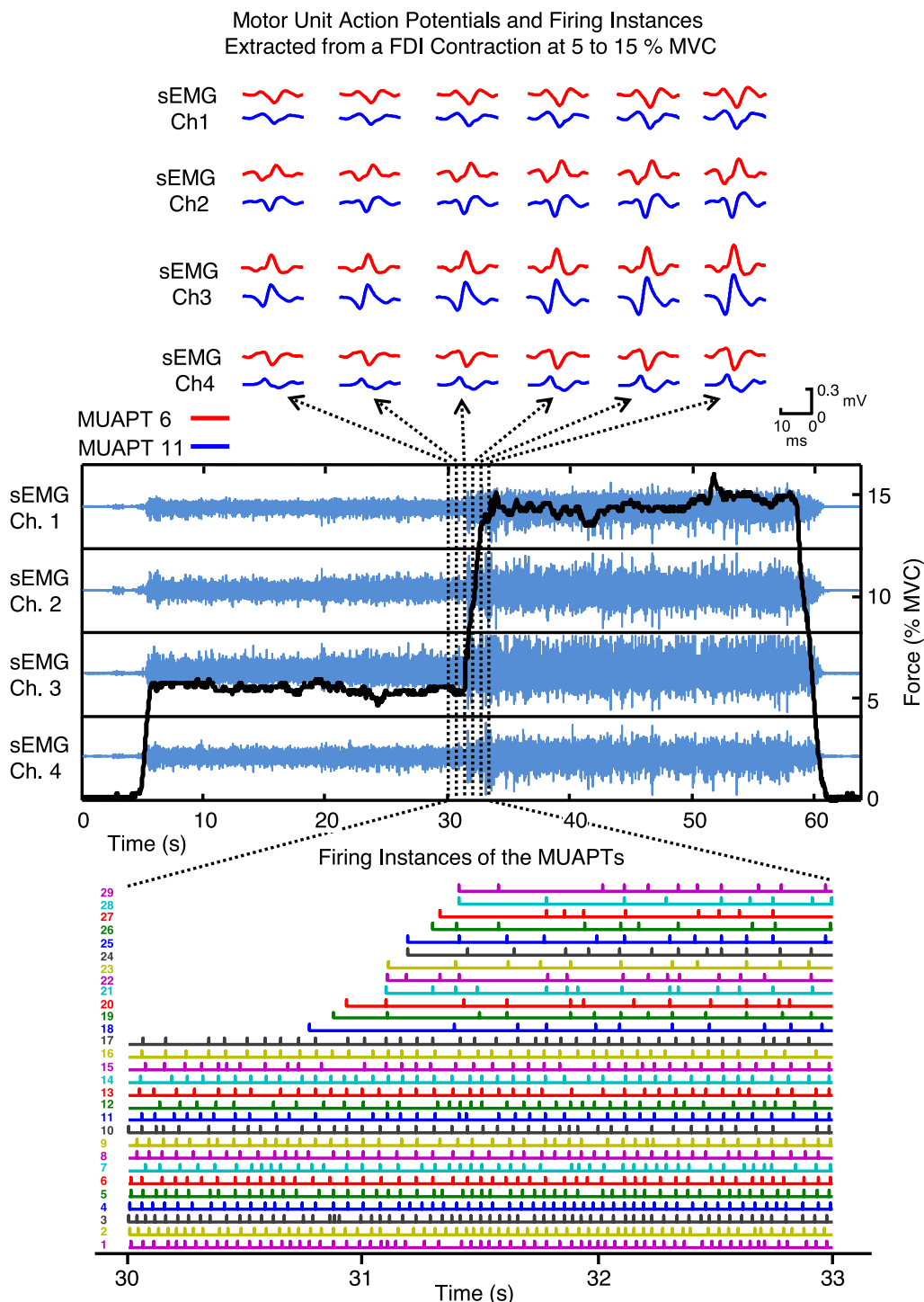


Fig. 1. The motor unit action potentials (MUAPs) and firing instances obtained from the decomposition and error reduction of a surface electromyography (sEMG) signal recorded during a voluntary isometric contraction of the first dorsal interosseus (FDI). The contraction consisted of 2 constant force regions: 1 at 5% maximal voluntary contraction (MVC) and the other at 15% MVC, as shown by the solid black line superimposed over 4 channels (Ch. 1–4) of the recorded sEMG signal (*middle*). The MUAPs observed in all 4 channels (Ch1-4) of the sEMG signal are shown in red and blue for MUAP trains (MUAPTs) 6 and 11, respectively (*top*). As the contraction progressed from the relatively low force level to the higher one, the MUAPs of the different MUAPTs progressively changed. These gradual changes in MUAP shapes were tracked by the decomposition algorithm to identify the firing instances of the same MUAPTs across changes in contraction force level. The firing instances of all 29 MUAPTs identified during this contraction are provided in an expanded view for visual clarity (*bottom*).

MVC for 25 s before it was decreased back to 0% MVC at a rate of 10% MVC/s (an example of this force paradigm may be seen in Fig. 1, *middle*). In the other two contractions of the FDI, the second force level was sustained at 20 or 25% MVC for 25 s. Two repetitions were performed for each of the three contractions.

For the VL muscle, three isometric trapezoidal trajectories were implemented but at relatively greater force levels to acquire sEMG signals with an adequate signal-to-noise ratio. The first trajectory started at 0% MVC, increased to a first constant force level at a rate of 10% MVC/s, was sustained at 20% MVC for 25 s, then again

increased to a second constant force level at a rate of 10% MVC/s, and was sustained at 30% MVC for 25 s before it was decreased back to 0% MVC at a rate of 10% MVC/s. In the other two contractions of the VL, the second force level was sustained at 35 or 40% MVC for 25 s. Two repetitions were performed for each of the three contractions.

Investigating associations between synchronization and motor unit firing characteristics. We implemented a protocol of six isometric trapezoidal trajectories, each with a single constant force region, to investigate associations among synchronization, motor unit firing characteristics, and different levels of voluntary activation. We implemented trajectories for the FDI muscle and separately for the VL muscle. For the FDI, the first trajectory started at 0% MVC, increased to a constant force level at a rate of 10% MVC/s, and was sustained at 5% MVC for 35 s before it was decreased back to 0% MVC at a rate of 10% MVC/s. In the other five contractions of the FDI, the constant force level was sustained at 10, 15, 20, 25, or 30% MVC for 35 s. Two repetitions were performed for each of the three contractions.

For the VL muscle, six isometric trapezoidal trajectories were implemented but at relatively greater force levels to acquire sEMG signals of an adequate signal-to-noise ratio. The first trajectory started at 0% MVC, increased to a constant force level at a rate of 10% MVC/s, and was sustained at 20% MVC for 35 s before it was decreased back to 0% MVC at a rate of 10% MVC/s. In the other five contractions of the VL, the constant force level was sustained at 25, 30, 35, 40, or 50% MVC for 35 s. Two repetitions were performed for each of the three contractions.

All contractions were performed in a pseudo-random order and followed by 5–10 min of rest. The recorded force output was low-pass filtered at 450 Hz, sampled at 20 kHz, and stored in computer memory for offline data analysis.

EMG Signal Decomposition

The four channels of sEMG signals were decomposed into constituent MUAPTs using the dEMG algorithm described by De Luca et al. (2006), improved in Nawab et al. (2010), and independently verified by Hu et al. (2013a, b, c, 2014). Further validation of our algorithm, using the two-source test, has been performed at different force levels ranging from 10 to 50% MVC and in different muscles, including the FDI and the tibialis anterior muscles (De Luca et al. 2006, 2014; Nawab et al. 2010).

The dEMG algorithm extracts the firing instances of different MUAPTs by identifying MUAPs throughout the sEMG signal, using a maximum likelihood estimator that is organized around the maximum a posteriori probability classifier described in LeFever and De Luca (1982). From the onset of its development, the classifier was designed with the inherent ability to track progressive changes in the shape of MUAPs. This is an essential requirement to track MUAPs that may change during the increasing contraction force level paradigm implemented in this study.

The decomposition output provided the firing instances of the identified MUAPs. The time occurrence of each firing instance, as measured by the algorithm, was defined by the time of the greatest absolute value of the MUAP. The accuracy of the decomposition result was calculated using the decompose-synthesize-decompose-compare method, described by Nawab et al. (2010). Occasional errors made by our decomposition algorithm were mitigated using the error-reduction technique described in Kline and De Luca (2014). Briefly, we obtained multiple independent decomposition estimates (each from the sEMG signal) after adding Gaussian white noise (equal in root mean square) to the residual signal remaining after the identified MUAPs have been extracted from the recorded sEMG signal. We implemented the error-reduction procedure using 60 dEMG estimates for each contraction. Only MUAPTs with >95% accuracy in their firing instances were retained for further analysis.

Figure 1 provides examples of the firing instances and MUAPs extracted from the recorded sEMG signal during an isometric con-

traction, first sustained at 5% MVC and subsequently, at 15% MVC in the FDI. As the contraction force (Fig. 1, *middle*) increased from the low force level to the higher level, the MUAPs of the two shown MUAPTs progressively changed, as is evident in Fig. 1, *top* (only 2 example MUAPTs are shown for illustrative clarity). The changes in the shape of the MUAPs were tracked by the dEMG algorithm to assist it in identifying the firing instances of each MUAPT across the changing force level. The firing instances of the complete set of 29 identified MUAPTs from the contraction are shown in Fig. 1, *bottom*.

Synchronization Computations

In the first set of experiments, designed to test the common input, synchronization was measured between MUAPTs active throughout a 20-s constant force region at the relatively low force level and compared with synchronization measured from the same pairs that were continuously active throughout a 20-s constant force region at the higher force level in the same contraction. Only MUAPTs active during both force levels of each contraction were included in the synchronization analysis. In the second set of experiments, designed to investigate associations between synchronization and the firing characteristics of different motor units, synchronization was measured from pairs of MUAPTs continuously active throughout a 25-s constant force region in each contraction.

We measured the degree of synchronization between MUAPTs using the SigMax synchronization detection method, developed by De Luca and Kline (2014). Our previous work demonstrated that alternative methods for calculating synchronization are prone to false detections and incorrect estimation of synchronization. SigMax overcomes these shortcomings by incorporating three essential statistical tests: 1) a test for stationarity of individual MUAPTs, 2) a test for statistical dependence between stationary MUAPTs, and 3) a test for the significant incidence of synchronous motor unit firing instances. Each test follows fundamental principles of basic statistics, and each is necessary for correctly measuring synchronization.

The first test in SigMax detects MUAPTs with statistically nonstationary firing instances using the Kwiatkowski-Phillips-Schmidt-Shin (KPSS) test (Andrews 1991; Kwiatkowski et al. 1992). MUAPTs with a statistically significant degree of nonstationary firing instances were identified as those that manifested a KPSS test statistic >0.463. MUAPTs that passed the stationary test were evaluated for statistically dependent firing instances. The dependence test was based on an analysis of recurrence times (Perkel et al. 1967), measured between each firing instance of a reference MUAPT and the immediate forward and backward firing instance of an alternate MUAPT. The reference MUAPT was selected as the one with fewer firing instances. To eliminate the effects of harmonics in our data, only recurrence times within \pm one-half of the mean interpulse interval (IPI) of the alternate MUAPT were included in the analysis (De Luca and Kline 2014). We tested each pair of MUAPTs for dependent firing instances by computing the goodness of fit between the empirical cumulative distribution of recurrence times and the uniform cumulative distribution of recurrence times predicted for independently firing MUAPTs (an example is provided in Fig. 2). The goodness of fit was quantified using the Cramér-von Mises test statistic (Cramér 1928; von Mises 1931), detailed by the equations in the Appendix of De Luca and Kline (2014). A Cramér-von Mises test statistic >0.461 indicated that the firing instances of the MUAPT manifested statistical dependence beyond the 0.05 significance level.

We measured synchronization between stationary MUAPTs with statistically dependent firing instances. Specifically, synchronization was detected from clusters of recurrence times with a temporal density that exceeded what would be expected due to chance. These clusters, or peaks, occurred at different latencies and lasted for different durations or peak widths. With the use of SigMax, we detected the most statistically significant occurrence of synchronization from each pair of MUAPTs by evaluating the likelihood of detecting synchro-

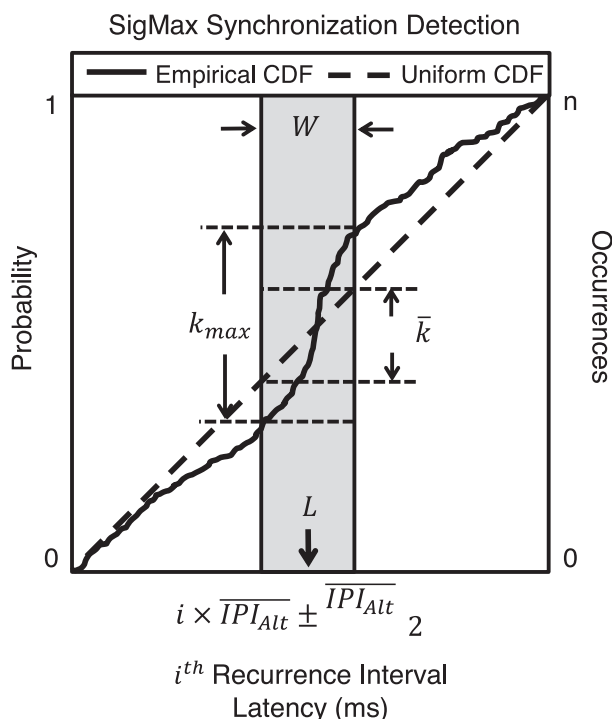


Fig. 2. An example of synchronization measured between MUAPTs using the SigMax detection method, described in De Luca and Kline (2014). The empirical cumulative distribution function (CDF) of recurrence times is shown by the solid line, and the uniform CDF, expected by chance, is given by the dashed line. The shaded region shows the synchronization peak width (W), the center of which indicates the average synchronization latency (L), of the most statistically significant detection of synchronization below the $P < 0.05$ significance level. The total number of synchronous firing instances (k_{max}) was measured from the total amplitude gained by the empirical CDF within the peak width. The number of synchronous firing instances expected due to chance (\bar{k}) was measured from the amplitude gained by the expected uniform CDF within the shaded region. Both k_{max} and \bar{k} were used to compute the synchronization index (SI) as the number of synchronous firing instances that occurred beyond what would be expected by chance (Eq. 1). The figure is modified from Fig. 2 in De Luca and Kline (2014). \overline{IPI}_{Alt} , mean interspike interval of the alternate MUAPT; i , recurrence interval number.

nization from all possible latencies and peak widths in the empirical cumulative distribution of the recurrence time data. We tested different peak widths in increments of 1 ms, ranging from 1 ms to one-half of the mean IPI of the alternate MUAPT. For each peak width, we identified the latency that produced the greatest number of recurrence time occurrences (k). The SigMax equations, detailed in the Appendix of De Luca and Kline (2014), were then used to evaluate the statistical significance of finding k occurrences within the given peak width. The peak width that produced the number of occurrences k with the greatest statistical significance beyond the 0.05 significance level was marked as a synchronization detection, an example of which is shown in Fig. 2. No more than one detection of synchronization was made for each pair of MUAPTs. The width of the shaded region indicated the final synchronization peak width, and its central location marked the average synchronization latency. We measured the degree of synchronization as the percentage of occurrences k_{max} that exceeded the average number of occurrences expected by chance, \bar{k} , using the synchronization index, SI

$$SI = \frac{k_{max} - \bar{k}}{n} * 100 \quad (1)$$

where n indicates the total number of possible occurrences, measured as the number of firing instances of the reference MUAPTs. The synchronization index provided the percentage of synchronous firing

instances between MUAPTs that occurred in excess of chance (De Luca et al. 1993).

RESULTS

Empirical Results from Testing the Common Input Notion

The synchronization analysis from 826 paired MUAPTs, active during the two-force level contractions designed to test the common input notion, demonstrates a major finding: as the MUAPTs progress in firing rate within a contraction from a relatively low force level to a higher one, the average degree of synchronization measured between MUAPTs decreases. Figure 3 provides an example from a voluntary contraction of the FDI. The synchronization index was measured between the same MUAPTs active during the 5% MVC force level and separately during the 20% MVC force level. The firing rate of the MUAPTs increases, as expected, when the force increases from a 5% to a 20% MVC level (Fig. 3A). Concurrently, the synchronization index measured between the MUAPTs decreases from 31.4 at the 5% MVC level to 9.1 at the 20% MVC level (Fig. 3B).

The net decrease in synchronization that occurred along with increases in contraction force is shown for multiple example contractions in Fig. 4. The synchronization indices are plotted as functions of the reference and alternate MUAPT mean firing rate (measured during the relatively low force region in the contraction paradigm) using the contour plotting function in the MATLAB curve-fitting toolbox (MathWorks, Natick, MA). Synchronization measured between MUAPTs active during the relatively low force level (Fig. 4, A–E) was compared with that measured between MUAPTs active during the relatively higher force level (Fig. 4, F–J). Generally, pairs of MUAPTs manifested a relatively high degree of synchronization during the relatively low force level (Fig. 4, A–E). However, as the MUAPTs progressed in firing rate to the relatively higher force level of the same contraction, the degree of synchronization measured between the MUAPTs was relatively lower (Fig. 4, F–J). We calculated the net change in synchronization from the same pairs of MUAPTs active during both force levels and plotted the difference in Fig. 4, K–O. On average, in all subjects and force levels shown, the synchronization index decreased as the contraction force increased. A Mann-Whitney-Wilcoxon significance test (Bergmann et al. 2000; Mann and Whitney 1947; Wilcoxon 1945) indicated that the decrease in synchronization was statistically significant in all five contractions shown ($P < 0.01$).

The synchronization behavior shown in Fig. 4 also indicates that the magnitude of the change in synchronization appears to vary for different pairs of MUAPTs depending on their mean firing rate. For example, reference and alternate MUAPTs with relatively similar but lower mean firing rates typically manifested the highest index of synchronization at the relatively low force level and the greatest decrease in synchronization as the force level increased; the percent change in the synchronization index among several of these MUAPTs declined by as much as a 50% (Fig. 4, K–O). Other reference and alternate MUAPTs with relatively similar and higher mean firing rates tended to exhibit relatively lesser degrees of synchronization at the low force level and relatively smaller and more variable changes in synchronization as the force level increased (Fig. 4, K–O).

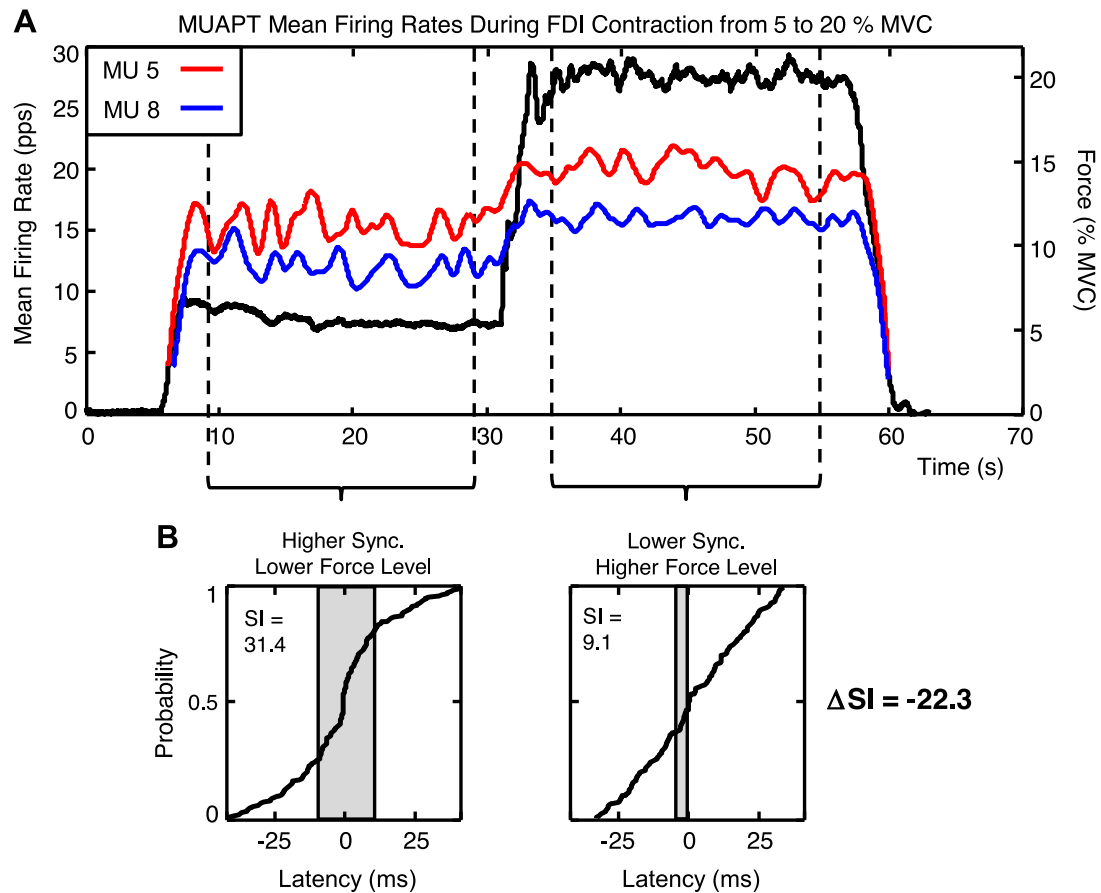


Fig. 3. Synchronization measured between MUAPTs active during an isometric contraction with 2 different force levels. *A*: superimposed over the contraction force (black line) are the mean firing rates (MFRs) of 2 MUAPTs in the FDI; only MU 5 (red line) and MU 8 (blue line) of the 16 MUAPTs extracted with >95% accuracy during decomposition are plotted. The vertical dotted lines at each force level indicate the 20-s region over which synchronization was measured between the MUAPTs. *B*: during the relatively low force level, an SI of 31.4 was measured. As the force increased to the relatively higher 20% MVC level, the SI decreased by 22.3 to 9.1 [change in SI (ΔSI)].

The changes in synchronization that occurred with increases in contraction force were consistent across subjects and force levels studied (Table 1). Because similar patterns of synchronization were found among the different subjects, we grouped all of the data to determine how synchronization varied across the entire population of MUAPTs studied from each muscle in Fig. 5. The synchronization index is plotted as a function of the reference and alternate MUAPT mean firing rate from FDI contractions in Fig. 5, *A*, *C*, *E*, and *G*, and from VL contractions in Fig. 5, *B*, *D*, *F*, and *H*. In both muscles, pairs of MUAPTs with relatively high degrees of synchronization at the relatively low force levels (Fig. 5, *A* and *B*) presented relatively lesser degrees of synchronization as the force level increased (Fig. 5, *C* and *D*). The net decrease in synchronization measured between all pairs of MUAPTs was statistically significant for both muscles ($P < 0.0001$).

Figure 5 also exemplifies that the changes in synchronization, observed across the two-force level contraction, vary depending on the mean firing rates of the MUAPTs. According to Fig. 5, *G* and *H*, pairs of MUAPTs with relatively higher mean firing rates presented relatively small increases or decreases in the degree of synchronization. These small changes in the synchronization index typically did not exceed the 95% confidence interval in each plot (Fig. 5, *G* and *H*). However, as the mean firing rate of the MUAPTs decreased, the magnitude

of the change in the synchronization index generally increased. In both muscles, MUAPTs with relatively lower mean firing rates presented relatively greater net decreases in the synchronization index beyond the 95% confidence interval.

Association between Synchronization and Motor Unit Firing Characteristics

We implemented the second contraction paradigm, where the force was maintained constant throughout the duration of the voluntary effort to study the association between synchronization and the firing characteristics of different motor units. In total, 786 stationary MUAPTs forming 6,280 pairs were from the 72 FDI contractions, and 1,204 stationary MUAPTs forming 11,266 pairs were from the 72 VL contractions. We observed three general manifestations of synchronization among three different categories of paired MUAPTs, depending on their mean firing rates (Fig. 6). *Category 1* pairs consisted of MUAPTs recruited at relatively high force thresholds with mean firing rates that averaged <20 pps and differed by <5 pps. They yielded a relatively high synchronization index, typically between 20 and 30. *Category 2* pairs consisted of MUAPTs recruited at relatively lower force thresholds with mean firing rates that averaged >20 pps and differed by <5 pps. They yielded a relatively lower synchronization index, typically between 10 and 20. Last, pairs in *category 3* consisted

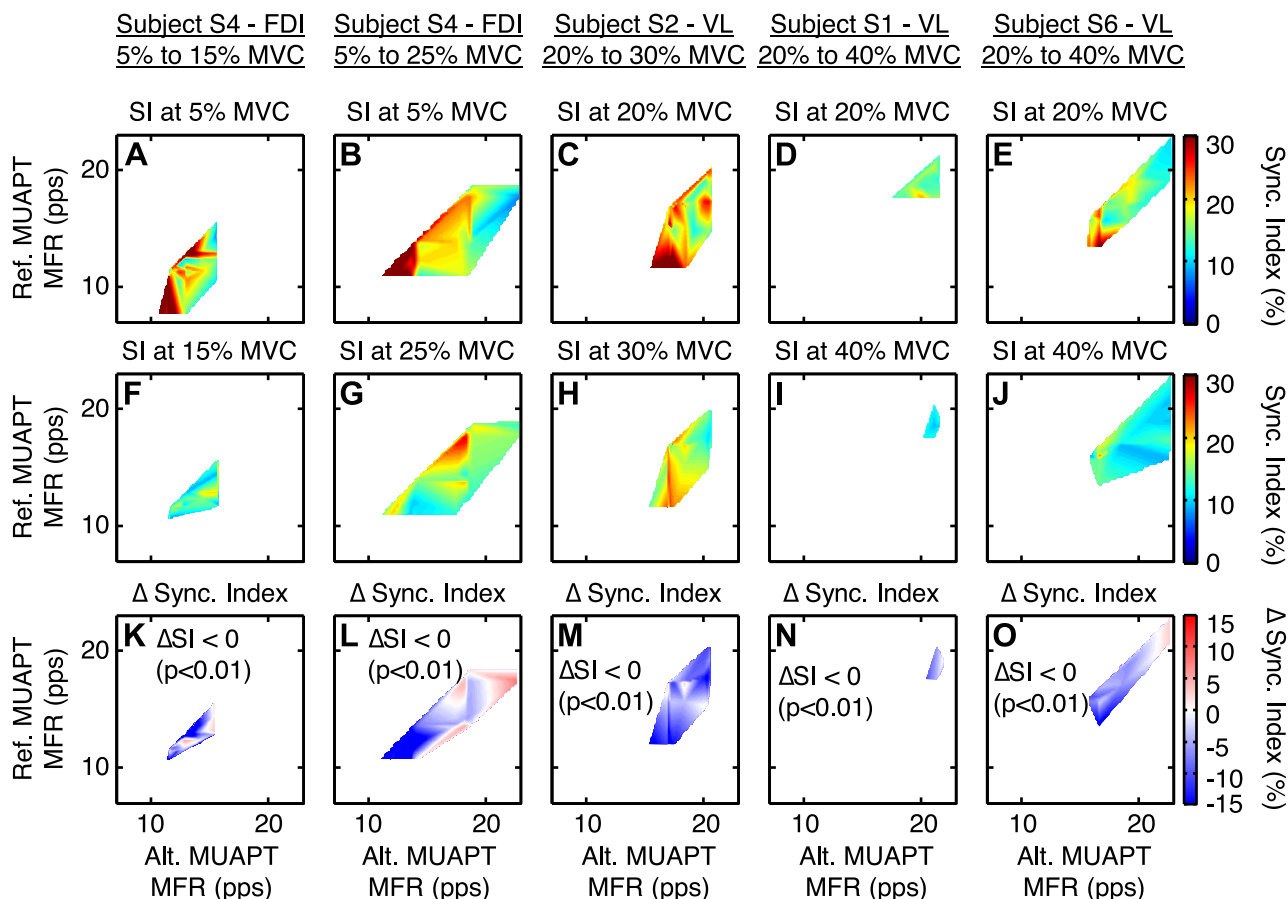


Fig. 4. The SI measured between MUAPTs active during 5 example contractions. *A, F, and K*: a 5–15% MVC FDI contraction in subject (S)4; *B, G, and L*: a 5–25% MVC FDI contraction in S4; *C, H, and M*: a 20–30% MVC vastus lateralis (VL) contraction in S2; *D, I, and N*: a 20–40% MVC VL contraction in S1; and *E, J, and O*: a 20–40% MVC VL contraction in S6. The synchronization data are plotted as contour functions of the reference (Ref.) and alternate (Alt.) MUAPT MFR. The color of the data in each plot indicates the magnitude of the SI quantified by the color bar on the right. For each contraction shown, the SIs observed during the relatively low force level (with pairs of MUAPTs manifesting a relatively high degree of synchronization, signified by the predominance of regions shaded dark red and orange; *A–E*) were compared with the SIs observed from the same pairs of motor units active during the relatively higher force level (where the degree of synchronization measured between the MUAPTs was relatively lower, signified by the predominance of regions shaded blue and yellow; *F–J*), and the difference between the 2 was evaluated (Δ Sync. Index; *K–O*). In all 5 contractions, the predominance of the blue shaded regions indicates that the average degree of synchronization significantly decreased as the contraction force level increased ($P < 0.01$).

of MUAPTs with relatively different recruitment thresholds and mean firing rates that differed by >5 pps. They presented little to no synchronization, with typical synchronization indices between 0 and 10. (Note that the specific mean firing rate values selected to group pairs of MUAPTs into different categories were based on high/low firing rates and similar/different firing rates while ensuring the number of MUAPT pairs in each category was sufficient to assess statistical differences.)

To determine how these manifestations of synchronization varied across a population of concurrently active motor units, we plotted the synchronization index as a contour function of the mean firing rate of the reference and alternate MUAPT for each contraction. Examples of our findings from two contractions of the FDI and two contractions of the VL are provided in Fig. 7. The synchronization index is indicated by the color of the plotted data, quantified by the color bars. The plots in each column contain data from contractions at different force levels, whereas those in each row provide data from different muscles. The same general manifestation of synchronization shown in Fig. 6 is also apparent in these data. For example, in the 10% MVC contraction of the FDI (Fig. 7A), pairs of

MUAPTs in *category 1* with relatively similar and lower mean firing rates produced relatively higher synchronization indices. Relatively lower synchronization indices were observed between MUAPTs in *category 2* having relatively similar and higher mean firing rates. The lowest degrees of synchronization were seen between MUAPTs in *category 3* having relatively different mean firing rates. The same pattern of synchronization was present across all four contractions in Fig. 7.

Synchronization Varies with Motor Unit Mean Firing Rate, Muscle, and Force Level

Notwithstanding the relatively small deviations among individual data points, the general pattern of synchronization shown in Fig. 7 remained seemingly invariant across contractions from different subjects. Therefore, to analyze trends better in synchronization values and because the patterns were closely similar among subjects, we grouped the synchronization index measurements into two plots for each muscle based on the contraction force level. For the FDI, synchronization data were plotted for 5–15% MVC contractions in Fig. 8A and separately for 20–30% MVC contractions in Fig. 8B. For the

Table 1. The magnitude and change in synchronization across different contraction force levels

Muscle	Force Range	Lower Force Level SI (n)	Higher Force Level SI (n)	Δ SI (n)
FDI	5–15% MVC	20.3 (128)	15.9 (128)	-4.2 (128)*
	5–20% MVC	19.2 (3)	10.1 (3)	-13.3 (3)†
	5–25% MVC	20.6 (63)	15.4 (63)	-5.6 (63)*
VL	20–30% MVC	15.3 (350)	13.5 (350)	-1.8 (350)*
	20–35% MVC	16.7 (163)	14.3 (163)	-2.7 (163)*
	20–40% MVC	16.9 (119)	12.8 (119)	-4.9 (119)*

SI, synchronization index; Δ SI, change in SI; FDI, first dorsal interosseous; MVC, maximal voluntary contraction; VL, vastus lateralis. *Statistically significant decrease in synchronization; †insufficient number of pairs to assess statistical significance.

VL, synchronization data were similarly plotted for two force ranges, one from 20 to 30% MVC in Fig. 8C and the other from 35 to 50% MVC in Fig. 8D. From these plots, we assessed differences in synchronization across different mean firing rates, force levels, and contracting muscles using a two-sided Mann-Whitney-Wilcoxon test (Bergmann et al. 2000; Mann and Whitney 1947; Wilcoxon 1945), implemented in MATLAB (MathWorks).

In all four grouped plots in Fig. 8, we observed significant differences in the degree of synchronization across the different categories of MUAPTs. Pairs of MUAPTs in *category 1* with lower mean firing rates had consistently greater synchronization indices than pairs of MUAPTs in *category 2* ($P < 0.0001$), and MUAPT pairs in *categories 1* and *2* with similar mean firing rates had significantly greater synchronization indices than those in *category 3* with different mean firing rates ($P < 0.0001$).

When comparing MUAPTs across the different contractions of the same muscle, we observed further that synchronization differed depending on the force level. For both the FDI and VL data, each of the three categories of MUAPTs active during the relatively low force level manifested significantly greater synchronization indices ($P < 0.0001$) than each of the three categories of MUAPTs active during relatively higher force levels (compare Fig. 8, A with B, and Fig. 8, C with D). Furthermore, when we compared MUAPTs across the different muscles at similar percent MVC levels, we also observed different degrees of synchronization. At the 20–30% MVC force level, each of the three categories of MUAPTs from the VL manifested significantly greater synchronization indices ($P < 0.001$) than each of the three categories of MUAPTs from the FDI (compare Fig. 8, B with C).

The patterns of synchronization observed among different categories of MUAPTs were generally invariant across the subjects and contractions studied. They give evidence to the association between synchronization and the mean firing rates of motor units in different muscles and for different voluntary contraction force levels.

DISCUSSION

Common Input Notion in Question

We set out to gather empirical evidence to test the validity of the notion that common inputs cause synchronization of motor unit firing instances during voluntary contractions in humans.

In recounting the current framework of the notion documented in the literature, we refer to Sears and Stagg (1976),

who initially proposed that synchronous firing instances observed between motoneurons in anesthetized cats are the result of excitation propagated by anatomical branches of common presynaptic inputs shared by the motoneurons. Later, Nordstrom et al. (1992) reported that the different magnitudes of synchronization in human subjects could be considered indicative of differing strengths of common inputs received by the

Synchronization Decreases with Increasing Force

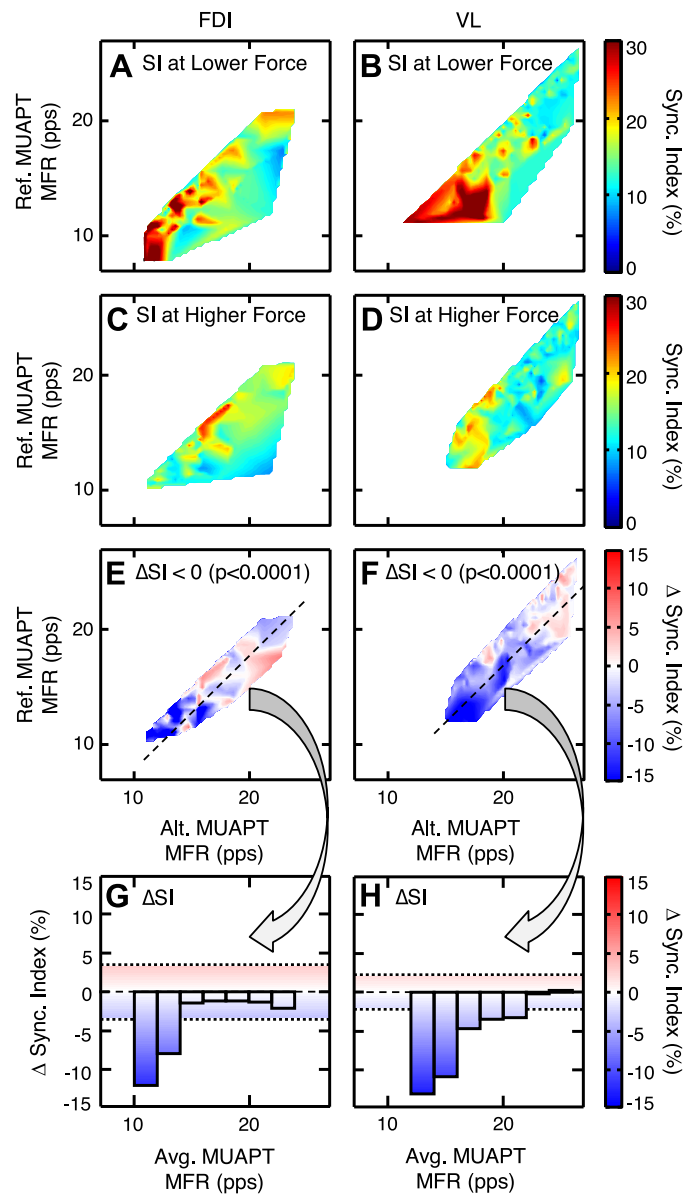


Fig. 5. The magnitude and change in the SI observed among all MUAPT pairs in the FDI (A, C, E, and G) and in the VL 2-force level contractions (B, D, F, and H). The SI is plotted as a contour function of the reference and alternate MUAPT MFR. The colors of the data represent the average value of the SI measured during the relatively low force level (A and B) compared with that measured from the same MUAPT pairs during the relatively higher force level (C and D). E and F: changes in the SI that resulted from changes in contraction force are plotted for the same pairs of MUAPTs active during both force levels. G and H: changes in the SI are provided in a bar plot for all MUAPTs as a function of the average (Avg.) of the MFRs of each pair. Horizontal dotted lines indicate the average 95% confidence interval across all bars for each muscle. As the contraction force increased, statistically significant decreases in the average SI were observed in both muscles.

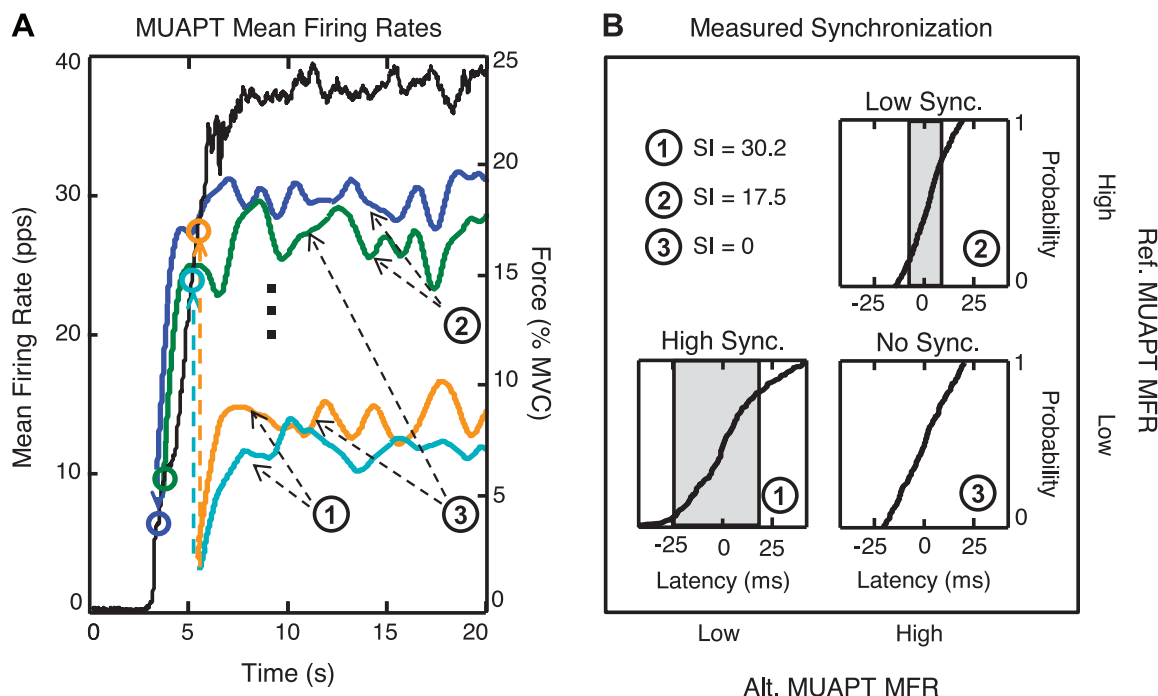


Fig. 6. Examples of 3 characteristic manifestations of synchronization detected from 3 different pairs of MUAPTs. *A*: the MFRs (shown by colored lines) are plotted with the force trajectory (black line) for a 25% MVC contraction of the FDI. Vertical dashed lines and arrows provide the recruitment threshold of each MUAPT. Only 4 of the 32 MUAPTs identified during the contraction are shown for illustrative clarity. Three categories of paired MUAPTs are indicated by the numbers on the plot. *B*: synchronization measured between the MUAPTs using the SigMax method. The degree of synchronization detected in each pair is marked by the shaded boxes and listed in the top left.

motoneurons. In our study, we investigated the behavior of synchronization from pairs of MUAPTs as their firing rates progressed within a contraction from a relatively low force level to a higher one. The changes in synchronization that were observed with increases in force depended on the characteristics of the mean firing rate of the MUAPTs. Pairs of MUAPTs with relatively higher mean firing rates presented relatively small net changes in synchronization. These changes were all within the level of variability or noise in the synchronization data. Other pairs of MUAPTs with higher firing rates manifested statistically significant decreases in synchronization by as much as 50%, with a 10–20% MVC increase in contraction force. Based on these observations and the currently accepted framework of the common input notion, the decreases in synchronization that occur with increases in contraction force indicate that the number and/or strength of the common inputs—of either central or peripheral origin—to motoneurons must correspondingly decrease. We examined the validity of this notion by considering the plausible scenarios in which changes in common inputs could account for decreases in the degree of synchronization between MUAPTs during increases in contraction force.

Common input plausibility 1. The decreased degree of synchronization could result from a reduction in the actual number of common inputs. For example, one could speculate that the number of common input changes as a result of varying contributions of input to the motoneuron pool from different descending pathways. However, no evidence has ever been presented to indicate that central neural pathways are systematically adjusted, such that the number of common inputs shared by motoneurons progressively decreases with increasing contraction force. It is similarly difficult to envision a

construct where anatomical inputs to motoneurons are remodeled during voluntary contractions. To date, this notion remains an unproven speculation with no evidentiary support.

Common input plausibility 2. The degree of synchronization measured between MUAPTs could decrease as a consequence of a decrease in the strength of the common inputs themselves. This scenario suggests that common inputs either decrease their rate of excitatory input or are selectively inhibited at synaptic terminals on the cell body of each motoneuron. However, increases in force output are the result of increases in the voluntary net excitation to motoneurons. No factual evidence has ever been presented to justify that common inputs exclusively decrease their excitation to motoneurons, whereas the vast majority of other inputs increases in excitation to meet the mechanical demands of increasing muscle force output. Without concurring empirical data, it is difficult to support such a construct.

Common input plausibility 3. The changes in the degree of synchronization could be a statistical consequence of different motor unit firing rates observed across the different contraction force levels. Indeed, it is well documented and widely accepted that under normal voluntary conditions, the firing rates of motor units increase as the contraction force increases. However, the index that we use to measure synchronization is robust to changes in motor unit firing rates, in part, because we normalize the number of synchronous firing instances by the number of firing instances in the reference MUAPT. This practice is not novel to our study, as it is well documented in the literature [for example, see the work of Datta et al. (1991), Datta and Stephens (1990), De Luca et al. (1993), among others]. In fact, in a comparative analysis of different synchronization metrics, Turker and Powers (2002) found that indices

Synchronization Between MUAPTs from Individual Contractions

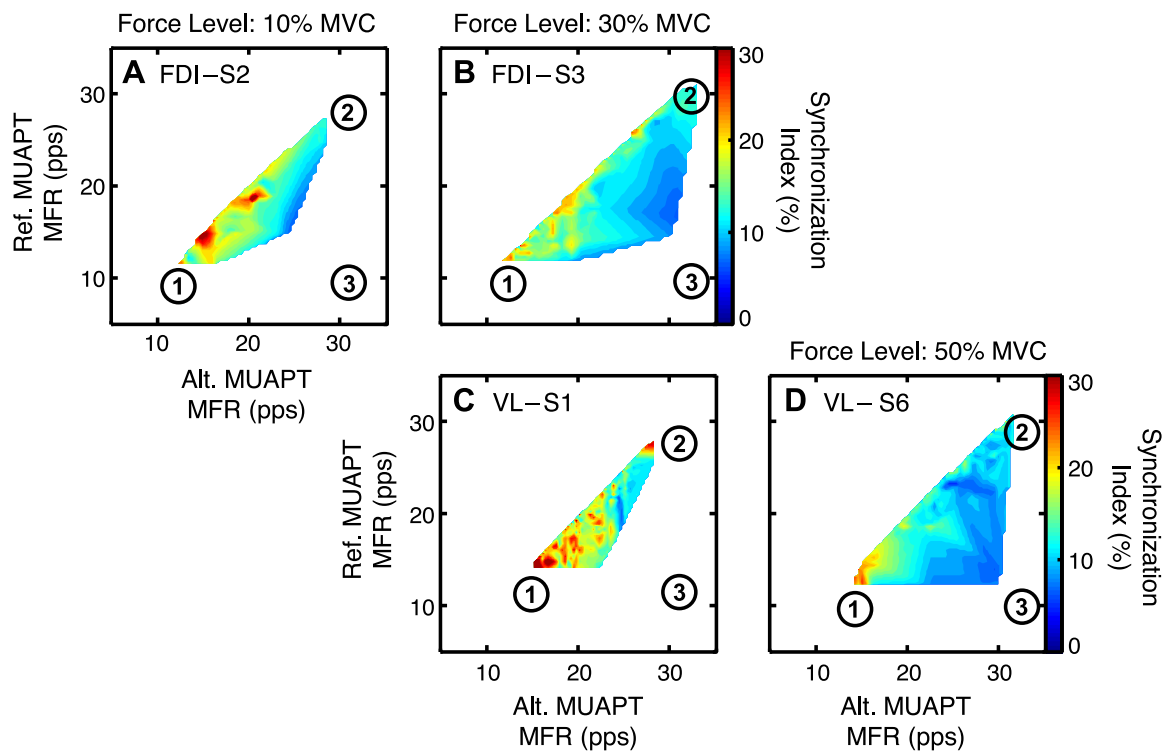


Fig. 7. The SI plotted as a contour function of the reference and alternate MUAPT MFR for a 10% MVC FDI contraction of S2 (A); a 30% MVC FDI contraction of S3 (B); a 30% MVC VL contraction of S1 (C); and a 50% MVC VL contraction of S6 (D). The color of the plot indicates the SI, quantified in the color bars on the right. The different columns and rows of plots provide data from contractions of different force levels and muscles, respectively. Numbered circles indicate categories of different pairs of MUAPTs based on their MFRs. The following same pattern of synchronization was present across all 4 contractions: pairs of MUAPTs in *category 1* with relatively similar and lower mean firing rates produced relatively higher synchronization indices (regions shaded red); relatively lower synchronization indices (regions shaded green) were observed between MUAPTs in *category 2* having relatively similar and higher mean firing rates; and the lowest degrees of synchronization (regions shaded blue) were seen between MUAPTs in *category 3* having relatively different mean firing rates.

similar to ours were the most robust to changes in motor unit firing rates.

These plausible scenarios demonstrate that the common input notion provides no empirically supported explanation to account for the synchronization behavior observed in this study. Thus the practice of inferring the presence of anatomical connections shared by motoneurons, based on observations of synchronization, remains empirically unsupported.

Of course, any number of explanations could be constructed to speculate manners in which common anatomical inputs elicit decreases in synchronization with increases in contraction force level. However, such explanations would also require corroborating empirical evidence to be considered valid.

Synchronization is not the only metric that has been used to make claims about the nature of common connections in the motoneuron pool. More recently, Farina et al. (2014) reported that the degree of common synaptic inputs to motoneurons can be calculated based on peaks in the coherence spectrum measured between groups of MUAPTs. However, the relationship that they reported between coherence and common inputs was based on models of motoneuron connections and simulated firing behavior.

It is important to note that although the use of models may have some use in exploring hypothetical scenarios of motor unit firing behavior, simulated data are not sufficient to prove or disprove the existence of real anatomical connections or physiological mechanisms of motor control. Proof requires

empirical evidence from specifically designed experimental tests.

Synchronization as an Epiphenomenon of the Onion Skin Property

Because of the lack of empirical evidence supporting the common input notion, we set out to identify an alternative explanation for synchronization more solidly based on empirical observations. We focused on the characteristics of the motor unit firing instances and found an association between the degree of synchronization and the characteristics of the motor unit firing rates in different muscles and at different contraction force levels. We propose that these associations are a natural consequence of the Onion Skin property of motor unit firing rates. Characterization of this property has evolved over the past three decades. Among others, we (De Luca et al. 1982) and others (Holobar et al. 2009; McGill et al. 2005; Monster and Chan 1977; Person and Kudina 1972; Tanji and Kato 1973) have shown that motor unit recruitment thresholds and firing rates have an inverse hierarchical relation that we have referred to as the Onion Skin property. That is, at any time and any force, the earlier recruited motor units have greater firing rates than later recruited ones. This property has been documented extensively by De Luca and Hostage (2010) for different muscles, force levels, and motor unit recruitment thresholds. More recently, De Luca and Contessa (2012)

Synchronization Between MUAPTs Grouped Across Multiple Contractions

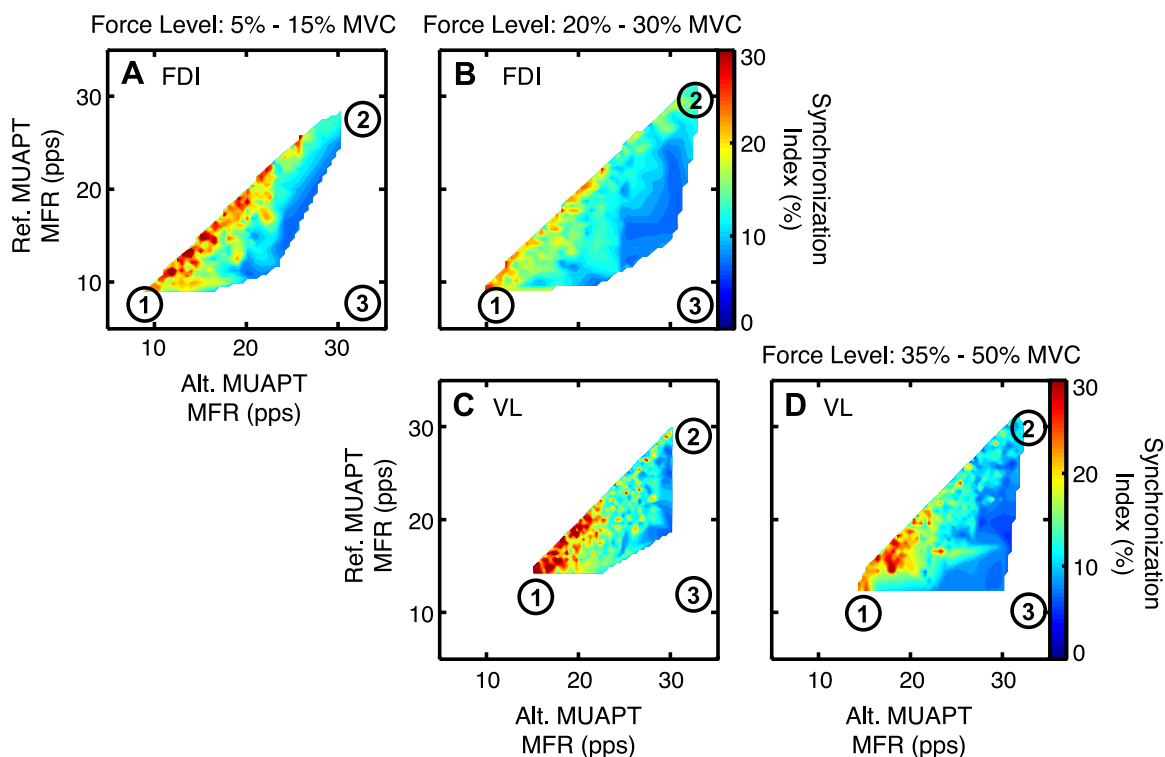


Fig. 8. The SI plotted as a contour function of the reference and alternate MUAPT MFR for all data from FDI contractions ranging from 5 to 15% MVC (A); FDI contractions ranging from 20 to 30% MVC (B); VL contractions ranging from 20 to 30% MVC (C); and VL contractions ranging from 35 to 50% MVC (D). The colors in each plot indicate the SI, quantified by the color bars on the right. Different columns and rows indicate data observed at different force levels and muscles, respectively. Numbered circles indicate categories of different pairs of MUAPTs based on their MFRs. These categories were compared within each plot, across plots of different muscles, and across contraction force levels to determine significant differences in the degree of synchronization among the different MUAPTs. Statistical significance was evaluated using the Mann-Whitney-Wilcoxon test and detailed in the text.

have described it as a spectrum of the firing rates of the motor units within a muscle, a representation of which is shown in Fig. 9 for the FDI and VL muscles.

A comparison of Figs. 8 and 9 demonstrates an association between synchronization and two characteristics of the motor unit firing rate: the similarity and the slope as a function of force. Consider the pairs of MUAPTs in *category 1* with relatively greater degrees of synchronization. These pairs are the ones with motor units that have relatively similar and steeper firing rate slopes. Other pairs of MUAPTs in *category 2*, which present relatively lesser degrees of synchronization, have relatively similar but shallower firing rate slopes, and pairs of MUAPTs in *category 3* that present little to no synchronization have the most dissimilar firing rate slopes.

The association between synchronization and the similarity and the slope of the motor unit firing rates can account for the synchronization behavior observed in contractions of different muscles and force levels. In both muscles, pairs of MUAPTs, active during relatively low force level contractions, have relatively greater degrees of synchronization and relatively steeper firing rate slopes. Yet, pairs of MUAPTs firing at similar rates, but at relatively higher force level contractions, have relatively lesser degrees of synchronization and relatively shallower firing rate slopes. When comparing across muscles active at the same 20–30% MVC force range, pairs of MUAPTs in the VL present greater synchronization indices and have steeper firing rate slopes than those firing at similar

rates in the FDI that present relatively lesser synchronization indices and shallower firing rate slopes.

It is important to point out that the association between synchronization and motor unit firing rate does not constitute definitive proof of a causal relationship between the parameters. Nonetheless, it is noteworthy that the association evidenced in Figs. 8 and 9 can account for the synchronous behavior of motor unit firing instances during low and high force level contractions; from different contracting muscles; and for motor units that have near-similar and dissimilar average firing rates. Thus the strong behavioral similarity between synchronization and the characteristics of the firing rates suggests a relationship between the two parameters that can be subjected to further investigation.

To understand the relationship between synchronization and motor unit firing rate in practical terms, consider the two-force level contraction paradigm implemented in this study. Figure 10 provides a stylistic representation of the firing rates, firing rate slopes, and synchronous firing instances of motor units, active during a voluntary contraction that progresses from a relatively low force level to a higher one. As the voluntary excitation increases, motor units are recruited, and the contraction force increases. The first to be recruited (*category 1* in Fig. 10) have firing rates with relatively steep slopes corresponding to a high sensitivity, where an incremental change in excitation results in a relatively larger incremental change in the motor unit firing rate. Pairs of motor units recruited at similar and low

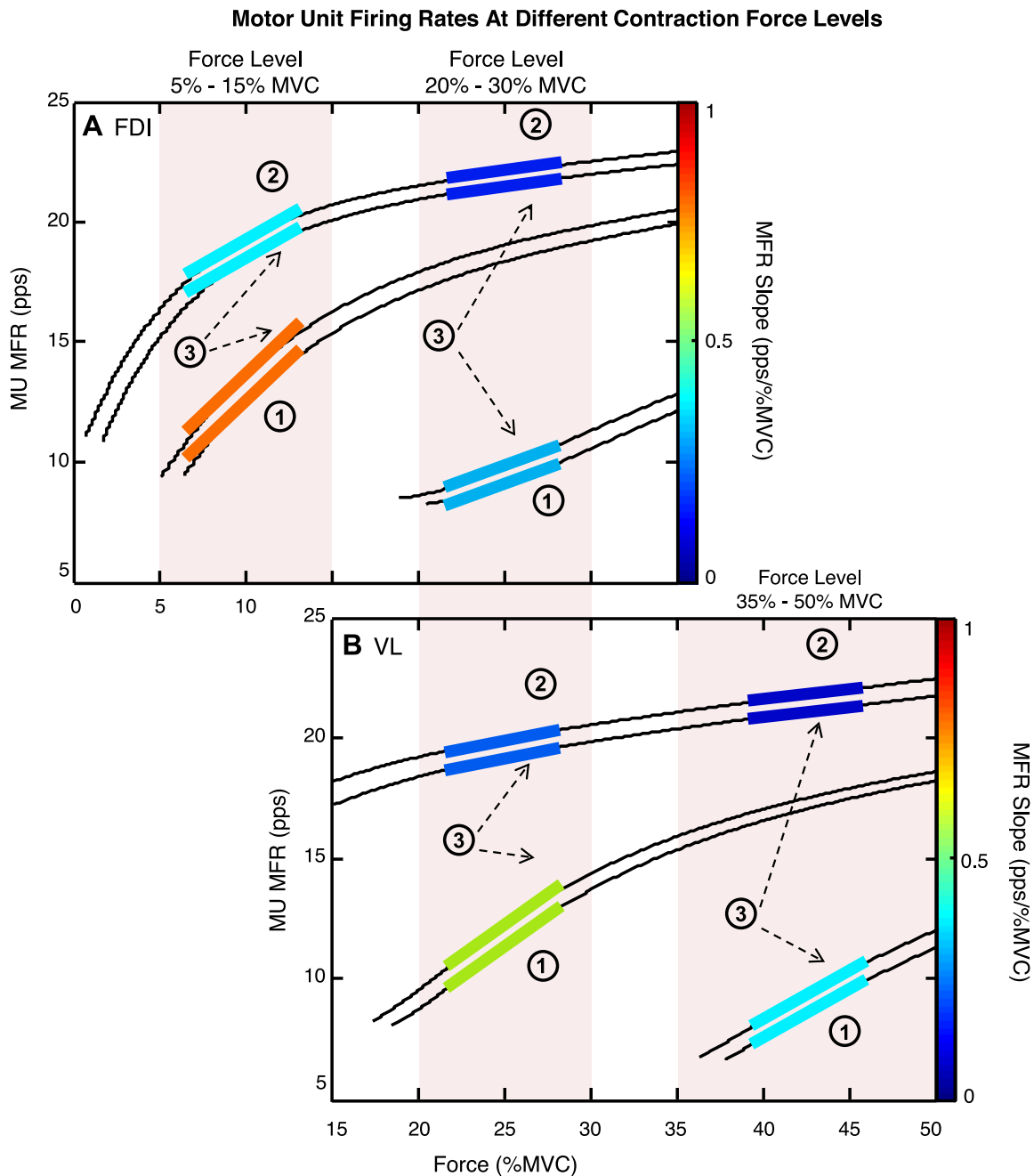


Fig. 9. A representation of the excitation plane adapted from De Luca and Contessa (2012), illustrating the association between synchronization and the slope of motor unit firing rates. The MFRs of several example motor units are plotted as a function of force for FDI data (A) and VL data (B) using the equations provided by De Luca and Contessa (2012). Slopes of the motor unit firing rates are illustrated by the tangential colored lines superimposed over MFR curves, quantified by color bars on the right. Different vertical shaded regions mark different contraction force levels. The numbers on the plots correspond to the different categories of MUAPTs and synchronization shown in Fig. 8.

force thresholds have similar firing rates that are relatively more sensitive to the voluntary excitation. Therefore, these motor units have a greater likelihood of producing coincident or near-coincident firing instances, resulting in relatively more synchronous firing instances. As the level of voluntary excitation increases, motor units recruited at the lower force threshold increase their firing rate and become progressively less sensitive to the voluntary excitation, thus reducing the incidence of synchronization. This is evidenced as *category 2* in Fig. 10 and explains the net decrease in synchronization that occurs with increases in contraction force observed in Figs.

3–5. The increase in the voluntary excitation also results in the recruitment of higher threshold motor units (*category 1* in Fig. 10). However, the firing rates of these motor units have relatively shallower slopes that lead to relatively fewer synchronous firing instances than occur between motor units firing at similar rates at the lower force level. For an example, compare *category 1* motor unit pairs between the two different force levels in Fig. 10. Throughout all force levels, some pairs of motor units fire at relatively different rates. These motor units respond more dissimilarly to voluntary excitation and are least likely to produce synchronous firing instances. Conse-

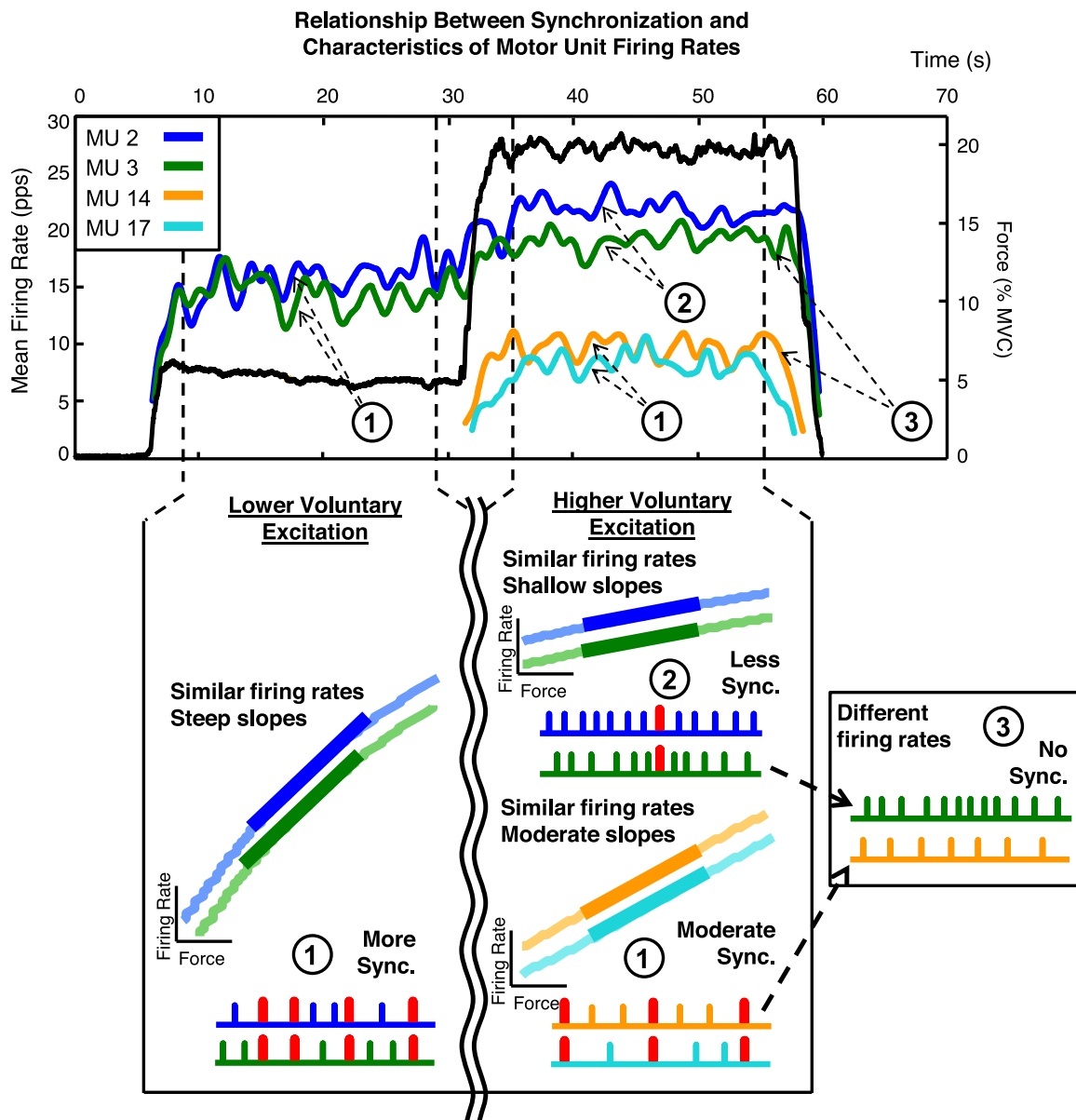


Fig. 10. A stylistic depiction of the relationship between synchronization and 2 characteristics of the motor unit firing rate: the similarity and the slope as a function of force. *Top*: the firing rates of 4 different motor units (colored lines), progressing within a contraction from a relatively low force (black line) level to a higher one. *Bottom*: different pairs of motor units are labeled by different numbered categories at each force level. These categories correspond to the same ones depicted in Figs. 6–8. For each motor unit, the slope of the firing rate as a function of the voluntary force is provided, as well as a stylistic illustration of several firing instances represented by repeated bars of the same color. Synchronous firing instances between pairs of motor units are represented by the red bars.

quently, they manifest the lowest synchronization indices (*category 3* in Fig. 10).

The relationship between synchronization and the characteristics of the firing rates is evidenced in both FDI and VL motor units, but the magnitude of synchronization and the firing rate slope differs among motor units of different muscles. For example, the high threshold motor units in the VL have firing rates with relatively steeper slopes, resulting in greater degrees of synchronization relative to the motor units active during similar force levels and firing at similar rates in the FDI. This may explain differences in the degree of synchronization reported from different muscles in the literature, among others (Bremner et al. 1991a, b; Datta et al. 1991; De Luca et al. 1993; Keen et al. 2012), and also illustrates an

important point for any synchronization analysis: synchronization can only be reliably assessed if the motor units being compared across muscles have similar firing characteristics and are active at similar force levels. Oversight of this fact could explain why Keen et al. (2012) reported correlations between the degree of synchronization and the proximity of the muscle to the spinal cord, even though the relationship had an $R^2 < 0.3$, meaning that muscle proximity fails to explain nearly 70% of the variance in synchronization data across the different muscles.

The relationship between synchronization and the characteristics of the firing rates indicates that occasionally coincident motor unit firing instances (synchronization) are likely a naturally occurring epiphenomenon of the Onion Skin property of

motor unit firing rates. Viewed from this perspective, the occurrence of synchronous firing instances is an innate consequence of the construct of motor unit firing characteristics.

Although the cause of synchronization requires further investigation, its existence raises the temptation of assigning some physiological and/or mechanical value to generating and controlling the force produced by a muscle. For example, Yao et al. (2000) used an artificial model to synthesize motor unit firing instances and argue that synchronization may have a purpose for increasing force fluctuations during voluntary contraction. However, under experimental conditions, Contessa et al. (2009) and De Luca et al. (1993) found no empirical evidence of a relationship between synchronization and force fluctuations. Instead, they concluded that synchronization is more likely epiphenomenal in nature, a position supported by our current work. As such, we believe that it may not be meaningful to attribute a specific physiological design or consequential purpose to synchronous occurrences of motor unit firing instances.

ACKNOWLEDGMENTS

The authors thank the subjects who painstakingly participated in the experiments.

GRANTS

Support for this work was provided by a grant from the National Institute of Neurological Disorders and Stroke (R43NS077526) and a grant from the Neuromuscular Research Foundation.

DISCLOSURES

C. J. De Luca is the president and chief executive officer of Delsys Inc., the company that developed the sEMG decomposition technology, and the president of the Neuromuscular Research Foundation.

AUTHOR CONTRIBUTIONS

Author contributions: J.C.K. and C.J.D.L. conception and design of research; J.C.K. performed experiments; J.C.K. analyzed data; J.C.K. and C.J.D.L. interpreted results of experiments; J.C.K. prepared figures; J.C.K. and C.J.D.L. drafted manuscript; J.C.K. and C.J.D.L. edited and revised manuscript; J.C.K. and C.J.D.L. approved final version of manuscript.

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