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HOW COMPUTATIONAL TECHNIQUE AND SPIKE TRAIN PROPERTIES AFFECT COHERENCE DETECTION

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Abstract

Spike train coherence is used to characterize common inputs that drive motor unit synchronization. However, data segmentation, overlap, and taper can affect coherence magnitude, thereby influencing the incidence at which significant coherence is detected. Also, the effect of spike train firing rate and common input variability on the detection of significant coherence is unknown. We used a pool of simulated synchronized spike trains with various firing rates (7–19 Hz), coefficients of variation (CV) (0.05–0.50), common input frequencies (10, 20, and 30 Hz, CV: 0.05–0.50), trial durations (30, 60, 90 and 120 sec.), and synchronization strength to explore the effects of segment length (1024 and 2048 1-ms samples), tapering (Hann, Nuttall, and rectangular), and overlap (0, 37.5, 50, 62.5, and 75%). Tapered segments overlapped by at least 50% maximized coherence, regardless of taper type. Coherence for 30-second trials revealed significant coherence for less than half of the motor unit pairs, demonstrating the advantages of longer trials. 2048-sample segments produced similar coherence values with twice the frequency resolution. Increasing the common input variability from 0.15–0.50 reduced coherence incidence by approximately 60%, indicating that synchronized physiological motor unit pairs may fail to show significant coherence if the common input frequency is sufficiently unstable.

Keywords

motor unit; coherence; synchronization; spike train

INTRODUCTION

Synchronized firing of motor units has been an area of increasing interest since it was first discovered. Studies have examined the relationship between motor unit synchrony and force steadiness (Semmler et al., 1995; Amjad et al., 1997; Santello and Fuglevand, 2004), task performance (Semmler et al., 1998; Maier et al., 1995a, b), and strength training (Semmler et al., 2004). Understanding these and other relationships regarding synchrony and neuromuscular performance could produce significant insights into human motor control regarding neuromuscular mechanisms, adaptation, and pathologies. However, to produce meaningful analyses, a consistent coherence calculation method and a better understanding of how it is affected by motor unit and common input properties must be established.

Coherence is derived from the auto- and cross- power spectral densities (APSD and CPSD) of the two spike trains and provides a different perspective of the synchronization process by examining the periodic firing patterns driven by one or more common inputs as opposed to

direct comparison of near-synchronous spike times used to measure short-term synchronization. Whereas short-term synchronization indices express the incidence of near-synchronous spike times, coherence reveals the strength and frequency of common inputs that drive these synchronous discharges, which are thought to be either oscillatory neural activity in the sensorimotor cortex (de Luca et al., 1993) or a subcortical region (Farmer et al., 1993), or branched common inputs at the spinal cord level (Sears and Stagg, 1976; Datta and Stephens, 1990). However, these two measures are inextricably linked mathematically since the cross-correlation used to find temporal synchrony indices is directly related to the CPSD by the Fourier transform. Therefore, if temporal synchronization exists as indicated by cross-correlation, it most likely is driven by a periodic common input, potentially detectable by measuring coherence of the same spike train data.

Comparison of motor unit coherence across studies involving the same muscles and similar tasks may be invalid if there are significant differences in coherence caused only by differences in the way it was calculated. Although there is a singular definition for coherence, there are several computational options regarding the way spike train times are processed. After the original spike train has been subdivided into segments of equal duration, individual coherence values for each segment are then averaged to find a mean coherence for the full trial duration. Larger segment size produces better frequency resolution, but also increases the variance by allowing for fewer segments. This inverse relationship between higher resolution and smaller variance forces a balance between these two factors and produces inconsistencies based on the priorities of the investigator. The number of segments can be increased by increasing the trial duration, but trial duration will be limited if fatigue effects are undesirable.

To improve resolution and reduce variance without increasing the trial duration, segment overlap and tapering can be applied (Bendat and Piersol, 2000). Overlapping segments increases the number of segments and thereby reduces the variance. Tapering data near the segment boundaries reduces the spectral leakage that produces false positive readings at other frequencies. A combination of both techniques has been shown to improve the accuracy for the analysis of continuous signals (Welch, 1967), but we do not know how effectively either process or the combination of these two processes will reduce spike train coherence variability and spectral leakage.

With no accepted standard for data segmentation parameters (size, taper, and overlap), there are notable differences in how they were applied across motor unit synchronization studies. For example, Rosenberg et al. (1989) used 1.024-second segments with no overlap or taper, Semmler et al. (2004) used 1.24-second segments with no overlap or taper, Farmer et al. (1993) used 1.024-second non-overlapped segments that were tapered with a Hann window, and Myers et al. (2004) used 2.048-second segments with 62.5% overlap and a Hann taper. Each of these studies used coherence to assess motor unit spike train synchrony, but their findings may have been partly dependent on which segment parameters were chosen.

In addition to issues related to coherence calculation, there are concerns about how coherence accuracy is affected by intrinsic motor unit properties. One prominent characteristic is synaptic noise, as indicated by firing rate variance (Nordstrom et al., 1992; Enoka et al., 1989), which may significantly reduce coherence as noise increases. This variance is likely a characteristic of the common input as well, which may also reduce the effectiveness of coherence in detecting the presence of common inputs. Modeling of synchronous motor unit discharges have been used to assess the effect of synchronization on force steadiness (Yao et al., 2000; Taylor et al., 2003; Moritz et al., 2005), however these models are inappropriate for the study of coherence since they did not include a true common input or represent a real physiological process. Another study used broad- and narrow-band common oscillatory inputs to represent the effect of cortical or subcortical drive that produced motor unit synchronization (Lowery et al.,

2007). This study found a linear relationship between coherence in the 15–30 Hz bandwidth and temporal synchronization indices, but did not examine synchronization driven by a branched common input, different coherence computation parameters, or the effect of synaptic and common input noise.

To assess the effects of segment size, taper, and overlap and the impact of spike train and branched common input frequency and variability, we used different combinations of segment parameters to measure spike train coherence for motor unit pairs with a range of firing rates and their variances, common input frequencies and their variances, and different trial durations. To create a broad database, we used a computational model to produce a large pool of spike train pairs with controlled features and synchronization levels. By comparing the coherence measurements that use different segmenting parameters on a pool of spike trains with known synchronization strength, we were able to find the segment parameters that maximized coherence and determine how spike train properties influenced its magnitude.

METHODS

EQUIPMENT

All computer code for spike simulation, coherence calculations, and statistical analyses were written in Matlab® version 7.1 with the Signal Processing and Statistics toolboxes. Computations were performed on a PC with a Pentium® D 3.0 GHz processor and Windows® XP OS.

SPIKE TRAIN GENERATION

For hand muscles exerting constant submaximal force, motor unit firing rates have been found to vary from 6–23 pulses per second (pps) (Kukulka and Clanann, 1981; Fruend et al., 1975; Moritz et al., 2005). Firing rate variabilities have been measured, as coefficients of variation (CV), from as low as 0.13 (Masakado et al., 2000) up to 0.49 (Enoka et al., 1989). Therefore, simulated spike trains were created using a range of firing rates from 7–19 pps and CVs varied from 0.05 to 0.50 for both the motor unit firing rate and the common input frequency. All spike trains were modeled as stationary signals with fixed firing rates and firing rate CVs for the entire trial duration. The set of firing rates was subdivided into reference and response pools, with each reference rate being paired with each response rate, for a total of 36 firing rate combinations. Because the computation of coherence is the ratio of the CPSD of both trains to the product of their individual APSDs, its magnitude is attenuated if the common input frequency, which is detected by the CPSD, is near or equal to the firing rate of either train, as detected by the APSD. Therefore, firing rates of 10 and 20 pps were excluded to minimize this effect for the 10 and 20 Hz common input frequencies.

To simulate the nature of motor unit firing, a “leaky integrator” model (Halliday, 1998) was used to create the spike trains. This model uses an integrate-to-threshold firing mechanism that sums all input voltages until a threshold voltage (v_{th}) is reached. Membrane voltage (v) increases at each time step as a function of the current membrane voltage and the independent input voltage (x). The voltage input for each time step (Δt) represents the graded potential contributed by an upper motor neuron or interneuron exclusive to one motor unit of the pair and was found using equation (1), where μ is the mean voltage input at each time step.

$$x_t = \mu + \sigma^* n_{\text{rand}}(t) \quad (1)$$

The input voltage variance (σ) for a given time step is a the product of the variance and n_{rand} , which is selected from an array of normally distributed random numbers (zero mean, unity variance). Membrane voltage at each successive time step (v_{t+1}) was then found using equation (2).

$$v_{t+1} = \frac{\tau}{\Delta t + \tau} v_t + \frac{\Delta t}{\Delta t + \tau} x_{t+1} \quad (2)$$

The time constant (τ) controls the rate at which the membrane voltage dissipates during each time step (Δt), creating an exponential charging curve, like that of a capacitor. The first term on the right-hand side of equation (2) represents the membrane voltage (v_t) retained during the current time step, while the second term is the contribution from the independent voltage input. For this study, a τ of 12.5 ms produced stable results for a time step of 0.5 ms, which provided sufficient temporal resolution for spike times that would eventually be assigned to 1 ms intervals (1 kHz sampling rate).

With known independent voltage inputs at all time steps established by equation (1) and the initial membrane voltage (v_0) assigned at a random value such that $0 \leq v_0 \leq v_{th} = 1.0$, membrane voltage was found by sequentially solving for v_{t+1} at each successive time step (equation 2). Each time v_{t+1} exceeded v_{th} , a spike firing time was recorded and v_{t+1} was reset to $v_{t+1} - v_{th}$ for the next time step. The process continued until t was equal to the desired trial duration (d), which produced a set of spike times, or spike train. Since firing rates and their variances cannot be directly calculated using this integrative approach, we wrote a goal-seeking algorithm to find appropriate μ and σ values for the desired firing rates and coefficients of variation. For each of the values and tolerances from Table I, the algorithm incrementally adjusted the values of μ and σ until values for all of the desired firing rate and CV combinations were obtained. These μ and σ values could then be used to produce synchronized spikes of a given firing rate and variance.

To induce synchrony, we used a common voltage that represented the activity of a branched common input of a last-order synapse at the spinal cord level. For a typical pair of motor neurons, the common input is the combined influence of all neurons that synapse onto both motor neurons. Although synchrony is likely driven by more than one neuron (Sears and Stagg, 1976), we modeled the simplest case, whereby a single branched common input influences the firing of a single motor unit pair. However, this model could be easily expanded to include more motor units and common inputs. The common input voltage is represented as y_{t+1} in equation (3), where it is modeled as a rectangular pulse train with a specified pulse width and amplitude (A_p).

$$v_{t+1} = \frac{\tau}{\Delta t + \tau} v_t + \frac{\Delta t}{\Delta t + \tau} (x_{t+1} + y_{t+1}) \quad (3)$$

$$y_{t+1} = A_p k_p \quad (4)$$

If the pulse was active during the $t+1$ time step, $k_p = 1$ and a voltage of A_p was added to the independent input ($y_{t+1} = A_p$). Otherwise, $k_p = 0$ and the membrane voltage would increase as though no common input existed. If the two motor units happen to be close to threshold when the input pulse was active, the additional input caused both membrane voltages to cross the threshold at or near the same time, producing a synchronous firing time for both motor units.

For our model, synchronization strength increased with larger pulse amplitude or width, but fixing one of these parameters simplified the model by reducing the degrees of freedom. In keeping with the original modeling work (Halliday, 1998), the pulse width was fixed at 2 ms (4 time steps) to simplify the process and approximate the duration of an action potential. The common input pulses were distributed with a given frequency (10, 20, and 30 Hz) and variance (CV = 0.05 – 0.50), which was fixed for the entire trial duration. Synchronization strength was controlled by varying common input pulse amplitude, but the correlation between synchronization strength and pulse amplitude is neither linear nor easily predictable because of the nonlinear nature of the model and the combined effect of pulse amplitude, frequency,

and frequency variance. Therefore, we used another goal-seeking algorithm that varied pulse amplitude at a given frequency and variance to attain a desired synchronization strength for each motor unit pair. However, this process required the selection of three independent variables to establish synchronization strength.

To reduce the three independent variables (pulse amplitude, frequency, and frequency variance) to a single independent variable, we used a simple cross-correlation index that allowed for direct comparison of motor unit pairs with similar short-term synchronization strength, without having to track multiple common input properties. Although physiological short-term (temporal) synchronization is not always driven by a branched common input, this model ensured that short-term synchronization would increase with a stronger common input, allowing for the use of a temporal synchronization index to assess the strength of synchronization driven by a single common input.

In addition to the basic requirement for accurate representation of the synchrony strength, the measure had to be kept simple to allow for computational efficiency, meaning that it needed to be independent of subjective criteria such as establishing cumulative sum deflection points, which would consume significant computational time. To address this issue, we took advantage of the knowledge inherent with simulated spike trains. First, the synchrony lag time typically seen in physiological motor unit pairs was nonexistent, which places the cross-correlation peak at zero lag time. Secondly, we knew that there was a single source of synchrony (a branched common input), so multiple peaks would not be an issue.

For the computational synchronization index (SI_{comp}), we used a cross-correlogram identical to those used for experimental synchrony indices (Bremner et al., 1991a; Datta and Stephens, 1990; Ellaway and Murthy, 1985; Logigian et al., 1988; Nordstrom et al., 1992; Wiegner and Wierzbicka, 1987), with lag times from -100 ms to 100 ms and 1-ms bins, with lag times centered at each bin. An event is counted each time a response spike from one train is within ± 100 ms of a reference spike in the other train. The event count for the bin corresponding to that lag time is then incremented by one for each of those occurrences. If synchrony existed, a peak would develop at the center of the cross-correlogram, allowing for the computation of a synchrony index based on the bin population within the peak region.

Instead of expending computational resources on finding peak regions by variance methods or visually identifying peak regions for thousands of motor unit pairs using CUSUM deflections (Ellaway and Murthy, 1985; Wiegner and Wierzbicka 1987, Nordstrom et al. 1992), the peak region was fixed at $-4.5 \text{ ms} \leq \text{lag time} \leq +4.5 \text{ ms}$ (the 9 central bins). The computational SI_{comp} index was then used to express synchrony as the ratio of the mean bin population within (binpop_{pk}) and outside (binpop_{npk}) the peak region, as shown in equation (5).

$$SI_{comp} = \frac{\text{mean}(\text{binpop}_{pk})}{\text{mean}(\text{binpop}_{npk})}. \quad (5)$$

To create spike train pairs with a selected synchronization strength, the pulse amplitude would be driven to a value that produced the desired SI_{comp} value for each set of motor unit pair parameters (firing rates, CVs, trial durations, etc.). This synchronization parameter is also much simpler to interpret physically than a common input pulse amplitude. For instance, synchronization strength was varied from $SI_{comp} = 1.0$, which corresponds to an unsynchronized motor unit pair, to an SI_{comp} of 1.5, which represents very strong synchronization with an average peak region bin population that is 150% of the average bin population outside the peak region. An example of coherence, cross-correlation plots and how they varied with SI_{comp} is shown in Figure 1.

For the simulated pool of motor unit pairs, there were 36 firing rate combinations, 10 firing rate CVs, 3 common input frequencies, 10 common input frequency CVs, 10 synchronization levels, and 4 trial durations (Table I). For a given spike train pair, the firing rate CVs were the same. Using all possible spike train property combinations, we created a pool of 432,000 synchronized spike train pairs from 144,000 unique spike trains with properties that approximated those of physiological motor units during submaximal contractions of hand muscles.

COHERENCE CALCULATION

Calculating the coherence between two stochastic point processes such as two spike trains (denoted as x and y), requires the Fourier transform of each spike train and the cross- and auto-spectra of these transforms (Rosenberg et al., 1989). To perform the transform summations, the spike train was subdivided into L segments of length T for the entire train duration ($d = L \cdot T$). Once the auto- and cross-spectra were calculated at each frequency, the spectral coherence was calculated using equation (6).

$$|R_{xy}(f)|^2 = \frac{|\Phi_{xy}(f)|^2}{\Phi_{xx}(f)\Phi_{yy}(f)} \quad (6)$$

$|R_{xy}(f)|^2$ represents the magnitude-squared coherence for spike trains x and y at frequency (f), which is the ratio of the squared magnitude of the cross-spectral density (Φ_{xy}) and the product of the auto-spectra of each spike train ($\Phi_{xx}(f)$ and $\Phi_{yy}(f)$). After digitizing each spike train at a sampling rate of 1 kHz, each sampling interval was coded. If a spike event occurred within the bounds of a 1-ms interval, the value for that interval was set to “1”, otherwise, it was set to “0”. Each train was then detrended to remove low-frequency responses caused by slow drifts in mean firing rates. Coherence for each pair was then found using the Matlab *mscohere* function, which uses the described technique and allows for convenient manipulation of segment size, taper, and overlap.

Random synchronization will cause coherence to be non-zero and results in some level of noise. To establish when coherence is significant, an upper confidence bound is found using an equation based on the chi-square distribution of coherence that assumes data stationarity (Brillinger, 1978). After selecting a type 1 error (α), the significance level (Z) is found using equation (7)

$$Z = 1 - \alpha^{\frac{1}{L-1}} \quad (7)$$

Our focus was on 1) finding the technique that maximized the incidence at which significant coherence was detected (coherence incidence) and 2) determining the effect of motor unit spike train parameters on the incidence value. The incidence of significant coherence was found by dividing the number of times the coherence rose above the significance level divided by the number of times coherence was calculated within the detection bandwidth for a group of motor unit pairs. For all techniques examined, the detection bandwidth comprises the frequency interval containing the common input frequency and the closest adjacent interval. Ideally, this coherence incidence should be 100% for all motor unit pairs in this study, which were all driven by a common input. Therefore, the incidence indicates how reliably coherence will detect a common input for a given set of motor unit properties and segmentation parameters.

After the coherence was computed for each motor unit pair, the coherence incidence values were calculated so that they could be compared when grouped by parameter (firing rate, variance, etc.). The volume of computations precluded the computation of multiple copies of motor unit pairs with the same fixed set of properties. Instead, each data group consisted of all motor unit firing rate combinations at each common input frequency across a range of property

values, as described and as shown in each figure. If a significant correlation was found between the dependent variable under study (usually coherence incidence) and either firing rate or common input frequency, then those groups were subdivided to remove that correlation and the results were presented. Unless otherwise noted, these groups comprised a set of 36×3 values for the 36 reference/response firing rate combinations and three common input frequencies.

SEGMENT TAPER AND OVERLAP

We examined two tapering windows, Hann and Nuttall, along with a non-tapered (rectangular) window. The Hann window is commonly used (Bendat and Piersol, 2000) because of its balance between narrow main lobe width (for resolution) and sidelobe suppression (for reduced spectral leakage). Compared to the Hann window, the Nuttall window has significantly better sidelobe suppression, which should make it more effective at reducing spectral leakage at the expense of poorer resolution caused by its wider main lobe (Nuttall, 1981). The equations for each window are:

Hann window—

$$W(k+1) = 0.5 \left(1 - \cos \left(\frac{2\pi k}{nfft-1} \right) \right) \quad (8)$$

for segment number $k = 0, \dots, nfft-1$, where $nfft$ is the number of segment elements ($T/\Delta t$)

Nuttall window—

$$W(k+1) = a_0 - a_1 \cos \left(\frac{2\pi k}{nfft-1} \right) + a_2 \cos \left(\frac{4\pi k}{nfft-1} \right) - a_3 \cos \left(\frac{6\pi k}{nfft-1} \right) \quad (9)$$

for $k = 0, \dots, nfft-1$, where $a_0 = 0.3635819$, $a_1 = 0.4891775$, $a_2 = 0.1365995$, and $a_3 = 0.0106411$. As these two windows are applied to spike train data in each segment, the magnitude of each spike is scaled down, or tapered, as the spike is located close to the segment boundary, until spike train magnitudes at the boundaries are scaled to zero. The shapes of the rectangular, Hann, and Nuttall windows are shown in Figure 2.

Equation (7) is viable for non-overlapping segments, even after tapering in the time domain. However, some accommodation must be made to account for overlapped segments. This modification (Welch, 1967) is:

$$Z_{ovlp} = 1 - \alpha^{\frac{1}{wL-1}} \quad (10)$$

where L^* is the number of overlapped segments, found using equation (11).

$$L^* = \text{floor}((L-1)/(1-ovlp)) + 1 \quad (11)$$

The variable $ovlp$ is the percentage of segment overlap and w is a weighting factor that is dependent on the amount of overlap and taper type. For tapered segments with window W and $nfft$ segment elements, w is found using equation (12).

$$\left[\sum_{k=0}^{nfft-1} W^2(k) \right]^2 \quad (12)$$

For this study, $nfft$ was set at 1024 and 2048, which produced frequency resolutions of 0.98 and 0.49 Hz, respectively, when using a 1 kHz sampling rate. The comparison of coherence incidence for these two segment lengths allowed us to examine the effect of temporal versus frequency resolution for different trial lengths and computational parameters.

Although tapering can improve accuracy and reduce spectral leakage, it can also eliminate synchronized spikes near segment boundaries and increase variability by reducing signal continuity. However, both of these issues can be ameliorated by overlapping tapered segments. For this study, we examined segment overlap of 0, 37.5, 50, 62.5, and 75%. A summary of all coherence segmentation parameters is given in Table II.

STATISTICS

Coherence is a bounded quantity ($[0,1]$) that follows a chi-squared distribution (Bendat and Piersol, 2000). Therefore, nonparametric tests were used to compare means between two or more groups. The coherence is expressed in terms of the incidence at which coherence exceeds the significance level at the common input frequency and is referred to as the incidence of significant coherence, which is the percentage of cases which show significant coherence within the detection bandwidth. Every simulated pair was synchronized to some degree, so the incidence would ideally be 100%. How far this value falls below 100% indicates the reliability of coherence measurements for the data segmentation and motor unit parameters.

For comparison of significant coherence incidence between two groups, a Wilcoxon rank-sum test was used to identify significant differences. In the case of comparisons involving more than two groups, Friedman's ANOVA based on ranks was used, along with a Tukey-Kramer post-hoc test to determine which group interactions were significant. Differences were deemed significant only if tests produced a p-value that fell below the type 1 error level (α) of 0.05. Unless otherwise stated, errors and error bars are equivalent to the 95% confidence interval (CI).

RESULTS

To provide an equitable comparison of data segmentation parameters, we fixed the sampling rate at 1 kHz and the frequency resolution at 0.97 Hz (1.024-second segment length) and varied only segment taper (rectangular or Hann) and overlap (0 or 50%), as shown in Figure 3. The combination of 50% overlap and a Hann taper yielded highly significant increases in coherence incidence ($p < 0.001$). The difference in mean coherence incidence between this technique and the baseline technique increased from 5.9% for 30-second trials ($p = 0.0009$) to 9.3% for a trial lengths of 90 and 120 seconds ($p = 0.0003$ and $p = 0.0004$, respectively). Although the combination of Hann taper and 50% segment overlap produced consistently significant advantages over the three other combinations, we also explored the possibility that other taper/overlap combinations might yield even higher coherence incidence.

To address this issue, we compared coherence incidence for a markedly different taper window (Nuttall) and overlap amounts (0, 37.5, 62.5, and 75%). For overlap amounts of 50% or less, coherence incidence for the Hann taper were higher than for the Nuttall taper, but these differences were not significant (Figure 4). The increase in coherence incidence was negligible for overlap amounts of 62.5 and 75% (Hann: 1.1%, Nuttall: 0.9%) and neither was significant. For the Hann window, the coherence incidence grew by 2.3% when overlap was increased from 50% to 75%, but this improvement was also not significant. However, this same increase in overlap produced a significant improvement of 6.2% for the Nuttall taper ($p = 0.018$). Of the combinations that produced coherence incidence not significantly different from the highest rate, the Hann taper/50% overlap combination produced the highest coherence incidence with the least overlap. Therefore, we continued to use this segment overlap for the remainder of the analyses.

A comparison of significant coherence incidence when using segment lengths of 1024- and 2048-sample segments showed that the mean coherence incidence values were slightly, but consistently, lower for the 2048-sample segments for all trial durations (Figure 5). However,

none of these differences were significant. To take advantage of the improved frequency resolution, we used the 2048-sample segment for the remaining comparisons.

Having established the segment parameters (size, overlap, and taper) that produced the highest coherence incidence values, we next explored the interactions between motor unit firing rate and common input frequency to ensure that they did not skew coherence incidence in a way that produced misleading trends. In spite of this precaution, there was still a noticeable interaction between these two properties (Figure 6). Each successively longer trial produced significantly higher coherence incidence values for all three input frequencies, with only one exception (10 Hz, 120-s trial). More importantly, the coherence incidence for all trial durations was significantly lower for common input frequencies of 10 Hz than that of the 30 Hz case ($p=0.0007$ for 120-second trial). There were also significant differences between the coherence incidence values for the 20 and 30 Hz cases for the 90- and 120-second trial ($p=0.0428$ and $p=0.0328$, respectively).

Unlike the relationship between motor unit firing rate and common input frequency, the correlation between coherence incidence and both trial length and synchrony level were predictable and proportional (Figure 7). These trends were confirmed with a few exceptions. Low synchrony ($SI_{comp} = 1.05$) incidence values failed to change noticeably for trial durations of 60, 90, and 120 seconds, which is why values for that synchrony level were not included in any of this study's previous comparisons. For synchrony levels as high as that corresponding to $SI_{comp}=1.2$, 120-second trials failed to yield average coherence incidence above 50%, making a false negative detection at and below this synchrony level highly likely. In general, the 30-second trials were too short to reliably detect synchronization, failing to produce coherence incidence above 50% even for the highest synchrony level studied. The correlation between synchrony level and coherence incidence was nearly linear for all trial durations ($R^2=0.975$ for 120-s trials), but this correlation became stronger as trial duration increased, as indicated by steeper regression slopes.

Coherence incidence for pairs with increasing motor unit firing rate variabilities (CV_{fr}) were adversely affected by this variance (Figure 8), especially when the CV_{fr} increased from 0.05 to 0.15. However, once CV_{fr} increased beyond this level, the correlation diminished. More significantly, the effect of variance of the common input frequency (CV_{com}) was far more pronounced in the range of 0.15 to 0.35, where a CV_{com} in excess of 0.25 causes coherence incidence values to drop below 50%, even for 120-second trials. This correlation weakened as CV_{com} increased beyond 0.4, but coherence incidence was below 30% at that point for all trial lengths.

Increasing common input and firing rate variance reduces coherence incidence, regardless of short-term synchronization strength (Figure 9). When both variances are 0.05, the incidence quickly rises above 85% as soon as synchronization strength rises above noise level ($SI_{comp}=1.05$) and is near or equal to 100% for SI_{comp} values of 1.2 or higher. However, coherence incidence rapidly degrades with an increase in both variances. Doubling the detection bandwidth from 2 to 4 frequency bins (0.98 to 1.96 Hz bandwidth) to capture significant coherence values that may have "leaked" to adjacent bins improves incidence somewhat. However, incidence is still less than 50% for a CV of 0.35. The possibility that coherence is significant outside of the 4-bin detection band is dispelled by the low incidence for this region, which is at or only slightly higher than that expected for a 5% significance level (Figure 10). For physiological CV values (0.15–0.35), these incidence values are all between 5 and 6%.

The effect of common input and firing rate variability also has a strong effect on the pulse amplitude needed to attain a desired level of short-term synchronization (Figure 11). Pulse

amplitude increased with synchronization strength, as indicated by the SI_{comp} index. However, this correlation was stronger for a common input frequency of 10 Hz and for higher firing rate variances (CV_{fr}). The difference between pulse amplitudes for a 10 Hz common input frequency and those for the other two frequencies was only significant when CV_{com} was fixed at 0.25 and CV_{fr} was in the low range ($p=0.024$). To attain the same level of short-term synchronization with a fixed common input frequency and CV_{com} (0.25), the pulse amplitude had to be 3–5 times larger for spike train pairs with a CV_{fr} in high (0.35–0.5) range, versus pairs with low CV_{fr} values (0.05–0.20). For each of the common input frequencies, the differences in pulse amplitudes were statistically different ($p<0.001$), but differences caused by CV_{com} values were not.

DISCUSSION

The impetus behind this study was to determine if spike train coherence was significantly affected by data segmentation, synchronization level, trial duration, and spike train parameters. Our study of the effects of segmentation parameters and spike train properties on coherence measurements have yielded some important conclusions: (1) calculating coherence using data segments tapered by a Hann window and overlapped by 50% maximized coherence incidence (2) 2048-sample segment lengths produce twice the frequency resolution of 1024-sample segments, while producing coherence incidence values that were not significantly different, (3) experimental protocols for motor unit synchrony studies should be as long as practical and no shorter than 30 seconds, and (4) the increase in firing rate variances over the range seen in physiological experiments did not significantly reduce coherence incidence. However, increases in common input variance across this same range caused a pronounced reduction in coherence incidence.

Previous motor unit coherence studies have used an inconsistent set of data segmentation parameters, which may have affected their outcome and our ability to make direct study comparisons. We reviewed the methods used for previous motor unit coherence studies (Table III). Of the 32 studies reviewed, 19 used no segment taper or overlap, 4 used only a taper, and 3 used a taper and overlap. None of the studies used overlapped segments exclusively and 6 studies did not identify any coherence calculation parameters. Also, several studies used trial durations far shorter than the shortest duration studied here, which would preclude detection of significant coherence except for very high levels. For example, coherence measurements have been applied to trials that were only 5 seconds (Kakuda et al., 1999) and 8 seconds (Kilner et al., 2002) long. The broad range of study parameters demonstrates the need to assess how these choices affect coherence incidence. Several findings in this study show that these choices could have significant impact on study results, which could mean the difference between a positive and negative finding of coherence. This effect may be particularly important for experiments with short trial durations or small subject pools, which are common in this field of study.

For the comparison of the four taper/overlap combinations, we found that the combination of segment taper and overlap produced significantly higher coherence incidence values as compared to the other three taper/overlap combinations. Most likely, the reduced leakage through tapering is countered by the attenuation of data that represents synchronized motor unit spikes. Similarly, overlapping may reduce the variance, but creates more leakage by introducing the additional discontinuities created by a larger number of segments. The drawbacks of each technique appear to be reduced by using them together. Overlapping tapered segments helps prevent portions of data from being marginalized by tapering and also removes the effect of discontinuities at the segment bounds caused by the larger number of segment boundaries generated by overlapping. The Hann window produced coherence incidence values that were consistently higher than those for the Nuttall window, but the differences were not

statistically significant, which indicates that window choice is not critical, considering these windows are near opposite ends of the spectrum in window design. Also, increasing the overlap beyond 50% did not produce significantly higher coherence incidence values for either window type, which shows that there is no need to use a segment overlap of more than 50%.

Regardless of segment overlap and taper, longer trials increased coherence incidence. Low synchrony ($SI_{comp} = 1.05$) produced coherence incidence values that were essentially insensitive to trial duration. Most likely, the synchrony was so small that the SI_{comp} index failed to consistently detect synchrony due to the variability of the index and the error level of 5%, which made random and stimulated synchrony indistinguishable. Even for motor unit pairs with double that synchrony ($SI_{comp} = 1.10$), the coherence incidence was still below 20% for 120-second trials, indicating that coherence studies will most likely fail to detect this level of synchrony. The synchrony level had to exceed an SI_{comp} of 1.2 in order to produce coherence incidence above 50% for a 120-second trial, which indicates that, regardless of how coherence is calculated, it may be less sensitive than cross-correlation techniques for detecting the presence of synchronization and may be unreliable in detecting lower synchrony levels. In general, increasing trial duration by 30 seconds always produced significantly higher coherence incidence values. However, since coherence incidence never exceeded 50% for the highest synchrony level, motor unit studies should be designed to record continuous spike trains that are longer than 30 seconds and as long as practical. If longer trials are not possible, an experiment incorporating the use of aggregate results, such as pooled coherence (Amjad et al., 1997), should be considered.

For studies of equal trial duration, the effect of using different segmentation parameters, the incidence differences were statically significant, but did not exceed 15% over a typical range of variabilities, firing rates, and synchronization levels similar to those that would be seen in a typical motor unit study. Whether the statistical differences in coherence incidence produce meaningful differences in a given motor unit coherence study will depend on the type of comparison made. A 10% improvement in coherence incidence for a study with 10 subjects will mean that coherence may be detected in one additional subject, which may or may not be important to the study's outcome. However, if comparisons of two studies that incorporate the same task performed by the same muscles produce different coherence values, there will be some question as to how much of that difference is attributable to the use of different coherence computation methods. Also, one should consider the effect of combined differences in segmentation parameters and trial duration, which could yield much larger discrepancies across study results.

Common input frequency and motor unit firing rates will always have some variability, which greatly reduced coherence incidence as variances increased. For the firing rate variability, this effect was minimal for CVs seen in physiological studies, which vary from 0.15 to 0.50 (Enoka et al., 1989; Masakado et al., 2000). However, the effect of common input frequency variability (CV_{com}) is larger than that of the CV_{fr} , particularly for values higher than 0.15. The combined effect of these variances on coherence incidence with regard to synchronization strength (Figure 8 and Figure 9) show that coherence measurements are extremely effective for motor units and common inputs with small variances, with incidences near or at 100% even for low synchronization levels. Unfortunately, this effectiveness rapidly diminishes with increasing variances to the point that incidence at the highest synchronization level was still below 50% for a CV_{com} of 0.35, which is still well within the physiological realm of synaptic noise. Assuming that the variabilities for the common input are equivalent to those for the firing rate, this property may create problems for studies that include highly variable common inputs, which cannot be measured directly, or for long-term studies, where the firing rate variance increased over time (Nordstrom et al., 1992; Enoka et al., 1989). The effect of this spike train parameter will be difficult to isolate, however, we may be able to reduce our uncertainty by

dividing long trials into shorter epochs and tracking mean coherence incidence and its variability in both magnitude and frequency for the entire trial, which is essentially an extension of the data segmentation techniques examined here.

As discussed previously, it was not possible to correlate coherence incidence directly to the strength of the common input because its strength was dependent on pulse amplitude, common input frequency, and frequency variability, at a minimum, which is why a single temporal index was used to quantify synchronization strength. The correlations shown in Figure 11 supports this statement and shows the complex relationship between these parameters. The correlation between pulse amplitude and short-term synchronization is important to verify, since it confirms the near-linear relationship between common input strength and short-term synchronization strength. This correlation is more sensitive to variance in the spike train firing rate than it is to common input frequency variance, which demonstrates that short-term synchronization is more difficult to achieve and detect with noisier spike trains, as opposed to the difficulty in detecting coherence for noisy common inputs. Although these relationships are restricted to a model that represents a simple branched common input, they are likely to extend to models incorporating narrow- and broad-band oscillatory common inputs used in a similar study (Lowery et al., 2007).

In addition to revealing the effect of data segmentation and motor unit properties on coherence measurements, this study also exposed some potential weaknesses of the coherence metric itself. Results of this pool of simulated motor unit pairs produced many cases for which temporal synchronization was present but coherence calculations failed to detect a common input more than half the time. And, if the common input was particularly noisy, there was a pronounced likelihood that even highly synchronized motor unit pairs would be classified as non-synchronous if only coherence was measured. These discoveries serve to emphasize the need to use the most reliable coherence calculations, complement them with temporal synchrony measurements, and fully understand the characteristics of the motor units under study. All of these findings should be carefully considered when planning and analyzing future motor unit studies so that we can produce reliable results and enable fair study comparisons that are critical to the advancement of this area of neuromuscular physiology.

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Figure 1.

Comparison of coherence and cross-correlation for motor unit pairs with the same properties but different synchronization strength, as quantified by SI_{comp} . For these pairs, the properties were a reference train firing rate of 12 pps, response train firing rate of 13 pps, firing rate CV of 0.20, common input frequency of 20 Hz and a common input frequency CV of 0.15. Trial duration was 120 seconds.

Figure 2.

Representative curves for each of the taper windows used in this study. While the rectangular data passes all data for processing, the Hann and Nuttall windows attenuate data located near the segment bounds while passing most of the data at the segment's center.

Figure 3.

Coherence incidence for calculation methods using the four segment taper and overlap combinations for the same pool of simulated spike train pairs. Using a combination of segment taper and overlap produces significantly higher coherence incidence than the baseline method (no taper/no overlap). An asterisk (*) indicates a significant difference between coherence incidence at that trial duration and the next shorter duration, whereas a plus (+) indicates a significant difference between that method and the no taper/no overlap method at that trial duration.

Figure 4.

Coherence incidence for Hann and Nuttall windows at different segment overlap. As compared to the Hann window, the Nuttall window yielded slightly lower mean coherence incidence for overlaps of 50% or less, but this difference was never significant. An asterisk (*) denotes a significant difference between the mean coherence incidence for that overlap and 75%, for the given window type.

Figure 5.

Comparison of coherence incidence for segments with 1024 and 2048 samples. Doubling the segment length produced small reductions in mean coherence incidence that were not significant for any trial duration.

Figure 6.

Comparison of coherence incidence for different common input frequencies. When coherence incidence values were categorized by common input frequency, they were significantly lower when the common input frequency was 10 or 20 Hz, as compared to those for 30 Hz, as indicated by the plus (+) sign. An asterisk (*) indicates a significant difference between coherence incidence values at that trial duration and the next shorter duration, for a given common input frequency.

Figure 7.

Linear regression of coherence incidence versus synchronization level (SI_{comp}). Coherence incidence was strongly correlated to the synchronization level of the simulated motor unit pair, as indicated by SI_{comp} . Higher regression slopes indicate this correlation strengthened as trial duration increased. For these comparisons, 2048-sample segments and all spike trains with CVs of 0.15–0.35 were used to measure coherence incidence.

Figure 8.

Variabilities of the firing rate (CV_{fr}) and common input frequency (CV_{com}) adversely affect coherence incidence as they increased. For the variabilities commonly seen in motor unit studies ($CV=0.15-0.50$), this effect was far more pronounced for the common input variability (CV_{com} - dotted lines) than the firing rate variability (CV_{fr} - solid line).

Figure 9.

Average coherence incidence for detection bandwidths of 2 bins (dashed) and 4 bins (solid) wide. The difference in incidence rates increased with CV, but incidence rates were still below 50% when CV=0.35. Note that coherence incidence exceeded 85% when both CVs were 0.05 and reached 100% for $SI_{comp} \geq 1.25$.

Figure 10.

Coherence incidence for significant coherence detected outside of the 4-bin (1.96 Hz) detection bandwidth. For physiologically representative CV values (0.15–0.35), the 5% significance level was well-matched by the noise level, however, there was a slight underprediction of the true 5% significance level as synchronization increased. Incidence is higher for a CV of 0.05, indicating that false positive detections outside the detection bandwidth were more common.

Figure 11.

Pulse amplitude of the simulated branched common input was correlated to short-term synchronization strength (SI_{comp}) and dependent on common input frequency and CV_{fr} (lower plot), but not CV_{com} (upper plot). Significantly larger pulses ($p=0.24$) were required to increase short-term synchronization strength (SI_{comp}) when the frequency was 10 Hz and CV_{fr} was low (0.05–0.20). For each of the common input frequencies, pulse amplitudes were significantly larger ($p<0.001$) for higher CV_{fr} values (0.35–0.50).

Table I

Summary of motor unit firing properties and synchrony parameters used for simulated spike trains.

Parameter	Range/Values	Increment	Tolerance
Firing Rate (pps)	Reference: 7,11,12,15,16,19	1.0	0.05
	Response: 8, 9, 13, 14, 17, 18		
Firing Rate Variance (CV_r)	0.05 – 0.50	0.05	0.005
Common Input Frequency (Hz)	10 – 30	10	-
Cmn. Input Freq. Variance (CV_{com})	0.05 – 0.50	0.05	0.005
Trial Length (seconds)	30 – 120	30	-
Synchrony (SI_{comp})	1.00 – 1.50	0.05	0.01

Table II

Data segmentation parameters for comparison of coherence calculation techniques.

Parameter	Value/Type
Segment Size (samples)	1024, 2048
Frequency Resolution (Hz)	0.98, 0.49
Detection Bandwidth (Hz)	1.96, 0.98
Taper	Rectangular, Hann, Nuttall
Overlap	0, 37.5, 50, 62.5, 75%

Table III

Coherence sampling and segmentation parameters for previous motor unit studies.

Segmentation	# of Studies	Samp. Rate (Hz)	Seg. Size (samples)	Freq. Res. (Hz)	Taper	Overlap
No Overlap No Taper	9	200	256	0.78	-	-
	5	1000	1024	0.97	-	-
	2	500	various	various	-	-
	1	200	1024	0.195	-	-
	1	300	512	0.59	-	-
	1	400	512	0.78	-	-
Taper w/o Overlap	1	500	4000	0.125	Hann	-
	1	500	various	various	Triangular	-
	1	1000	1024	0.97	Hann	-
	1	2000	4096	0.49	Hann	-
Taper w/ Overlap	1	1000	2048	0.49	Hann	62.5%
	1	1000	not spec.	not spec.	not spec.	not spec.
	1	not spec.	not spec.	not spec.	not spec.	not spec.
Not Specified	6	-	-	-	-	-