

A peer-reviewed version of this preprint was published in PeerJ on 10 December 2019.

[View the peer-reviewed version](https://doi.org/10.7717/peerj.7907) (peerj.com/articles/7907), which is the preferred citable publication unless you specifically need to cite this preprint.

Tweedell AJ, Tenan MS. 2019. motoRneuron: an open-source R toolbox for time-domain motor unit analyses. PeerJ 7:e7907
<https://doi.org/10.7717/peerj.7907>

motoRneuron: an open-source R toolbox for time-domain motor unit analyses

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Motor unit synchronization is the tendency of motor neurons and their associated muscle fibers to discharge near-simultaneously. It has been theorized as a control mechanism for force generation by common excitatory inputs to these motor neurons. Magnitude of synchronization is calculated from peaks in cross-correlation histograms between motor unit discharge trains. However, there are many different methods for detecting these peaks and even more indices for calculating synchronization from them. Methodology is typically laboratory-specific and requires expensive software, like Matlab or LabView. This lack of standardization makes it difficult to draw definitive conclusions about motor unit synchronization. To combat this, we have developed a freely available, open-source toolbox, “motoRneuron”, for the R programming language. This toolbox contains functions for calculating time domain synchronization using different methods found in the literature. Our objective is to detail the program’s functionality and provide a clear use-case for implementation. The programs primary function “mu_synch” automatically performs the cross-correlation analysis based on user input. Automated peak detection methods such as the cumulative sum method and the z-score method, as well as subjective, visual analysis are available. Users can also define other parameters like the number of recurrence intervals to be used and histogram bin size. The function outputs six common synchronization indices, the common input strength (CIS), k' , $k'-1$, E, S, and Synch Index. This toolbox allows for better standardization of techniques and for more comprehensive data mining in the motor control community.

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Abstract

Motor unit synchronization is the tendency of motor neurons and their associated muscle fibers to discharge near-simultaneously. It has been theorized as a control mechanism for force generation by common excitatory inputs to these motor neurons. Magnitude of synchronization is calculated from peaks in cross-correlation histograms between motor unit discharge trains. However, there are many different methods for detecting these peaks and even more indices for calculating synchronization from them. Methodology is typically laboratory-specific and requires expensive software, like Matlab or LabView. This lack of standardization makes it difficult to draw definitive conclusions about motor unit synchronization. To combat this, we have developed a freely available, open-source toolbox, “motoRneuron”, for the R programming language. This toolbox contains functions for calculating time domain synchronization using different methods found in the literature. Our objective is to detail the program’s functionality and provide a clear use-case for implementation. The programs primary function “mu_synch” automatically performs the cross-correlation analysis based on user input. Automated peak detection methods such as the cumulative sum method and the z-score method, as well as subjective, visual analysis are available. Users can also define other parameters like the number of recurrence intervals to be used and histogram bin size. The function outputs six common synchronization indices, the common input strength (CIS), k' , $k'-1$, E, S, and Synch Index. This toolbox allows for better standardization of techniques and for more comprehensive data mining in the motor control community.

Introduction

Motor unit synchronization is the tendency of separate motor units (i.e. motor neurons and their associated muscle fibers) to discharge near-simultaneously (within 1 – 5 ms of each other) more often than would be expected by chance (Farmer et al. 1997; Semmler 2002). It is often interpreted as an indicator of functional connectivity between motor neurons through common excitatory post-synaptic potentials (Sears & Stagg 1976). Typically, cross-correlation analyses are employed, whereby the discharge times of one motor unit are correlated against those of another concurrently active motor unit (*Fig. 1*) and a histogram is created based on these recurrence intervals. Peaks in the histogram represent a higher probability of a discharge from the response motor unit around that latency of the reference motor unit discharge (seen in *Fig. 2B*). Various indices are calculated from these peaks and their magnitude indicates the level of synchronization (for review see (Farmer et al. 1997; Semmler 2002; Farina & Negro 2015)). This time-domain synchronization appears to be a critical factor in force modulation. For example, synchronous activation of muscle fibers produces longer and greater twitch forces than if they were activated asynchronously (Merton 1954). In practice, this phenomenon is evidenced in strength-trained individuals, who display higher motor unit synchrony than untrained individuals do (Semmler & Nordstrom 1998; Fling et al. 2009). Although beneficial for producing high forces, synchronization has been shown to be detrimental to force steadiness (Yao et al. 2000). Thus, understanding motor unit synchronization seems to be important for modeling neuromuscular performance.

Over the last few decades, it has become much easier and cheaper to collect motor unit action potentials with either intramuscular or decomposed surface electromyography. Researchers have gone from examining synchronization in 2-3 motor units to 15+ in a single contraction (Schmied & Descarreaux 2010; Defreitas et al. 2014). Unfortunately, while data collection technology has improved and multiplied, so have the options for synchronization analysis. Reconciling results from different types of analyses remains difficult. Concerning the cross-correlation analysis, there are numerous ways in which to determine the size and location of peaks present in histograms. Methodology is largely laboratory specific, with some groups using automated methods like the z-score method or the cumulative sum method. Before automated methods were developed, subjective, visual analysis was used. Within these methods, parameters such as the number of orders of recurrence intervals used and histogram bin size are likely to vary as well. Additionally, there are a number of indices available to characterize synchronization magnitude. Common input strength (CIS) and k' (“k prime”) are most often reported; however, the Synch Index (SI) and others are available. The lack of standardization in respect to motor unit synchronization hinders ability to make definitive conclusions. Therefore, we have developed the open-source toolbox “motoRneuron” in the statistical programming language R (henceforth referred to as R) for the calculation of time-domain synchronization using various peak determination methods. This toolbox provides a list of functions to calculate recurrence intervals, create and plot cross-correlation histograms, and ultimately, calculate synchronization indices.

R has quickly risen in popularity recently because of its very active user/developer base that rapidly iterates to improve the functionality of the language. Typically, programs that perform synchronization analyses are handed down through laboratories using paid software like Matlab and LabView. Meanwhile, R and our toolbox are freely available. Source code for all R

functions are available to the user. MotoRneuron was created as a free, open-source platform with which users can perform all necessary functions to calculate synchronization, or alter to suit their unique needs. With the numerous ways to calculate synchronization, this toolbox allows for better standardization of techniques and for more comprehensive data mining in the motor control community. The objective is to detail the functionality of the motoRneuron toolbox for investigating motor unit time-domain synchronization.

Functionality and Application

R Programming Environment

MotoRneuron was developed in RStudio (v 1.1.453) using R (v 3.5.0) (R Development Core Team 2015). It was configured on a Windows 10 computer (Enterprise V. 1703. Intel® Core™ 2.80 GHz, x64-based processor). The toolbox is under the GNU General Public License version 3. This paper will discuss general methods used and applications for the motoRneuron toolbox. For readers unfamiliar with the R language, sample motor unit data and R scripts with instructions are provided in the Supplementary Material. In addition, packages will always include help files for specific details about their functions. It is highly recommended to download R and RStudio in order to follow along with the sample scripts provided. Briefly, R uses command-line scripting to perform functions on data within the working environment. Common data formats for R are vectors, matrices, lists, and data frames, which can be imported into the working environment from any number of formats, including text or csv files. MotoRneuron leverages many functions not included in base R which are automatically incorporated by downloading the following add-on packages from Github or the Comprehensive R Archive Network (CRAN): ‘dplyr’, ‘ggplot2’, ‘dygraphs’, ‘magrittr’, and ‘tseries’ (Milton-Bache & Wickham 2014; Trapletti & Hornik 2018; Vanderkam et al. 2018; Wickham et al. 2018a; Wickham et al. 2018b).

To access motoRneuron through R and all the functions, sample data, and help files wherein, the following functions are called in the console of RStudio. “*install.packages*” will automatically download the package from CRAN. “*library*” will attach the packages items to your working environment for use.

```
> install.packages("motoRneuron")
> library(motoRneuron)
```

Package Implementation

In general, there are three steps involved in calculating time-domain synchronization. First, the cross-correlation histogram is created. Second, the size and location of the peak is determined. Last, synchronization indices are calculated based on the size of the peak. The primary function of motoRneuron is *mu_synch*, which completes all three steps based on the user’s inputs. The function’s syntax and five formal arguments are:

122

123 `mu_synch(motor_unit_1, motor_unit_2, method, order, binwidth, plot)`

124

125 *Motor_unit_1* and *motor_unit_2* arguments are vectors of the discharge times of two motor unit
126 action potential trains. Included in *motoRneuron* is a real world data set that will be used to
127 reduce and analyze motor unit synchronization. The data set was selected from a previous study,
128 with informed consent and in accordance with the United States Army Research Laboratory
129 Institutional Review Board (approval number ARL 16-099), using fine wire electromyography
130 from the flexor digitorum superficialis during a 30-second isometric finger flexion task. The data
131 format is a data frame time series of two concurrently active motor units, named *motor_unit_1*
132 and *motor_unit_2*. Here we provide the code to read in the data and reduce into the constituent
133 motor units discharge times for further use in the package.

134

```
135 > Sample_data <- motoRneuron::motor_unit_data
136 > motor_unit_1 <- as.vector(subset(Sample_data, select = Time, motor_unit_1
137 == 1))
138 > motor_unit_2 <- as.vector(subset(Sample_data, select = Time, motor_unit_2
139 == 1))
```

140

141 Below is sample output from R for the two motor unit discharge vectors showing the first six
142 time points by calling the function *head*. For example, the first three discharge times for
143 *motor_unit_1* are at 0.035, 0.115, and 0.183 seconds, while *motor_unit_2* discharged at 0.1,
144 0.205, and 0.298 seconds.

145

```
146 > head(motor_unit_1)
147 ## [1] 0.035 0.115 0.183 0.250 0.306 0.377 ...
148 > head(motor_unit_2)
149 ## [1] 0.100 0.205 0.298 0.377 0.471 0.577 ...
```

150

151 The motor unit with fewer discharges is called the reference unit, while the other is referred to as
152 the event unit. This distinction is made automatically within the function and is output as a part
153 of the motor unit characteristics. *Method* indicates which method(s) of cross-correlation peak
154 determination is to be used, while *order* and *binwidth* specifies how many orders of recurrences
155 intervals to calculate and the size of the bins for the histogram, respectively. Some researchers
156 argue that only first order intervals should be used for analysis, as the presence of harmonics
157 within the cross-correlation may cause non-physiological peaks to appear in the long latency
158 portions of the histogram (De Luca et al. 1993). Therefore, the default argument of *order* is set at
159 1, indicating only first order intervals are to be used; however, the function is flexible enough to
160 handle any order input by the user. Additionally, the *binwidth* argument is set at a default of
161 0.001 sec or 1 ms. This allows for appropriate resolution in short-term synchronization

measurements. What is returned from this function is a list of individual motor unit characteristic data along with a list of all synchronization indices (detailed below). Characteristics for each motor unit included are the number of discharges, the mean interspike interval (ISI), all ISI's, and the intervals for each specified recurrence order. The *plot* argument takes a TRUE or FALSE to indicate whether the resulting histogram will be displayed or not.

Peak Determination Methods Available

The three methods employed in this toolbox reflect the three broad classes of cross-correlation histogram peak determination in the current motor unit synchronization literature. The *methods* argument allows the user to choose how a peak in the histogram will be determined. More specifically, this code automatically computes the boundary bins of the peak based on certain criteria.

The visual method - The bins of the histogram are progressively summed across from -100 to +100 ms and subsequently divided by the baseline mean count (considered as ≤ -60 and ≥ 60 ms) to produce a "normalized" cumulative sum graph. Large increases or decreases in bin counts are seen as large deflections in the cumulative sum graph. The user is asked to identify the peak as the beginning and end of this large deflection (Nordstrom et al. 1992). An example of this plot is shown in Fig. 2A, with boundaries chosen by a user highlighted. *Visual_mu_synch* is the function syntax to call this method separately.

The cumulative sum (cumsum) method - The bins of the histogram are progressively summed across from -100 to +100 ms to produce a cumulative sum. Peak boundaries are determined as the bins associated with 10 and 90% of the range (maximum - minimum) of this cumulative sum. The peak is considered significant if it's mean bin count exceeds the sum of the mean and 1.96 times the standard deviation of the baseline bins (considered as ≤ -60 and ≥ 60 ms). If no significant peak is detected, a default peak is used as ± 5 ms (Keen et al. 2012). *Cumsum_mu_synch* is the function syntax to call this method separately.

The z-score method - First, a random uniform distribution is used to create a cross-correlation based on parameters from the experimental data. This new, "shuffled" histogram depicts the correlation of two motor unit trains that are completely independent (i.e. flat). It is used to calculate a significance threshold (Equation A) to compare with the experimental data.

$$A) \text{ Significance Threshold} = \text{mean bin count} + (1.96 * \text{standard deviation of bin count})$$

Any bins in the experimental histogram within ± 10 ms of 0 that crosses this threshold are considered to be significantly greater than expected due to chance and subsequently used for analysis. If no peak is detected, synchronization indices of 0 are returned. Because the z-score method tests each bin individually, peak bins are not necessarily adjacent (Defreitas et al. 2014). *Zscore_mu_synch* is the function syntax to call this method separately.

203

204 The following R scripts calls the *mu_synch* function to perform all three methods for first order
205 recurrence intervals with a bin size of 1 ms. Each individual method can also be called separately
206 with their respective functions.

207

```
208 > mu_synch(motor_unit_1, motor_unit_2, method = c("Visual", "Zscore",  
209 "Cumsum"), order = 1, binwidth = 0.001, plot = FALSE)
```

210

211 *Recurrence_intervals* and *bin* are support functions used within the synchronization functions to
212 compute the recurrence intervals and discretize the data for the histogram, but they can also be
213 called separately for individual use. A *plot_bins* function is also available that will display the
214 associated histogram in the Plot window of RStudio (*Fig. 3D*). This is useful for visually
215 checking data for abnormalities prior to calculating synchronization. The code below creates an
216 R list named '*first_order_intervals*' that contains the motor unit characteristic data along with
217 the first order recurrence intervals.

218

```
219 > first_order_intervals <- recurrence_intervals(motor_unit_1, motor_unit_2,  
220 order = 1)
```

221

222 To access just the intervals, we need to index them using the '\$' operator. Below, we use the
223 *head* function again just to view the first six elements of the first order intervals.

224

```
225 > head(first_order_intervals$`1`)
```

```
226 ##      [1] -0.065 0.015 -0.022 0.045 -0.048 0.008 ...
```

227

228 Now these intervals are input to the *bin* function, along with the user-defined bin width, to
229 discretize the intervals into bins for the detection peaks. A data frame '*binned_data*' is created
230 with the code using a bin width of 1 ms. The resulting data frame contains a column depicting
231 the bin, or the amount of time in second before (negative) or after (positive) the reference motor
232 unit discharge, and the frequency of occurrence at that interval. This data frame can be put
233 directly into the *plot_bins* function to display the histogram (such as in *Fig. 3D*).

234

```
235 > binned_data <- bin(first_order_intervals$`1`, binwidth = 0.001)  
236 > head(binned_data)
```

```
237 ##   Bin Freq
```

```
238 1 -0.101    1
```

```
239 2 -0.100    0
```

```
240 3 -0.099    0
```

241 4 -0.098 0

242 5 -0.097 0

243 6 -0.096 0

244 > plot_bins(binned_data)

245 *Synchronization Indices*

246 Once the boundaries of the peak are established, the synchronization indices are calculated. Six
 247 indices that are commonly found throughout the literature are automatically returned (Nordstrom
 248 et al. 1992; De Luca et al. 1993; Kamen & Roy 2000). The peak of the histogram can be
 249 considered two different regions; the region of counts that are expected due to chance and the
 250 region containing “extra” counts more than what is expected due to chance (*Fig. 2B*). These
 251 extra counts are typically the number of counts in the peak bins over a certain threshold. In the
 252 Z-score method, this is the significance threshold calculated from the shuffled histogram
 253 (Defreitas et al. 2014). In the Visual method, the threshold is the baseline mean bin count
 254 (Nordstrom et al. 1992). The total counts in peak consists of the summation of the regions.
 255 Synchronization indices attempt to quantify the relationship between these different regions. The
 256 larger the magnitude of the indices, the higher the chances that the motor units are firing in
 257 synchronization. The CIS index is commonly used because it allows for normalization with
 258 respect to trial duration. Nordstrom *et al.* developed the CIS because most other indices available
 259 at that time were influenced by discharge rate (Nordstrom et al. 1992). The equations used to
 260 calculate the various indices are below.

261

$$262 \quad CIS = \frac{\text{extra counts in peak}}{\text{duration of trial (s)}}$$

$$263 \quad k' = \frac{\text{total counts in peak}}{\text{expected counts in peak}}$$

$$264 \quad k' - 1 = \frac{\text{extra counts in peak}}{\text{expected counts in peak}}$$

$$265 \quad E = \frac{\text{extra counts in peak}}{\text{number of discharges from reference motor unit}}$$

$$266 \quad S = \frac{\text{extra counts in peak}}{\text{total number of discharges from both motor units}}$$

$$267 \quad SI = \frac{\text{extra counts in peak}}{\frac{\text{total counts}}{2}}$$

268

269 Along with the synchronization indices listed above, also reported is the peak duration and peak
 270 center. These refer to the width of the peak and the bin location of the center of the peak,
 271 respectively, to help characterize the latency of synchronization. Below is some example R

272 output of the synchronization results of data obtained from the Cumulative Sum technique for
273 motor unit synchronization. Here we see the CIS between the example motor units is 2.16, which
274 indicates 2.16 synchronous discharges per second. The peak duration was 10 ms centered at bin
275 0.

```
276
277 `$Cumsum Indices`
278 `$Cumsum Indices`$CIS
279 [1] 2.163168
280 `$Cumsum Indices`$kprime
281 [1] 3.79096
282 `$Cumsum Indices`$kminus1
283 [1] 2.79096
284 `$Cumsum Indices`$E
285 [1] 0.2110322
286 `$Cumsum Indices`$S
287 [1] 0.08638251
288 `$Cumsum Indices`$SI
289 [1] 0.2117218
290 `$Cumsum Indices`$Peak.duration
291 [1] 0.01
292 `$Cumsum Indices`$Peak.center
293 [1] 0
```

294
295 An advantage to R, as alluded to before, is its robust statistical computing. Using the R
296 environment allows for direct access to many statistical packages. The “stats” package comes
297 included in base R so many statistical tests are immediately available for testing synchronization
298 metrics. This eliminates the need for transforming and importing data into 3rd party statistical
299 software, such as SAS and SPSS. Simple tests such as t-tests and ANOVAS are common, while
300 more complex, multi-level models are available.

301
302 Bugs or errors in software are common in open-source scripted codes like R. As this is the first
303 stable version of the motoRneuron package, it is possible that users will notice performance
304 issues or errors stemming from R version fragmentation or other sources. Users are urged to

email any errors or issues found in motoRneuron to the package maintainer (Andrew Tweedell andrew.j.tweedell.civ@mail.mil). Errors that can be fixed will be updated in new versions of the package as they are found. As such, it is important to update the package continually to guarantee efficient performance.

Discussion

MotoRneuron is a free package containing a list of functions capable of performing many different cross-correlation analyses for calculating many time-domain synchronization metrics for use in the motor control field. This free, all-inclusive software package enables researchers to easily examine the many options for calculating and reporting synchronization indices. Additionally, new data can be quickly reconciled with results from previous studies for better physiologic interpretation. MotoRneuron is written in the R programming language, which provides an open-source platform to perform data and statistical analysis on motor unit data. MotoRneuron's simplistic function syntax and detailed output allow for easy comprehension. The package also allows for the visualization of these analyses through R's powerful and flexibility graphics capabilities. In the future, the package will be expanded to include frequency-domain characterization as well.

Citation

Researchers using motoRneuron in a published paper should cite this article and indicate the used version of the package.

Acknowledgements

The authors would like to acknowledge Courtney Haynes for her work as technical reviewer for the manuscript. The authors would also like to acknowledge the entire R community for providing a free platform for the creation and distribution of this package to the greater scientific community.

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- 379

Figure 1(on next page)

Recurrence Intervals Diagram

Schematic representation of the recurrence intervals between two concurrently active motor units. Each discharge from one motor unit is used as a reference point to determine forward and backward latencies to the discharges of the second motor unit. The first order intervals are the latencies to the *first* forward and backward discharges (noted in red). The second order are the *second* forward and backward discharges (noted in purple).

Reference Spike

Motor Unit 1



n Order Intervals

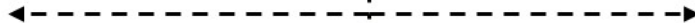


2nd Order Intervals



1st Order Intervals

Motor Unit 2



Backward

0

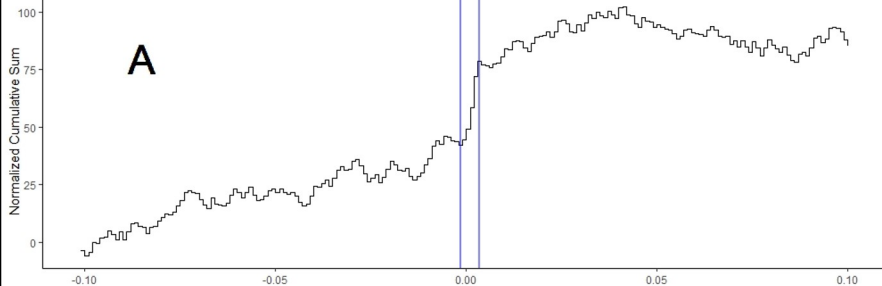
Forward

Time

Figure 2(on next page)

Cumulative Sum and Histogram

(A) Example of cumulative sum graph of bin counts. (B) Cross-correlation histogram rendered in RStudio using the “ggplot” package. Peak boundaries (blue lines) were determined by visual analysis of the cumulative sum graph, where a peak is seen as a large deflection near time 0. Synchronization indices are calculated based on the relationship between the counts of the histogram expected due to chance (in red) and the counts that are in excess of what is expected (in blue). In most cases, this threshold for determination (red line) is the baseline mean count of the histogram.



Cross-Correlation Histogram

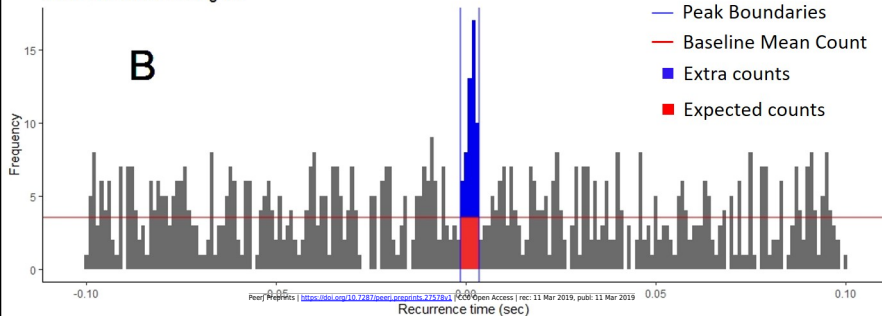


Figure 3(on next page)

RStudio Graphical User Interface

R integrated development environment RStudio's graphical user interface. The interface is made up of four panels: (A) R script panel, (B) Console, (C) Global Environment, (D) Plot panel depicting an example cross-correlation histogram.

Untitled1*

```
1 This is where you write your own code!
```

A

Environment History Connections

Global Environment

Values

motor_unit_1	num [1:443]	0.035 0.115 0.183 0.25 0.306 0.377 0.455 0.512 0.577 0.6...
motor_unit_2	num [1:307]	0.1 0.205 0.298 0.377 0.471 0.577 0.664 0.739 0.843 0.94...

C

1:39 (Top Level) R Script

Console Terminal

```
> This is where you execute your functions!
```

B

