Published in final edited form as:

Behav Brain Res. 2017 January 15; 317: 226–236. doi:10.1016/j.bbr.2016.09.022.

MUSIC-Expected Maximization Gaussian Mixture Methodology for Clustering and Detection of Task-Related Neuronal Firing Rates

Alexis Ortiz-Rosario [Ph.D. Student],

Departments of Biomedical Engineering, The Ohio State University

Hojjat Adeli [Dist. Member ASCE, Fellow IEEE, Fellow AAAS, Fellow American Neurological Assoc. Professor], and

Departments of Biomedical Engineering, Biomedical Informatics, Civil and Environmental Engineering and Geodetic Science, Electrical and Computer Engineering, Neurology, and Neuroscience, The Ohio State University, 470 Hitchcock Hall, 2070 Neil Avenue, Columbus, Ohio 43210

John A. Buford [Associate Professor and Director]

Physical Therapy Division, School of Health and Rehabilitation Sciences, The Ohio State University, 453 W 10th Ave, Rm. 516E, Columbus, Ohio 43210

Abstract

Researchers often rely on simple methods to identify involvement of neurons in a particular motor task. The historical approach has been to inspect large groups of neurons and subjectively separate neurons into groups based on the expertise of the investigator. In cases where neuron populations are small it is reasonable to inspect these neuronal recordings and their firing rates carefully to avoid data omissions. In this paper, a new methodology is presented for automatic objective classification of neurons recorded in association with behavioral tasks into groups. By identifying characteristics of neurons in a particular group, the investigator can then identify functional classes of neurons based on their relationship to the task. The methodology is based on integration of a multiple signal classification (MUSIC) algorithm to extract relevant features from the firing rate and an expectation-maximization Gaussian mixture algorithm (EM-GMM) to cluster the extracted features. The methodology is capable of identifying and clustering similar firing rate profiles automatically based on specific signal features. An empirical wavelet transform (EWT) was used to validate the features found in the MUSIC pseudospectrum and the resulting signal features captured by the methodology. Additionally, this methodology was used to inspect behavioral elements of neurons to physiologically validate the model. This methodology was tested using a set of data collected from awake behaving non-human primates.

Corresponding author: Hojjat Adeli, adeli.1@osu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

multiple signal classification; Gaussian Mixture Model; Expectation-Maximization algorithm; firing rate; neurons; signal processing; empirical wavelet transform

INTRODUCTION

Within the nervous system, the primary method of information exchange is synaptic transmission. The neuron's action potential, the event that triggers this information exchange, is a uniform waveform that, by itself, carries no information (Kostal et al., 2007). However, a neuron's firing rate, or the rate at which action potentials are generated, is known to be information-rich. This firing rate is used to decode or interpret complex behaviors of a subject such as movement (Carmena et al., 2003; Lebedev et al., 2005; Abou Zeid and Chau, 2015; Suberbiola et al., 2015; Villar et al., 2016), slow eye movements (Takemura et al., 2001; Ghahari and Enderle, 2014), attention (Ecker et al., 2016), or memory recall (Janetsian et al., 2015) among others. The relationship between firing rate and behavior can be made directly via correlation (Takemura et al., 2001), or indirectly using triggered averaging schemes to observe the contribution of a neuron to the recruitment of a particular muscle (Davidson et al., 2007).

At the simplest level, neural activity can be divided into two classes: task related and not task related. These two classes represent a neuron's participation in (or correlation to) an observed behavior. This classification is often done by simply assessing the increase/decrease of firing rate of the neuron over a statistical threshold such as the standard deviation from the mean of a baseline firing rate (Chase et al., 2012). This approach, although rudimentary, works when action potentials are recorded for a large population of neurons on the order of hundreds or thousands due to the fact that multiple neurons within this population often share the desired information content (Carmena et al., 2003). When the action potential data can be obtained for only a small population of neurons on the order of a few dozen, the aforementioned simple statistical approaches often miss important information. Some work has been performed to improve classification by applying signal processing techniques such as Fourier transform (Urrestarazu et al., 2006) but this problem still remains a challenge.

This paper presents a methodology to automatically classify and detect groups of neurons which are related to a particular motor task, a reaching task in this particular research, by studying their firing patterns. A new methodology is presented for clustering and detection of firing rate patterns recorded from an awake behaving primate model. It consists of a pseudospectrum technique called multiple signal classification (MUSIC) (Jiang and Adeli, 2007) that will extract features from firing rate profiles and a Gaussian mixture algorithm (Tian et al., 2011) that clusters them in a three dimensional feature space created from the extracted features.

MATERIALS AND METHODS

Subject and Task

Data collected from two male macaque monkeys (*Macaca Fascicularis*), identified as H and N, were used in this study. The subjects were trained to perform a bimanual reaching task with the goal of reaching with one hand to a target projected on a touch screen monitor while the other hand remained at rest. The task started with the subject placing his two hands on the two pressure plates. Immediately, a circular cyan-colored cue (Figure 1A) was displayed, instructing the subject to wait. After one second, one of the two targets, left or right, was displayed, prompting him to reach and touch that target (Figure 1B). The color of the target represented which hand the subject was to use: red for right hand or green for left hand. The subject was trained over a period of months to perform one of the four possible acts: left hand left target (LHLT), left hand right target (LHRT), right hand left target (RHLT), and right hand right target (RHRT). The subjects were cared for as required by the NIH Guide for Care and Use of Laboratory Animals, and the laboratory protocol approved by the Institutional Animal Care and Use Committee at The Ohio State University.

Data Acquisition

To acquire the neurological signals, the subjects were implanted with recording chambers over the left parietal bone near the precentral dimple to access the motor cortex, and over the occipito-parietal region to access the ponto-medullary reticular formation (PMRF). Electrodes were positioned over cortical motor areas and advanced intracortically during each experimental session, and removed in between sessions. These electrodes, typically two to four individual glass coated tungsten microelectrodes (Alpha Omega, Alpharetta, GA), were placed in regions that displayed shoulder/elbow responses to stimulation. Surgical details can be seen in (Herbert et al., 2010; Ortiz-Rosario et al., 2013). A single tungsten microelectrode was used to reach the PMRF in the brainstem with access to either the left or right side while avoiding the midline with to a ten degree lateral tilt of the chamber. To properly assess the location of the PMRF in the brainstem, neural activity was recorded and an audio was played out during electrode descent. Electrophysiological landmarks, such as the abducens nucleus, were identified prior to recording to ensure accurate electrode placement.

Signals from each electrode were amplified by 5,000 and filtered with a bandpass of 100 Hz to 3 kHz with a differential amplifier. The data were sent to a computer via a 1401 data acquisition unit and processed using the Spike2 software, both manufactured by Cambridge Electronic Design, Cambridge, UK. Figure 2A presents the neuron isolation procedure to identify the individual neurons. The continuous signals from the amplifiers were converted into single action potentials with a -0.75 mV trigger. This trigger ensured that only action potentials that were below -0.75 mV were accepted for isolation. The individual action potentials were then analyzed using a principal component analysis (PCA) algorithm (Ghosh-Dastidar et al., 2008; Ortiz-Rosario et al., 2015). The first two components of PCA are plotted in Figure 2A. Each action potential in the 2D feature space was clustered using the Gaussian mixture model (GMM) (Ortiz-Rosario et al., 2015). The resulting clusters, in this example 3, were identified as individual neurons. Once each neuron was isolated, its

firing frequency was obtained. Figure 2B shows the procedure for the firing rate averaging approach of these individual neurons over the four task types in a 2 s window with 50 ms bins. The 2 s window included preparation of the subject (getting ready to move mentally), as well as movement to reach the target. Since the subject was exposed to four different task types, the firing rate of each neuron (457 neurons in total) was averaged across trials for each of the four task types (resulting in 1828 observed events). A pre-filtering criterion for noisy signals with too much variability or no detectable features was implemented in order to eliminate them from the data. After this initial filtering, all the signals were visually inspected and classified as task-related or not task-related. This visual inspection was used as the benchmark to compare the performance of the proposed methodology.

Multiple Signal Classification (MUSIC) Pseudospectrum

MUSIC is a method for computing the pseudospectrum of a time series. In the present study, it was used as the feature extraction technique for the methodology. A pseudospectrum is a power density spectrum for finding eigenvalues of a non-normal matrix (a matrix with non-orthogonal eigenvectors) which is challenging to solve using a conventional eigenvalue solution method due to failed resonance or asymptotic behaviors of the matrix (Marple, 2014). These non-normal matrices show up in transient non-linear and chaotic systems typical of measured neuroscience data (Adeli et al., 2007; Adeli et al., 2008; Rosselo et al., 2014; Martens et al., 2014). This method provides a representation of a signal's frequency components from a set of eigenvectors extracted from an autocorrelation matrix of the data. Compared with other methods such as the fast Fourier transform (FFT), MUSIC can yield accurate results, even for signals with low signal-to-noise ratios (Jiang and Adeli, 2007).

The firing frequency profile shown at the right of Fig. 2B is placed in an N_a -dimensional vector $\mathbf{R}$ where N_a is the length of each signal (1000 in this research). The pseudospectrum was estimated using the following five steps:

1. Divide $\mathbf{R}$ into N_w length sections resulting in $N_{aw} = \lfloor N_a / N_w \rfloor$ vectors, where the i^{th} length section is denoted as N_w -dimensional row vector $\mathbf{U}_i$ and $\lfloor \cdot \rfloor$ is the rounding operator.
2. Construct a N_w by N_{aw} matrix $\mathbf{Y}$ as follow:

$$\mathbf{Y} = [\mathbf{U}'_1, \mathbf{U}'_2, \dots, \mathbf{U}'_i, \dots, \mathbf{U}'_{N_{aw}}] \quad (1)$$

where $\mathbf{U}'_i$ represents the transpose of $\mathbf{U}_i$.

3. The value of the j^{th} row and k^{th} column of the correlation matrix, $c(\mathbf{Y})_{jk}$, is computed as follows:

$$c(\mathbf{Y})_{jk} = \frac{\text{Cov}(\mathbf{Y})_{jk}}{\sqrt{\text{Cov}(\mathbf{Y})_{jj} \text{Cov}(\mathbf{Y})_{kk}}} \quad (j=1, 2, \dots, N_w; k=1, 2, \dots, N_{aw}) \quad (2)$$

where $\text{Cov}(\mathbf{Y}) = (\mathbf{Y}^T \mathbf{Y})^{-1}$ is the covariance matrix.

4. Create a rectangular matrix V with all the eigenvectors of the correlation matrix (Eq. 2) as its columns.
5. Transform matrix Y in Eq. (1) into the frequency domain using the FFT method with a length $2(L-1)$ as follows where L represents the dimension of the eigenvectors (number of rows in V):

$$e(Y) = [1, \exp(j2\pi Y), \exp(j4\pi Y), \dots, \exp(j2(L-1)\pi Y)]^H \quad (3)$$

in which $\exp(\cdot)$ represents a complex sinusoid of the FFT and the conjugate transpose operator is represented by superscript H .

6. Compute the pseudospectrum defined as follows:

$$P_M(Y) = \frac{1}{\sum_{l=N_{aw}+1}^L |V_l^H e(Y)|^2} \quad (4)$$

where V_k is the k^{th} eigenvector (k^{th} column in V). Selecting an effective FFT length K is not critical in this analysis as long as it is an integer number with a power of 2 and not larger than N_a .

The values of the pseudospectrum were converted into the decibel (dB) for ease of comparison between observed events:

$$P_Y = 10 \log_{10} [P_M(Y)] \quad (5)$$

In the pseudospectrum, the most prevalent signal features are represented as peaks in the Y axis (or power). The first three highest frequencies in the spectrum, sorted in descending order of power (dB), were selected as the features to be clustered by EM-GMM. These are placed in a 1×3 vector of X_i where $i \in \{1, 2, \dots, N\}$, N being the total number of data points.

Expectation-Maximization Gaussian Mixture Model (EM-GMM)

Clustering methods are employed to group similar data into clusters with common threads (Coletta et al., 2015; Cai et al., 2015; Wang et al., 2015; Peng et al., 2016; Rachidi et al., 2016; Mammone et al., 2011). In this research, two different clustering methods were explored: k-means (Siddique and Adeli, 2013) and the expected maximization gaussian mixture model (EM-GMM). The latter was found to accurately encompass task-related activity compared to K-means and therefore was selected in this research. A mixture model is a type of statistical unsupervised learning, in this case with Gaussian properties, where subpopulations, or clusters, are extracted based on statistical properties of the population, or data set (Tian et al., 2011). The user of this methodology needs to provide only the number of clusters for a given data set. Such a model is useful in data sets where classes cannot be readily identified.

Let $\mathbf{X}_i$ be a D -dimensional data point (feature vector), $P_c(\mathbf{X}_i|\boldsymbol{\theta}_c)$ be the conditional probability of $\mathbf{X}_i$ belonging to a cluster with centroid $\mathbf{M}_c$ (D -dimensional vector) and covariance matrix $\mathbf{S}_c$ (a D by D dimensional square matrix) where $i \in \{1, 2, \dots, N\}$, and $\boldsymbol{\theta}_c = \{\mathbf{M}_c, \mathbf{S}_c\}$. In GMM the total probability distribution corresponding to $\mathbf{X}_i$ is expressed as the summation of weighted conditional probabilities as follows:

$$P_{total}(\mathbf{X}_i) = \sum_{c=1}^C A_c P_c(\mathbf{X}_i|\boldsymbol{\theta}_c) \quad (6)$$

where A_c is a weight and represents the contribution of the C_{th} conditional probability to the total probability distribution, C is the total number of clusters defined by the user, and

$\sum_{c=1}^C A_c = 1$. In this research a Gaussian probability density function is used to compute $P_c(\mathbf{X}_i|\boldsymbol{\theta}_c)$:

$$P_c(\mathbf{X}_i|\boldsymbol{\theta}_c) = \frac{1}{(2\pi)^{\frac{D}{2}} |\mathbf{S}_c|^{-\frac{1}{2}}} e^{-0.5(\mathbf{X}_i - \mathbf{M}_c)' \mathbf{S}_c^{-1} (\mathbf{X}_i - \mathbf{M}_c)} \quad (7)$$

where $|\mathbf{S}_c|$ is the determinant of covariance matrix. The probability of $\mathbf{X}_i$ belonging to the c^{th} cluster is:

$$W_{ic} = \frac{A_c P_c(\mathbf{X}_i|\boldsymbol{\theta}_c)}{P_{total}(\mathbf{X}_i)} \quad (8)$$

where W_{ic} is the *membership value* and $\sum_{c=1}^C W_{ic} = 1$

EM-GMM uses expectation-maximization (EM) algorithm (Segovia et al., 2010) to optimize the parameters of GMM (i.e. $\boldsymbol{\theta}_c$ and A_c). Figure 3 presents a flowchart of EM-GMM consisting of four steps where the second and third steps correspond to *expectation* and *maximization*, respectively. In the first step, $\boldsymbol{\theta}_c^{t=0}$ and $A_c^{t=0}$ are generated randomly for each

cluster such that $\sum_{c=1}^C A_c^{t=0} = 1$. The superscript $t \in \{1, 2, \dots, T\}$ represents the iteration number, where T is the total number of iterations. Unlike the nature-inspired optimization techniques, T is not defined by user and is determined by the EM-GMM algorithm. In the second step, W_{ic}^t is computed for each feature vector $\mathbf{X}_i$ and each cluster. In the third step, A_c^t and $\boldsymbol{\theta}_c^t = \{\mathbf{M}_c^t, \mathbf{S}_c^t\}$ are updated as follows:

$$A_c^{t+1} = \frac{N_c^t}{N} \quad (9)$$

$$\mathbf{M}_c^{t+1} = \left(\frac{1}{N_c^t} \right) \sum_{i=1}^N W_{ic}^t \mathbf{X}_i \quad (10)$$

$$\mathbf{S}_c^{t+1} = \left(\frac{1}{N_c^t} \right) \sum_{i=1}^N W_{ic}^t (\mathbf{X}_i - \mathbf{M}_c^{t+1})' (\mathbf{X}_i - \mathbf{M}_c^{t+1}) \quad (11)$$

where $N_c^t = \sum_{i=1}^N W_{ic}^t$, represents the total number of data points in cluster C , and A_c^{t+1} , $\mathbf{M}_c^{t+1}$ and $\mathbf{S}_c^{t+1}$ are the weight, vector of centroid, and covariance matrix in iteration $t + 1$. In the fourth step, *log-likelihood*, defined as sum of the $\log P_{total}(\mathbf{X}_i)$ over all data points is computed as follows:

$$G^{t+1} = \sum_{i=1}^N \log P_{total}(\mathbf{X}_i)^{t+1} \quad (12)$$

If $|G^{t+1} - G^t| < E$, where E is a predefined error limit, set to 0.00001 for this research, the algorithm stops (Bishop, 2007). In that case, $T = last\ t + 1$, and $\mathbf{X}_i$ belongs to the cluster with maximum membership value in iteration T .

Automatic clustering of similar firing rate using MUSIC + EM-GMM

The first part of the methodology is to convert the average neuronal firing rates into the MUSIC pseudospectrum as described above. The most prevalent signal features are represented as peaks in the Y axis, representing power, of the pseudospectrum, while the X axis represents frequency. To create a feature space, for each observed group of events, the first three highest frequencies in the spectrum, sorted in descending order of power (dB), are projected in a three-dimensional space where x, y, and z represent the highest, the second highest, and the third highest frequency (Figure 4). Once the feature space is created the EM-GMM clusters the firing rate data into C clusters (*in this research* $C = 4$). The size of C was selected to match the number of observable clusters.

Validating GMM-clustered MUSIC pseudospectrum using the empirical wavelet transform

In recent years wavelet transform (WT) has been used extensively for denoising signals as well as for feature extraction in various fields such as biomedical engineering and computational neuroscience (Perez et al., 2014; Hsu, 2015; Vahabi et al., 2016), image processing, health monitoring of structures (Su et al., 2014), seismology (Ghodrati Amiri et al., 2014), and reliability analysis (Dai and Wang, 2014; Dai et al., 2015). The empirical wavelet transform (EWT) is an adaptive version of the WT where the wavelet basis is extracted from the signal itself instead of using a predefined wavelet (Gilles, 2013). The EWT was employed in this research to validate the prominent features found in the MUSIC pseudospectrum and clustered by EM-GMM. In WT, a predefined wavelet basis, called

mother wavelet, is used to represent a time-series signal, akin to cosines and sines used as the basis of the Fourier transform (FT) (Adeli and Karim, 2005; Adeli and Ghosh-Dastidar, 2010). A challenge in WT is that mother wavelets often do not share similarities with the signals being processed thus impacting the overall effectiveness of the technique (Amezquita-Sanchez and Adeli, 2015; Gilles, 2013). Researchers rarely justify the selection of the mother wavelet and those who do justify the selection based on superficial similarities with the signal (Rafiee et al., 2009; Shalchyan et al., 2012). The wavelet packet transform (WPT) (Jiang et al., 2007; Jiang and Adeli, 2004; Maheshwari et al., 2016; Singh and Sunkaria, 2015) provides an improvement over WT but it also has limited adaptability in the way it divides frequency bands in the frequency domain. Compared to WT and WPT, EWT can adapt both its basis and frequency bands to model the analyzed signal properly.

Figure 5 displays an example of a MUSIC pseudospectrum being divided into frequency bands using both WT and EWT. The frequency bands subdivision scheme of WT is non-adaptive irrespective of the actual frequency content of the signal while that of EWT is adaptive and reflects the actual frequency content of the signal. In Figure 5 it can be seen that features in the MUSIC pseudospectrum are better represented by EWT compared with WT. Example time domain signals for each decomposition methodology are also included at the bottom of Figure 5 to show signal features. EWT has been previously validated in various synthetic and biological signals in conjunction with a MUSIC pseudospectrum (Amezquita-Sanchez and Adeli, 2015). The mathematical details of EWT and the creation of boundaries can be seen in Gilles (2013) and Amezquita-Sanchez and Adeli (2015).

Figure 6 shows an example of the specific decomposition used in this research. The MUSIC pseudospectrum was divided into three frequency bands by EWT. This subdivision was performed in order to understand what kind of firing rate time-series components were clustered by the proposed methodology. The three frequency bands were selected based on features found in the MUSIC pseudospectrum and the general understanding that too many frequency bands create artifacts. The first three frequency bands were determined to show relevant features, as it is understood that too many frequency bands do not capture relevant signal information and created artifacts. The first three frequency bands contained specific amplitude/frequency properties of the signal. Figure 6 shows that frequency band 1 contained high amplitude and slow frequency aspects of the signal. Frequency band 2 contained mid-range amplitude and frequencies, while frequency band 3 contained the lowest amplitude, but fastest frequencies found in the signal.

RESULTS

MUSIC+GMM Generated Clusters

There were a total of 1828 observed events (944 for subject H and 848 for subject N). An event is a waveform representing the average firing rate of a particular neuron across trials of a particular task. Thus, with four different tasks, each neuron produced four events. In the initial analysis, these were treated independently to discover task-related and non-task related patterns. This allowed for detection of neurons that had task-related activity for reaching with one arm but not the other, or only a certain target. At the end of this section,

the events are recombined into a set of four tasks for each neuron to determine whether the approach yields physiologically meaningful results.

In order to provide a dataset with well-modulated activity, events with a maximum firing rate below 30 Hz and events without any detectable firing rate in the 2 second time window were removed from the dataset. Among the 1828 events, only 364 (or roughly 20%) met these criteria. The MUSIC pseudospectrum was obtained for the selected events followed by the application of the EM-GMM to find the clusters. The clustered feature spaces for subjects H and N are presented in Figure 7 A and B, respectively, where each cluster represents firing rates with similar signal features obtained from the MUSIC pseudospectrum.

For both subjects, these clusters were compact and linearly separable, showing the capability of the proposed methodology. To determine if events in a particular cluster were related to the reaching task, they were compared with the visual classification of firing rate signals by an expert electrophysiologist based on the increase of activity above a baseline threshold. Approximately 70% and 58% of the events in cluster 2 were determined to be task-related for subject H and subject N, respectively, the highest accuracy among all clusters. The corresponding numbers for cluster 3 are 20% and 24% and cluster 1 are 10% and 20%. Events in cluster 1 and cluster 3 were found to be noisier than those events in cluster 2. Cluster 4 gathered no visually classified events.

Analysis of pseudospectra of MUSIC+GMM Clusters

Figure 8A and B present four sets of sample signals from the four clusters for subjects H and N, two for each, respectively. Cluster 2, which had the highest accuracy among all clusters, captured firing rates with prominent high amplitude features and with the lowest levels of noise. In contrast, cluster 4 captured firing rates with the highest levels of noise and no features. The amounts of noise in clusters 1 and 3 were comparable, and were between clusters 2 and 4.

Figure 9 displays the MUSIC pseudospectrum (on the left) and EWT decomposition (on the right) corresponding to the examples shown in Figure 8A. The EWT decomposition aids in the visualization of the equivalent time-series signals (firing rate) represented by the MUSIC pseudospectrum. In Figure 9, each example corresponds to the two signals in the two rows of Figure 8A. The vertical lines represent the boundaries of the frequency bands. Each frequency band corresponds to a time-series signal, similar to Figure 6. The frequency bands are such that the signals are selected around prominent spectral features (or peaks) in the MUSIC pseudospectrum where the power is high. The first, second, and third frequency bands contained low, mid, and high range frequencies, and high, mid, and low range firing rate amplitudes, respectively. The first and second frequency bands contained the smaller amount of noise compared to the third one, hence, representing relevant event information in the spectrum. In Fig. 9, it can be seen how the peaks in the MUSIC pseudospectrum were different among clusters, thus validating the capability of MUSIC for detecting different signal features.

Observing Neuronal Behaviors using EM+GMM

To this point, the results demonstrated that the proposed EM-GMM methodology could be used to identify task-related v. non-task related features in neural firing rates. After classifying events as task-related or not, the results were evaluated to determine whether certain neurons had certain features, such as being related to one arm or the other, or to a particular hand-target combination, etc. As noted previously, each event in the dataset represented a neuronal response to one of four different motor reaching tasks. In this section, each neuron's pattern of responses for the four tasks was evaluated to classify neurons by their neurophysiological behavior.

Figure 10A displays a sample of this functional evaluation. In this figure, for sample neuron #187, four averaged firing rate profiles are shown with their trial-by-trial record of the action potentials, known as rasters. Based on the results of the EM-GMM clustering, it may be concluded that this neuron was most involved in tasks requiring reaching with the right hand. Figure 10B displays an example of neurons involved in reaching with both hands (neuron 13 in Fig. 10B), and another example involved in only right target reaching (neuron 36 in Fig. 10B).

This type of behavioral analysis can be used to validate the effectiveness of the methodology to detect physiologically relevant information from the data set. Table 1 presents the count of neurons detected to have preferences by hand and by target. These are also divided by brain region. From this analysis, it was observed that PMRF neurons were predominantly bilateral, with 41% of neurons found to be active for reaching with both hands, and about 30% each were active with reaching with the left arm or the right arm. Neurons in the cortex were most frequently involved with reaching with the right (contralateral) hand (40%), but a sizeable proportion (37%) were involved with reaching with the left (ipsilateral) hand. The remainders of the neurons (23%) were involved in both hands.

DISCUSSION

This paper presents a novel methodology for the categorization and grouping of firing rate profiles found in neurons involved in a motor task. This methodology is general and could be used in any situation where multiple neurons are recorded, whether there be a single type of trial or multiple trial types, as considered here. For example, this application could be used in the study of motor commands for gaze control (Takemura et al., 2001), neuronal oscillators (Schwemmer and Lewis, 2011; Geertsema et al., 2015; Aydin et al., 2016) or other brain studies such as deep brain stimulation (Florin et al., 2013). Further, it could be used for research in brain-computer interfaces (Carmena et al., 2003; Ortiz-Rosario and Adeli, 2013). Many studies using neuron firing rates often rely on detecting events by evaluating departure of the firing rate from a baseline threshold value, for example, based on the standard deviation of the mean baseline firing rate (Takemura et al., 2001). In this conventional approach, the researcher must make decisions about which neurons to accept as task related and which to discard as spurious, which is error-prone. The proposed methodology automates the identification of task-related neurons. This methodology was tested using a set of data collected from awake behaving primates and validated by EWT.

The 70% accuracy of the methodology may not appear high, but this is partly due to the nature of the problem, because the visual inspection used as the *gold standard* is not necessarily accurate itself. The methodology, however, achieves obvious separation among clusters, which is encouraging. With further development of this approach, there may be room for improvement in the accuracy by optimizing the parameters of MUSIC. For example, by selecting a different length section, N_w , it may be possible to focus the extraction on relevant features to achieve more accurate clustering. In this research, this parameter was selected by trial-and-error, but this parameter could be optimized using an optimization technique such as the neural dynamic optimization model of Adeli and Park (Adeli and Park H.S., 1995a; Adeli and Park H.S., 1995b).

With linearly separable and compact clusters, the EM-GMM algorithm was deemed appropriate for the current application. Additional efforts towards improving the proposed methodology could be in the form of applying a classification technique such as the enhanced probabilistic neural network (EPNN) (Ahmadlou and Adeli, 2010) using already identified classes of firing rates. The fact that the approach was able to separate cells into physiologically relevant groups demonstrates its utility. In the present results, about 40% of the cells were found to be bilateral for the PMRF, and about 30% each were found to relate to the ipsilateral or contralateral arm. This is strikingly similar to results previously obtained in studies using electrical stimulation (Davidson and Buford, 2006). Likewise, in the cortical motor areas, most cells were best related to the contralateral hand, but a reasonably large portion were related to the ipsilateral hand. From electrical stimulation studies in the same subjects, we also found relatively high proportions of motor outputs ipsilaterally; this relatively high incidence of ipsilateral hand representation in the cortex can be explained by the recordings being concentrated in areas related to recruitment of proximal muscles and by the inclusion of recordings from PMd and SMA in addition to M1 (Montgomery et al., 2013). Hence, this unbiased neural classification methodology produces results that match well with the findings from electrical stimulation in the PMRF and in the cortex. This lends validity to the approach because, in comparison to examining neural firing frequencies and attempting to interpret their relationship to a task, detecting EMG responses to stimulation is relatively objective.

In sum, the present paper presents an objective means of classifying neurons recorded in association with behavioral tasks into groups. By identifying characteristics of neurons in a particular group, the investigator can then identify functional classes of neurons based on their relationship to the task. The historical approach has been to inspect large groups of neurons and subjectively separate cells into groups based on the expertise of the investigator. The more objective approach presented here warrants further investigation and might prove particularly useful in understanding new neural systems where types of neural activity patterns have yet to be identified. A future study comparing this approach in well-defined systems to compete with classification by groups of experts might be useful to further test the applicability of this approach.

Acknowledgments

The authors thank Stephanie Moran, Rebecca Slattery, Amanda Jellick, Wendy Herbert, and Lynnette Montgomery for assistance in collecting these data. The authors would like to also thank Ph.D. Candidate Mohammad Hossein Raffei for his input to improving the presentation of the paper. This work was supported in part by NIH NINDS R01 NS37822.

References Cited

- Abou Zeid E, Chau T. Electrode Fusion for the Prediction of Self-initiated Fine Movements from Single Trial Readiness Potentials. *International Journal of Neural Systems*. 2015; 25(4):1550014. (14 pages). [PubMed: 25903225]
- Adeli, H.; Ghosh-Dastidar, S. *Automated EEG-based Diagnosis of Neurological Disorders – Inventing the Future of Neurology*. CRC Press; Taylor & Francis, Boca Raton, Florida: 2010.
- Adeli H, Ghosh-Dastidar S, Dadmehr N. A wavelet-chaos methodology for analysis of EEGs and EEG subbands to detect seizure and epilepsy. *Ieee Transactions on Biomedical Engineering*. 2007; 54(2): 205–211. [PubMed: 17278577]
- Adeli, H.; Karim, A. *Wavelets in Intelligent Transportation Systems*. John Wiley and Sons; West Sussex, United Kingdom: 2005.
- Adeli H, Park HS. A neural dynamics model for structural optimization—Theory. *Computers & Structures*. 1995a; 57(3):383–390.
- Adeli H, Park HS. Optimization of space structures by neural dynamics. *Neural Networks*. 1995b; 8(5):769–781.
- Ahmadlou M, Adeli H. Enhanced Probabilistic Neural Network with Local Decision Circles: A Robust Classifier. *Integrated Computer-Aided Engineering*. 2010; 17(3):197–210.
- Amezquita-Sanchez JP, Adeli H. A new music-empirical wavelet transform methodology for time-frequency analysis of noisy nonlinear and non-stationary signals. *Digital Signal Processing*. 2015; 45:55–68.
- Aydin S, Demitras S, Ates K, Tunga MA. Emotion Recognition with Eigen Features of Frequency Band Activities Embedded in Induced Brain Oscillations Mediated by Affective Pictures. *International Journal of Neural Systems*. 2016; 26(3):1650013. (16 pages). [PubMed: 26971786]
- Bishop, CM. *Pattern Recognition and Machine Learning*. New York: Springer; 2007. p. 461–522.
- Cai XT, He FZ, Li WD, Li XX, Wu YQ. Encryption based partial sharing of CAD models. *Integrated Computer-Aided Engineering*. 2015; 22(3):243–260.
- Carmena JM, Lebedev MA, Crist RE, O’Doherty JE, Santucci DM, Dimitrov DF, Patil PG, Henriquez CS, Nicolelis MAL. Learning to control a brain-machine interface for reaching and grasping by primates. *Plos Biology*. 2003; 1(2):193–208.
- Chase SM, Kass RE, Schwartz AB. Behavioral and neural correlates of visuomotor adaptation observed through a brain-computer interface in primary motor cortex. *J Neurophysiol*. 2012; 108(2):624–644. [PubMed: 22496532]
- Coletta LFS, Hruschka ER, Acharya A, Ghosh J. Using metaheuristics to optimize the combination of classifier and cluster ensembles. *Integrated Computer-Aided Engineering*. 2015; 22(3):229–242.
- Dai H, Wang W. An adaptive wavelet frame neural network method for efficient reliability analysis. *Computer-Aided Civil and Infrastructure Engineering*. 2014; 29(10):801–814.
- Dai H, Wang W, Zhang H. A Multiwavelet neural Network-based Response Surface Method for Structural Reliability Analysis. *Computer-Aided Civil and Infrastructure Engineering*. 2015; 30(2): 151–162. 2015.
- Davidson AG, Buford JA. Bilateral actions of the reticulospinal tract on arm and shoulder muscles in the monkey: stimulus triggered averaging. *Exp Brain Res*. 2006; 173(1):25–39. [PubMed: 16506008]
- Davidson AG, Schieber MH, Buford JA. Bilateral spike-triggered average effects in arm and shoulder muscles from the monkey pontomedullary reticular formation. *J Neurosci*. 2007; 27(30):8053–8058. [PubMed: 17652596]

- Ecker AS, Denfield GH, Bethge M, Tolias AS. On the Structure of Neuronal Population Activity under Fluctuations in Attentional State. *J Neurosci*. 2016; 36(5):1775–1789. [PubMed: 26843656]
- Florin E, Dafsari HS, Reck C, Barbe MT, Pauls KAM, Maarouf M, Sturm V, Fink GR, Timmermann L. Modulation of Local Field Potential Power of the Subthalamic Nucleus During Isometric Force Generation in Patients with Parkinson's Disease. *Neuroscience*. 2013; 240:106–116. [PubMed: 23454540]
- Geertsema EE, Visser GH, Velis DN, Claus SP, Zijlmans M, Kalitzin SN. Automated seizure onset zone approximation based on non-harmonic high-frequency oscillations in human interictal intracranial EEGs. *International Journal of Neural Systems*. 2015; 25(5):1550015. (18 pages). [PubMed: 25986751]
- Ghahari A, Enderle JD. A Neuron-based Time-Optimal Controller of Horizontal Saccadic Eye Movements. *International Journal of Neural Systems*. 2014; 24(6):1450017. (19 pages). [PubMed: 25081425]
- Ghodrati Amiri G, Abdolahi Rad A, Khanmohamadi Hazaveh N. Wavelet based Method for Generating Non-Stationary Artificial Pulse-Like Near-Fault Ground motions. *Computer-Aided Civil and Infrastructure Engineering*. 2014; 29(10):758–770.
- Ghosh-Dastidar S, Adeli H, Dadmehr N. Principal component analysis-enhanced cosine radial basis function neural network for robust epilepsy and seizure detection. *Ieee Transactions on Biomedical Engineering*. 2008; 55(2):512–518. [PubMed: 18269986]
- Gilles J. Empirical Wavelet Transform. *Ieee Transactions on Signal Processing*. 2013; 61(16):3999–4010.
- Herbert WJ, Davidson AG, Buford JA. Measuring the motor output of the pontomedullary reticular formation in the monkey: do stimulus-triggered averaging and stimulus trains produce comparable results in the upper limbs? *Exp Brain Res*. 2010; 203(2):271–283. [PubMed: 20379705]
- Hsu WY. Assembling a Multi-feature EEG Classifier for Left-Right Motor Data Using Wavelet-based Fuzzy Approximate Entropy for Improved Accuracy. *International Journal of Neural Systems*. 2015; 25(8):1550037. (13 pages). [PubMed: 26584583]
- Janetsian SS, Linsenbardt DN, Lapish CC. Memory impairment and alterations in prefrontal cortex gamma band activity following methamphetamine sensitization. *Psychopharmacology (Berl)*. 2015; 232(12):2083–2095. [PubMed: 25572530]
- Jiang XM, Adeli H. Wavelet packet-autocorrelation function method for traffic flow pattern analysis. *Computer-Aided Civil and Infrastructure Engineering*. 2004; 19(5):324–337.
- Jiang X, Adeli H. Pseudospectra, MUSIC, and dynamic wavelet neural network for damage detection of highrise buildings. *International Journal for Numerical Methods in Engineering*. 2007; 71(5):606–629.
- Jiang X, Mahadevan S, Adeli H. Bayesian wavelet packet denoising for structural system identification. *Structural Control and Health Monitoring*. 2007; 14(2):333–356.
- Kostal L, Lansky P, Rospars JP. Neuronal coding and spiking randomness. *Eur J Neurosci*. 2007; 26(10):2693–2701. [PubMed: 18001270]
- Lebedev MA, Carmena JM, O'Doherty JE, Zacksenhouse M, Henriquez CS, Principe JC, Nicolelis MA. Cortical ensemble adaptation to represent velocity of an artificial actuator controlled by a brain-machine interface. *J Neurosci*. 2005; 25(19):4681–4693. [PubMed: 15888644]
- Maheshwari S, Pachori RB, Acharya UR. Automated Diagnosis of Glaucoma Using Empirical Wavelet Transform and Correntropy Features Extracted from Fundus Images. *IEEE J Biomed Health Inform*. 2016
- Mammone N, Inuso G, Foresta FL, Versaci M, Morabito FC. Clustering of entropy topography in epileptic electroencephalography. *Neural Computing and Applications*. 2011; 20(6):825–833.
- Marple SL. *Digital Spectral Analysis With Applications* Dover Publications. 2014
- Martens MB, Schubert D, Chiappalone M, Tiesinga PHE. Separating burst from background spikes in multichannel neuronal recordings using return map analysis. *International Journal of Neural Systems*. 2014; 24(4):1450012. (15 pages). [PubMed: 24812717]
- Montgomery LR, Herbert WJ, Buford JA. Recruitment of ipsilateral and contralateral upper limb muscles following stimulation of the cortical motor areas in the monkey. *Exp Brain Res*. 2013; 230(2):153–164. [PubMed: 23852324]

- Ortiz-Rosario A, Adeli H. Brain-computer interface technologies: from signal to action. *Rev Neurosci*. 2013; 24(5):537–552. [PubMed: 24077619]
- Ortiz-Rosario A, Adeli H, Buford JA. Wavelet methodology to improve single unit isolation in primary motor cortex cells. *J Neurosci Methods*. 2015; 246:106–118. [PubMed: 25794461]
- Ortiz-Rosario A, Berrios-Torres I, Adeli H, Buford JA. Combined corticospinal and reticulospinal effects on upper limb muscles. *Neurosci Lett*. 2013
- Peng H, Wang J, Shi P, Perez-Jimenez MJ, Riscos-Nunez A. An extended membrane system with active membranes to solve automatic fuzzy clustering problems. *International Journal of Neural Systems*. 2016; 26(3):1650004. (17 pages). [PubMed: 26790484]
- Perez G, Conci A, Moreno AB, Hernandez-Tamames JA. Rician Noise Attenuation in the Wavelet Packet Transformed Domain for Brain MRI. *Integrated Computer-Aided Engineering*. 2014; 21(2):163–175.
- Rafiee J, Tse PW, Harifi A, Sadeghi MH. A novel technique for selecting mother wavelet function using an intelligent fault diagnosis system. *Expert Systems with Applications*. 2009; 36(3):4862–4875.
- Rashidi TH, Rey D, Jian S, Waller ST. A Clustering Algorithm for Bi-Criteria Stop Location Design with Elastic Demand. *Computer-Aided Civil and Infrastructure Engineering*. 2016; 31(2):117–131.
- Rosselló JL, Canals V, Oliver A, Morro A. Studying the Role of Synchronized and Chaotic Spiking Neural Ensembles in Neural Information Processing. *International Journal of Neural Systems*. 2014; 24(5):1430003. (11 typeset pages). [PubMed: 24875785]
- Schwemmer MA, Lewis TJ. Effects of dendritic load on the firing frequency of oscillating neurons. *Phys Rev E Stat Nonlin Soft Matter Phys*. 2011; 83(3 Pt 1):031906. [PubMed: 21517524]
- Segovia F, Gorriz JM, Ramirez J, Salas-Gonzalez D, Alvarez I, Lopez M, Chaves R, Padilla P. Classification of functional brain images using a GMM-based multi-variate approach. *Neurosci Lett*. 2010; 474(1):58–62. [PubMed: 20227464]
- Shalchyan V, Jensen W, Farina D. Spike Detection and Clustering With Unsupervised Wavelet Optimization in Extracellular Neural Recordings. *Ieee Transactions on Biomedical Engineering*. 2012; 59(9):2576–2585. [PubMed: 22772975]
- Siddique, N.; Adeli, H. *Computational Intelligence – Synergies of Fuzzy Logic, Neural Networks and Evolutionary Computing*. Wiley; West Sussex, United Kingdom: 2013.
- Singh O, Sunkaria RK. Powerline interference reduction in ECG signals using empirical wavelet transform and adaptive filtering. *J Med Eng Technol*. 2015; 39(1):60–68. [PubMed: 25412942]
- Su WC, Huang CS, Chen CH, Liu CY, Huang HC, Le QT. Identifying the Modal Parameters of a Structure from Ambient Vibration Data via the Stationary Wavelet Packet. *Computer-Aided Civil and Infrastructure Engineering*. 2014; 29(10):738–757.
- Suberbiola A, Zulueta E, Lopez-Guede JM, Etxeberria-Agiriano I, Grana M. Arm Orthosis/Prosthesis Movement Control Based on Surface EMG Signal Extraction. *International Journal of Neural Systems*. 2015; 25(3):1550009. (12 pages). [PubMed: 25851029]
- Takemura A, Inoue Y, Gomi H, Kawato M, Kawano K. Change in neuronal firing patterns in the process of motor command generation for the ocular following response. *J Neurophysiol*. 2001; 86(4):1750–1763. [PubMed: 11600636]
- Tian G, Xia Y, Zhang Y, Feng D. Hybrid genetic and variational expectation-maximization algorithm for gaussian-mixture-model-based brain MR image segmentation. *IEEE Trans Inf Technol Biomed*. 2011; 15(3):373–380. [PubMed: 21233052]
- Urrestarazu E, Jirsch JD, LeVan P, Hall J, Avoli M, Dubeau F, Gotman J. High-frequency intracerebral EEG activity (100–500 Hz) following interictal spikes. *Epilepsia*. 2006; 47(9):1465–1476. [PubMed: 16981862]
- Vahabi Z, Amirfattahi R, Ghassemi F, Shayegh F. Online Epileptic Seizure Prediction Using Wavelet-Based Bi-phase Correlation of Electrical Signal Tomography. *International Journal of Neural Systems*. 2015; 25(6):1550028. (22 pages). [PubMed: 26126613]
- Villar JR, Vergara P, Menendez M, de la Cal E, Gonzalez VM, Sedano J. Generalized Models for the Classification of Abnormal Movements in Daily Life and Its Applicability to Epilepsy Convulsions Recognition. *International Journal of Neural Systems*. 2016; 26(6) (21 template pages).

Wang H, Yajima A, Liang RY, Castaneda-Lopez H. Bayesian Modeling of External Corrosion in Underground Pipelines Based on the Integration of Markov Chain Monte Carlo Techniques and Clustered Inspection Data. *Computer-Aided Civil and Infrastructure Engineering*. 2015; 30(4): 300–316.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Research Highlights

- A new methodology for classification of neurons recorded in behavioral tasks
- The data set was recorded from non-human primates during a reaching task
- The methodology is capable of identifying and clustering similar firing rate profiles
- It produces physiologically meaningful classifications of neurons.

Figure 1.

Task setup. A) The subject had to press both pressure plates before starting. A target (either on the left or the right) would show up and depending on the color the subject would reach with his right or left hand depending on the color red (solid) or green (crosshatch) respectively. B) Sample trial RHLT where the subject is depicted reaching a left target with his right hand.

Figure 2. Neuron isolation procedure and firing rate averaging. A) steps to isolate neurons via PCA and GMM algorithms. B) averaging of each neuron over the four tasks per recording session

Figure 3.
The macro flowchart of the 4-step EM-GMM algorithm

Figure 4.

Feature space creation. The firing rate is transformed to a pseudospectrum using MUSIC. The first three highest frequencies in the spectrum, sorted by power (dB), are represented in a three-dimensional space where x, y, and z represent the highest, the second highest, and the third highest frequency.

Figure 5.

An example of decomposition comparing WT and EWT. The frequency bands subdivision scheme of WT is non-adaptive irrespective of the actual frequency content of the signal while that of EWT is adaptive and reflects the actual frequency content of the signal. Features in the MUSIC pseudospectrum are better represented by EWT compared with WT. (Definitions: *a1* – approximation, *d1–d3* – details, and *fb1–fb3* – frequency bands). Example time domain signals for each decomposition methodology are included to show signal features.

Figure 6. Example Signal Decomposition. The firing rate of an event is converted in the MUSIC pseudospectrum and decomposed using EWT. Three frequency bands are extracted with different amplitude/frequency components.

Figure 7.

A) clustered feature space corresponding to subject H and B) clustered feature space corresponding to subject N

Figure 8.
Two sets of sample signals from the four clusters for subjects H (A) and N (B).

Figure 9.

Decomposition of Subject H Sample Signals. The pseudospectrum and three frequency bands for subject H's sample signals. The figure is organized to mimic the organization of Figure 8 for ease of comparison.

Figure 10.

Example of Detected Behavioral Information using EM-GMM. A) Four firing rate profiles, each from a single neuron, are shown. Each event is classified using the EM-GMM algorithm and interpreted based on the classification. B) Two examples of other possible combinations found in the data.

Table 1

Results for Behavioral Validation of EM+GMM.

Area	Hand	Target			Grand Total
		Both Targets	Left Target	Right Target	
Cortex	Both Hands	17	3	1	21
	Left Hand	7	16	12	35
	Right Hand	10	23	5	38
PMRF	Both Hands	25	3	0	28
	Left Hand	4	9	7	20
	Right Hand	3	10	7	20
	Grand Total	66	64	32	162