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A Real-Time Spike Classification Method Based on Dynamic Time Warping for Extracellular Enteric Neural Recording with Large Waveform Variability

Yingqiu Cao^{a,*}, Nikolai Rakhilin^a, Philip H. Gordon^a, Xiling Shen^b, and Edwin C. Kan^a

^aSchool of Electrical and Computer Engineering, Cornell University, Ithaca, New York 14853, USA

^bDepartment of Biomedical Engineering, Duke University, Durham, NC 27708, USA

Abstract

Background—Computationally efficient spike recognition methods are required for real-time analysis of extracellular neural recordings. The enteric nervous system (ENS) is important to human health but less well-understood with few appropriate spike recognition algorithms due to large waveform variability.

New Method—Here we present a method based on dynamic time warping (DTW) with high tolerance to variability in time and magnitude. Adaptive temporal gridding for fastDTW in similarity calculation significantly reduces the computational cost. The automated threshold selection allows for real-time classification for extracellular recordings.

Results—Our method is first evaluated on synthesized data at different noise levels, improving both classification accuracy and computational complexity over the conventional cross-correlation based template-matching method (CCTM) and PCA + k-means clustering without time warping. Our method is then applied to analyze the mouse enteric neural recording with mechanical and chemical stimuli. Successful classification of biphasic and monophasic spikes is achieved even when the spike variability is larger than millisecond in width and millivolt in magnitude.

Comparison with Existing Method(s)—In comparison with conventional template matching and clustering methods, the fastDTW method is computationally efficient with high tolerance to waveform variability.

Conclusions—We have developed an adaptive fastDTW algorithm for real-time spike classification of ENS recording with large waveform variability against colony motility, ambient changes and cellular heterogeneity.

*Corresponding author: School of Electrical and Engineering, Cornell University, Ithaca, New York, 14853, USA, yc923@cornell.edu, Phone: +1 607 379 0076, Fax: +1 607 254 3508 .

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Keywords

dynamic time warping; enteric nervous system; FET sensors; extracellular action potentials

1. Introduction

Enteric nervous system (ENS) is composed of 200-600 million neurons found in the gastrointestinal tract and plays a vital role in upholding gut functions of motility, epithelial secretion, and intestinal barrier (Savidge et al. (2007); Furness (2014)). Disruption of ENS can cause Crohns disease (Lakhan and Kirchgessner (2010)), diabetes (Yarandi and Srinivasan (2014)), irritable bowel disease (IBD) (Straub et al. (2006)), and ulcerative colitis (Bassotti et al. (2014)). Different from those in the central nervous system (CNS), which is situated in the largely stationary brain, neural signals in the ENS are coupled to gut motility and peristalsis, interacting with gastrointestinal endocrine cells (Szurszewski et al. (2002)) and intestinal longitudinal muscles (Thuneberg and Peters (2001); Huizinga and Chen (2014)). These movements not only alter neural activities, but also cause electrode drift during recordings. Activities of the ENS is also influenced by the immune system via cytokines and mast cell tryptase (De Giorgio et al. (2004); Lomax et al. (2006)). Furthermore, the ENS consists of various types of enteric neurons embedded in dense mesh-like 2-D ganglia, called myenteric and submucosal plexuses, and hence an extracellular electrode will pick up signals from an ensemble of heterogeneous neurons with different action potential waveforms. These factors render ENS recordings large waveform variability, which is compounded by the lack of knowledge of the ENS. A spike classification method with high non-stationary tolerance is needed to reliably interpreting the ENS extracellular action potential (EAP) recording.

The recent decade has seen enormous progress in spike recognition and classification methods for CNS. Such methods typically include three steps: (1) detection of candidate spikes, (2) waveform feature extraction, and (3) clustering of spikes. Different combinations of feature extraction, e.g. principle component analysis (PCA) (Adamos et al. (2008)), wavelet transform (Quiroga et al. (2004)) and first and second derivative extrema (FSDE) (Paraskevopoulou et al. (2014)), and clustering, e.g. k-means (Chan et al. (2008)), superparamagnetic (Quiroga et al. (2004)) and hierarchical adaptive means (HAM) (Paraskevopoulou et al. (2014)), have been proposed for better accuracy. Overlapping spikes can be handled by either iteration and subtraction (Lewicki (1994); Zhang et al. (2004); Prentice et al. (2011); Pillow et al. (2013); Ekanadham et al. (2014)) or independent component analysis (Takahashi et al. (2003); Takahashi and Sakurai (2005); Jäckel et al. (2012)). Most of these methods are off-line, requiring the collection of all spikes before running the analysis, unsuitable for real-time closed-loop applications. Additionally, these methods require off-chip processing due to the high computational cost, limited by the stringent power density on chip for safe neural implants (Kim and McNamara (2007)).

A special case of the spike classification method for CNS that can be done in real-time is the template matching (TM) method, which uses the waveform in time domain directly in contrast to the extracted feature. TM compares each candidate spike to the template library

and makes assignment by the largest similarity. The template library must be formulated in advance by feature extraction and clustering either from the initialization phase of the same experiment (Navajas et al. (2014)) or from earlier experiments. The key step in TM is the similarity calculation, for which Euclidean distance (Rutishauser et al. (2006); Karkare et al. (2013)) and cross correlation (Kim and McNames (2007)) are most widely used. Both similarity calculations can be implemented in neural implant with real-time, low-power on-chip processing to render a reduced data transmission rate (Lopez et al. (2012); Rizk et al. (2009); Wattanapanitch and Sarpeshkar (2011)).

While many of these spike classification methods are used in CNS, they cannot be used as effectively in ENS. Minimal waveform variability in phase, duration and magnitude was often assumed, which is invalid due to electrode drift and non-stationary waveforms (Bar-Hillel et al. (2006)). Electrode drift is caused by the neuron movement relative to the recording electrode, as well as the change in the electrolytic property of the biological environment (Quiroga (2009)). Non-stationary waveforms refer to the change of the spike shape over time (Wolf et al. (2009)). In CNS, Such variability problems have been investigated previously by modeling the source neurons as a mixture of Gaussians. Bayesian clustering (Bar-Hillel et al. (2006)) was employed to calculate the candidate clusters in short-time frames, and the transition probabilities among each cluster mixture determine the cluster choice as the maximum-a-posteriori solution. Simplification based on Kalman filtering (Calabrese and Paninski (2011)) can be utilized to enhance efficiency. Bayesian optimal TM (Franke et al. (2015)) was also suggested for online applications. However, the main focus of these methods was on the variability of the spike magnitude caused by the movement of the cluster centers, but not on the time course, which proves critical for ENS EAP recordings due to large colony motility (Thuneberg and Peters (2001)) in the complex environment.

Here we propose dynamic time warping (DTW) as a similarity measure with high tolerance to the spike variability in both magnitude and time. DTW has proven to be very effective in speech recognition under source and ambient variations (Zhang et al. (2014)) and ECG profile characterization (Huang and Kinsner (2002)), which are similar to the ENS EAP analysis at hand. We established a fastDTW method with automatic thresholding for ENS recordings with large spike variability for real-time applications. During the similarity calculation, we have also implemented adaptive temporal gridding (Salvador and Chan (2004)) with linear complexity. The performance of fastDTW is first evaluated on synthesized ENS EAP data at various noise levels, showing remarkable enhancement in accuracy and computational complexity in comparison to cross-correlation based TM (CCTM) and PCA + k-means clustering without time warping. To demonstrate the manner and degree how our fastDTW method can impact neural recording, experimental EAP of the mouse enteric neurons under different stimuli are analyzed, where our fastDTW method was able to successfully classify biphasic and monophasic spikes when the waveform variability is more than millisecond in width and millivolt in magnitude. In addition to more precise spike counting than the TM method, fastDTW also directly provides specific waveform parameters with associated changes in time for further statistical processing and behavior recognition.

2. Methodology

We will first introduce dynamic time warping (DTW) as a similarity measure with high tolerance to the spike variability. Automatic thresholding and fastDTW are then added to enhance real-time performance. We use synthesized ENS EAP recordings for accuracy benchmark. We will then describe the experimental setup for EAP recordings of mouse enteric neurons by different stimuli and inhibition with an emphasis on how variability can be captured in the presence of high noise level.

2.1. DTW in similarity calculation

We will show how DTW can be effectively and efficiently employed for similarity calculation in the neural spike classification algorithm when large variability is expected.

Let X denote the time series of the spike template with length n , and Y denote the time series of the candidate spike segment with length m (X and Y are normalized in magnitude before similarity calculation):

$$X = x_1, x_2, \dots, x_i, \dots, x_n \quad (1)$$

$$Y = y_1, y_2, \dots, y_j, \dots, y_m \quad (2)$$

where i, j are time indexes of the series X and Y , respectively.

The similarity matrix $[a]_{nm}$ is constructed where each element is the normalized Euclidean distance between a pair of points in series X and Y :

$$a_{ij} = d(x_i, y_j) = \frac{|x_i - y_j|}{m+n} \quad (3)$$

A warp path P is defined on the similarity matrix $[a]_{nm}$

$$P = p_1, p_2, \dots, p_k, \dots, p_l \quad (4)$$

where l is the length of the warp path and the k^{th} element of the warp path is

$$p_k = (i, j) \quad (5)$$

The warp path starts at $p_1 = (1, 1)$, and ends at $p_l = (n, m)$, with the time indexes increasing monotonically:

$$p_k = (i, j), \quad p_{k+1} = (i', j') \quad i \leq i' \leq i+1 \quad j \leq j' \leq j+1 \quad (6)$$

The minimum-distance warp path P is then found, with a cumulative distance of:

$$d(P) = a_{11} + \sum_{k=1}^{K-1} d(p_k, p_{k+1}) = \sum_{p_k=(i,j) \in P} a_{ij} \quad (7)$$

The minimum-distance warp path P in Eq.(7) is usually obtained by dynamic programming:

$$\begin{aligned} D(i, j) &= d(p_1, p_k) \\ &= a_{ij} + \min(D(i, j-1), D(i-1, j-1), D(i-1, j)) \end{aligned} \quad (8)$$

The minimum $d(P)$ is between 0 and 1, which is used as an indicator of the similarity ξ between the template and the candidate spike :

$$\xi = 1 - d(P) \quad (9)$$

Based on the minimum-distance warp path P , a new time series Y' with length n can be created, which is the candidate spike time warped (TW) to the template:

$$Y' = y_{s_1}, y_{s_2}, \dots, y_{s_h}, \dots, y_{s_n} \quad (10)$$

The time index s_h of the time warped candidate spike Y' is the 2nd coordinate of the points on P , when the 1st coordinates go from 1 to n :

$$S = s_1, s_2, \dots, s_h, \dots, s_n, \quad (h, s_h) \in P, \quad h=1, 2, \dots, n \quad (11)$$

Figure 1 shows an example of the similarity calculated with DTW. The candidate in Fig. 1(a) is the template parallel-shifted by 25% of the segment width. If we denote the segment width to be 360° in phase, a 25% shift corresponds to a 90° phase shift. The candidate spike in Fig. 1(b) is the template scaled to half the duration. The similarity matrices are plotted in Figs. 1(c)(d), respectively. Darker color indicates a larger Euclidean distance and less similarity. The minimum-distance warp path P is plotted in a red line on top of the similarity matrix. P consists of two parts: the distance from the waveform similarity independent of the warping in time domain, and the distance from spike misalignment, stretching or shrinking. The similarities ξ in Figs. 1(a)(b) are 0.945 and 0.95, respectively, very close to 1 for exact matching. The matched pairs of points in the template and the candidate spike are connected by red dash lines.

DTW in Eq. (8) has a complexity of $O(N_2)$, where N is the size of the input series. In order to expedite the algorithm, we adopted fastDTW in our work (Salvador and Chan (2004)) with a linear complexity $O(N)$. FastDTW accelerates DTW by coarsening and refinement, as demonstrated in Fig. 2. P is first found in the lowest resolution by coarsening the two input series. The path searching returns to high resolution by refining the area containing the present path in a quad-tree manner. The search continues until the original series resolution is reached.

2.2. Overview of the fastDTW method with automatic thresholding

The algorithm for our proposed fastDTW has three main parts as shown in Fig. 3: pre-processing for one time before each recording setup, spike candidate detection, and spike classification by fastDTW as a similarity measure. The latter two steps can be pipelined to decrease the overall computational time.

2.2.1. Pre-processing—First, the recorded data are passed through a bandpass filter from 1 Hz to 30 kHz in Fig. 3(a) to remove the slow voltage drift and high frequency noise. Then the filtered data is cut into short time bins to collect statistics for further automated processing. The bin size needs to be sufficiently large to expect to encompass more than 10 spikes under stimulation for better statistical collection and automated decision making. Although the choice of bin location and size can readily be adaptive, we have used a fixed size of 250 ms for the synthesized data, and 500 ms for the experimental data for simplicity in this work. Different bin size can also be chosen depending on the application. The spikes in each time bin will then be detected and classified.

2.2.2. Spike candidate detection—The candidate spikes in each time bin are detected by automatic thresholding. The threshold is determined by the statistics of local maxima. The magnitude distribution of the local maxima is extracted for its probability density in Fig. 3(c). The largest maximum of the estimated probability is most likely due to noise, while the other maxima by actual EAP (Kim and McNames (2007)). The threshold is determined automatically by locating the first minimum to the right of the first maximum.

Candidate spikes with peak magnitude larger than the threshold are saved in segments, with the segment width that is larger than but close to the anticipated spike duration. If the spike duration cannot be easily estimated beforehand, or the duration varies significantly over time, multiple segments with different widths covering the duration range are saved for each spike candidate.

Although the peak location of the candidate spike is estimated in the threshold determination procedure, the candidate spike can still have temporal misalignment with the template. In conventional TM where cross-correlation or sum of Euclidean distance is used as the similarity measure, either peak alignment or a sliding window scheme needs to be applied according to the misalignment tolerance. Accurate peak alignment to the template usually requires up-sampling (Rutishauser et al. (2006)), and is difficult in many situations. For example, if the spikes are aligned to the peak with the largest absolute value, biphasic spikes that have similar magnitudes for the positive and negative peaks can be easily misaligned. “Sliding window” saves multiple shifted versions of each candidate spike with equal width. Spike recognition is then performed on the multiple segments for each candidate. In our fastDTW method, which can tolerate large duration and alignment variation, omission of peak alignment or “sliding window” greatly reduces the computational complexity.

2.2.3. Spike classification—When no spike template is available, the template estimator first calculates the averaged spike waveform in the first few time bins from the saved spike candidates by taking the median of the spikes (Fig. 3(d)). The estimated templates are used

to start the algorithm and are modified adaptively to deal with the evolution of spike shapes over time.

When the spike templates are known from cellular model construction (Fromherz (1999)) or feature extraction and clustering in earlier experiments, the candidate spikes in the time bins can be classified by similarity directly using fastDTW (Fig. 3(e)). The similarity distribution is formed with the probability density estimator and the similarity threshold (Fig. 3(f)) is automatically determined in the same way as the spike magnitude threshold. Spike candidates with similarities above the similarity threshold to a template are classified to that group, and the rest are further checked by other templates or are discarded. With the classified spikes in the time bin, the template estimator can iteratively modify the template by learning over time (Fig. 3(g)). The classification process is repeated for all templates and then proceeds to the next time bin. If a spike candidate is recognized by more than one template group, it should be classified to the group with highest similarity.

A hard threshold of similarity can be alternatively set for each template to reduce the false positive rate as well. If the automatic similarity threshold by statistics is smaller than the hard threshold, the similarity threshold is forced to the hard threshold. In conventional TM method, the choice of the hard threshold greatly influence the accuracy. In contrast, fastDTW has high tolerance to the threshold variation and in most case the automatic similarity threshold is sufficient without the need for a hard threshold. Our proposed fastDTW algorithm is thus fully automatic, suitable for experimental recordings with pixel-array recording sites (Eickenscheidt et al. (2012); Jäckel et al. (2012); Hutzler et al. (2006)), where manual intervention of the threshold for each pixel would be impractical.

2.3. Accuracy benchmark on synthesized EAP recording

The accuracy of fastDTW as described in Section 2.2 is evaluated on synthesized recordings in comparison with CCTM and PCA + k-means clustering methods with and without time warping, as the ground truth is known.

The recording is synthesized as the sum of known EAP waveforms and white noises. Two spike waveforms are used in the simulation: biphasic and monophasic (Fig.4), which had been observed in ENS EAP experiments (Spencer and Smith (2004); Cornelissen et al. (2000); Nurgali et al. (2004)). The duration of the synthesized spikes follows Gaussian distribution with 1 ms mean and different variances are applied to mimic the duration variation in ENS.

Each recording contains 100 biphasic spikes and 100 monophasic spikes at random firing time (spacing larger than the refractory period of 3 ms) at signal-to-noise ratios (SNR) from 10 to 3.

In comparison, CCTM, PCA + k-means clustering with and without time warping are also implemented. CCTM follows the same procedure in Section 2.2 except that cross-correlation in Eq.(12) is used as the similarity measure instead of DTW. For each candidate spike, CCTM runs on 3 segment widths. At each segment width, sliding window of 10 shifted segments is employed.

$$\xi = \sum_{i=1}^n \left(x_i - \bar{x} \right) \left(y_i - \bar{y} \right) \quad (12)$$

For PCA + k-means clustering, all candidate spikes in the whole recording are collected, aligned to the largest peak and segmented before processing. Spike detection is done in a similar manner as described in Section 2.2.2. Candidate spike segments with or without TW are used as inputs of PCA. With TW, each candidate spike is time warped to the most similar template using DTW before running PCA. The minimum distance warping path P between the candidate spike and the template is found as described in Section 2.1, and the candidate spike is time warped using Eqs. (10) and (11). The principle components are then utilized for k-means clustering.

The accuracy of the methods are evaluated by the true positive rates (R_{TP}) and the sum of false positive (FP) and false negative (FN) at different noise levels. When the method reports a spike of certain template group and the spike truly belongs to that group, a TP is recorded. Otherwise the reported spike is a FP. When a spike in the synthesized data is not reported by the method, a FN is recorded.

$$R_{TP} = \frac{\text{number of TP spikes}}{\text{total number of spikes in the recording}} \quad (13)$$

R_{TP} is between 0 and 1, when 1 represents all spikes being correctly classified by the method. There are two sources for FP spikes: by the background noise mistaken as a spike, and by misclassification to the wrong template group. An accurate method provides a high R_{TP} and a small sum of FP and FN (FP+FN).

2.4. Experimental methods

We used a non-amperimetric CuMOS sensor (Jayant et al. (2013)) for the experimental EAP recording of mouse ENS neurons in the jejunum tissues. As large ambient variability is expected from motility and frequent vesicle release, CuMOS can use its control gate to pin the transistor operating point for less waveform distortion and reduce reliance on the large reference electrode which is impractical for future *in vivo* monitoring. The operating principle of the CuMOS sensor was described in the supplementary material. Intestine tissues were harvested from C57BL/6J mice (Jackson Laboratory) about 20 minutes before the recording. Longitudinal muscle-myenteric plexus (LMMP) tissue was isolated and kept in phosphate buffered saline (PBS) of pH 7.4 at room temperature. A $1\text{ mm} \times 1\text{ mm}$ section of LMMP was placed onto the sensing electrode chip with $200\mu\text{m} \times 200\mu\text{m}$ gold electrodes, which was secured by a 3D-printed fluidic well of epoxy. The electrode chip was wire-bonded to the sensing gate of the CuMOS sensor as in Fig. 5. The neural activities were then captured by the output drain current of CuMOS . A Keithley 2400 was used to set the transistor operating point. The drain current was converted to voltage by the transimpedance amplifier (TIA) (Stanford Research System SR570, CA, USA) with a sensitivity of $100\mu\text{A/V}$ and a band-pass filtering from 1 Hz to 300 kHz. The TIA output was collected on the computer through a data acquisition test board (NI BNC 2110 and NI USB 6259). The tissue

was chemically (300mM KCl) or mechanically (tweezers stretching) stimulated to induce action potentials. To verify the action potential signal origin, the LMMP neural signal was later suppressed through the addition of 1mM tetrodotoxin (TTX).

3. Resultes and discussion

3.1. Spike alignment and duration variation on similarity

The effect of timing variability on similarity calculation and the resulting spike recognition ability is compared between fastDTW and CCTM. Two spike templates of biphasic and monophasic waveforms are adopted in the synthesized recording (Fig. 4). The candidate spike segments are of the same waveforms, either shifted to mimic the misalignment, or shrunk or expanded to mimic the duration variation (similar to Fig. 1).

In order to achieve correct spike recognition, the similarity between the candidate spike and the true template group must be larger than the similarity threshold, and the similarity with all other groups should be smaller than the threshold. In the ideal case without noise, the minimum similarity threshold for correct spike discrimination is 0.95 for fastDTW and 0.82 for CCTM (Fig. 6).

The similarity is a function of the spike misalignment, as calculated by fastDTW (Fig. 6(a) top) and by CCTM (Fig. 6(b) top). Using the similarity threshold criteria, fastDTW can correctly detect and discriminate both waveforms in a 201° window out of 360° for misalignment. In contrast, CCTM only allows a 18° window. In other words, at most 2 shifted segments are needed (and in many situations one segment works reasonably well) for each candidate spike by fastDTW, in comparison to 20 segments by CCTM. Because of the tolerance to misalignment, fastDTW is expected to have less computational cost than the CCTM to achieve the same classification accuracy, by avoiding repetitions on shifted segments.

The similarity is also a function of the spike duration variation, as calculated by fastDTW (Fig. 6(a) bottom) and by CCTM (Fig. 6(b) bottom). FastDTW can detect and discriminate the biphasic and monophasic spikes accurately in mosy duration variation from 0.5 ms to about 1.45 ms. In comparison, CCTM can only tolerate the duration variation of about 0.2 ms for monophasic spikes and 0.12 ms for biphasic spikes. As a result, CCTM will need to run on more than 6 segment widths for the duration variation between 0.5 and 1.5 ms so as to achieve same accuracy as fastDTW.

3.2. Accuracy analysis

The accuracy of fastDTW is compared with those of CCTM and PCA + k-means clustering with and without TW as described in Section 2.3. The accuracy as a function of SNR is plotted in Fig. 7. FastDTW has a R_{TP} only slightly smaller than PCA + k-means clustering with TW, which is an off-line method requiring data collection of all spikes before processing. Among these methods, fastDTW shows the smallest FP + FN at all noise levels.

Fig. 8 demonstrates how time warping of candidate spikes increases the accuracy of PCA + k-means method at two noise levels SNR=10, and 3. The candidate spikes assigned to

Cluster 1 (monophasic) and 2 (biphasic) are plotted in blue and red, respectively. Without TW, it's difficult to separate the two clusters even at SNR=10 because the shapes are non-Gaussian due to spike waveform variability (Fig. 8(a) left). The estimation of cluster centroids is rather hard and about 50% of biphasic spikes are misclassified as monophasic. The average waveforms of the true and misclassified spikes can be found in Supplementary Material. With TW, both R_{TP} and the FP + FN improved noticeably with clearly separable clusters (Fig. 8(a) right). Most of the misclassified spikes are outliers far away from the cluster centroids, which may be removed by applying constraints on the largest distance to the centroids to reduce FP. At SNR=3, the clusters are further corrupted by noise (Fig. 8(b) left). However TW is still able to improve the accuracy of PCA + k-means clustering.

The waveform dependency of accuracy by different methods is depicted in Fig. 9. Using highly waveform dependent method, the classification of one (or a few) cluster(s) can have many errors, even the overall FP+FN is low. The variation of accuracy with different template waveforms by fastDTW is the smallest. With fastDTW, the biphasic spikes demonstrate slightly higher R_{TP} than the monophasic spikes (Nguyen et al. (2014)).

The influence of the similarity hard threshold on accuracy is investigated for fastDTW and CCTM (Fig. 10). As described in Section 2.2.3, besides the automatically determined similarity threshold by statistics Th_{auto} , a hard threshold Th_{hard} can be alternatively set to reduce the FP rate. If Th_{auto} is smaller than Th_{hard} , the similarity threshold Th_{sim} is forced to the Th_{hard} in Eq.(14).

$$Th_{sim} = \max(Th_{auto}, Th_{hard}) \quad (14)$$

The relative hard threshold is defined in Eq. (15).

$$\text{relative hard threshold} = \frac{Th_{hard} - Th_{hard,min}}{Th_{hard,max} - Th_{hard,min}} \quad (15)$$

Where $Th_{hard,max}$ and $Th_{hard,min}$ are as follows.

$$Th_{hard,max} = \min [Th_{hard}: TP \rightarrow 0] \quad (16)$$

$$Th_{hard,min} = \max [Th_{hard}: FP \rightarrow FP_{max}] \quad (17)$$

When Th_{hard} is as high as $Th_{hard,max}$, no spike is classified to the template group and the relative hard threshold is 1. When Th_{hard} is $Th_{hard,min}$, FP is the largest and the relative hard threshold is 0. $Th_{hard,max}$ and $Th_{hard,min}$ are determined by sweeping Th_{hard} .

R_{TP} (FP + FN) decreases (increases) more slowly with increasing relative hard threshold by fastDTW than by CCTM. The accuracy is less sensitive to the change of the relative hard threshold by fastDTW. Even if it goes to zero, fastDTW still gives a R_{TP} of about 0.92 (0.8) and a FP + FN of about 20 (100) at SNR=10 (SNR=3). In other words, when fastDTW is

used, TR_{auto} is sufficient to reject most false spikes, with no need to choose an optimized hard threshold. FastDTW is thus fully automatic, suitable for experimental recordings with pixel-array recording sites (Eickenscheidt et al. (2012); Jäckel et al. (2012); Hutzler et al. (2006)), where setting Th_{hard} manually for each pixel would be impractical.

3.3. Computational complexity

The computational complexity is important to realize on-chip spike classification due to the stringent constraints on power, bandwidth and area. The computational complexity of fastDTW is compared with those of CCTM and PCA + k-means clustering in Table 1. The computational complexity is defined as the number of operations performed by the algorithm (eg. addition, multiplication) (Sipser (2005)), where each operation takes a fixed amount of time to perform. PCA can be transformed to a problem of singular-value decomposition (SVD) of a matrix (Householder (1958)). The complexity of k-means clustering is estimated assuming Lloyds algorithm (Lloyd (1982)) which runs iteratively for the local optimum. For fastDTW and CCTM, the complexity is estimated on the similarity calculation, because other steps of the algorithm run only once for the whole time bin.

The actual running time is subject to the hardware platform and different schemes have been introduced to determine a single complexity figure-of-merit (CFOM). For example, Gibson et al. (2008) and Paraskevopoulou et al. (2013) define CFOM as the sum of the number of additions and ten times the number of multiplications. Here we assume ARM Cortex M4 as the hardware platform and define CFOM as the weighted sum of operation numbers, where the weight is the number of clock cycles for each operation:

$$CFOM = N_{\text{addition}} + N_{\text{multiplication}} + N_{\text{shift}} + N_{\text{condition}} + 10N_{\text{division}} + 14N_{\text{squareRoot}} \quad (18)$$

Figure 11 demonstrates the trade-off between the error rates and CFOM. The value of the variables are shown in Table 2. It can be clearly seen fastDTW offers the lowest error rate with a very small complexity (3000 clock cycles per spike). Assuming a micro-controller frequency of 10 MHz, each spike is classified within 0.3 ms, making fastDTW a real-time method. Assuming 100 recording channels, each with a firing rate of 20 Hz, this corresponds to 6 MIPS and 2 μW /recording channel in power consumption (ARM Cortex M4 consumes 33 μW /MHz).

Furthermore, as can be seen in Table 1, fastDTW only requires addition, condition and right/left shift to run, in contrast to PCA + k-means clustering requiring multiplication/division and square root. The operations needed by fastDTW can potentially be implemented more efficiently in hardware (just like matched filter can be used for CCTM), so that the recording in each channel is processed in parallel with less than 2 μW /recording channel.

3.4. Variable spike waveforms of experiment data

Mouse ENS EAP recording with high variability from cellular motility and vesicle release was performed on a non-amperimetric CMOS sensor with stabilized biasing Jayant et al. (2013) and analyzed by fastDTW and CCTM. The segment width is chosen to be 6.4ms to

allow for large variation in spike widths. The time bin to learn the average spike templates in the initialization phase is 1s and is changed to 0.5s in the later classification phase.

Two major spike waveforms were recognized: the biphasic (BP) and the monophasic (MP) spikes. EAP waveforms reflect the time profiles of the total membrane current as the summation of sodium, potassium, capacitive and leakage currents (Gold et al. (2006)). The magnitude and the time course are further determined by the neuron type and morphology.

The bandpassed experimental recordings are plotted for mechanical stimulation (Fig. 12(a) top), chemical stimulation (Fig. 12(b) top), and TTX inhibition (Fig. 12(c) top). Mechanical stimulation by tissue stretching was applied twice at about 1s and 35s, which mimics stretching by intestinal peristalsis and stimulates serotonin-mediated enteric neuron firing. The peaks with magnitude over 3mV (Fig. 12(a) top around 40s) are most likely caused by movements between tissues and electrodes during the tweezers touching and stretching procedure. The captured BP and MP spikes are zoomed in during a 0.5s segment under the mechanical stimulation, which has magnitude below 0.25 mV (Fig. 12(d)). Chemical stimulation and inhibition was performed by KCl (Fig. 12(b)) and TTX (Fig. 12(c)) addition about 5 minutes before the recording, limited by diffusion.

The firing rates sorted by fastDTW over time are shown in Fig. 12 2nd row. The firing rate of BP spikes increased immediately after the mechanical stimulation applied at 35s. Both the BP and MP spikes ebbed away gradually after 90s. The magnitude (Fig. 12(a) 4th row) of the captured spikes peaked immediately after 35s, and returned to a smaller value after 50s. This suggests that mechanical stimulation increased enteric neuron firing, which subsided after a period. In contrast to the mechanical stimulation, neuronal firing stimulated by chemical stimulation sustained much longer, and the magnitude remained almost unchanged. TTX shuts down the sodium channel, and accordingly we observed the BP spike stopped, followed by the MP spikes (Only first 10s of the 120s long recording shown, no spike was present after the first 10s).

In comparison with CCTM (Fig. 12, 3rd row), fastDTW can not only count and capture firing more accurately, but also report the waveform evolution over time. The half-widths of the first and second phases of the BP spikes, and that of the MP spikes before, during and after the mechanical stimulation at 35s are depicted in Fig.13(a). Comparing before and right after stimulation, the half-width of the 1st phase of the BP spikes increased from about 0.75 ms to 1 ms. The half-widths of both the 2nd phase of the BP spikes (0.71 ms) and the MP spikes (0.53 ms) remained relatively constant. These waveform features are also demonstrated by the re-aligned median waveforms over time (Fig. 13(b)(c)), with clearly observable variations. For chemical stimulation, the BP spikes demonstrated a more constant 1st phase half-width of about 1 ms (Fig. 14(a)). The half-width of the 2nd phase of the BP spikes, as well as that of the MP spikes, decreased gradually from 0.9 ms to 0.63 ms, and from 0.79 ms to 0.6 ms, respectively. The re-aligned waveforms are shown in Figs. 14(b)(c).

FastDTW captures approximately 3 times more spikes than CCTM overall, which can cause inaccurate interpretation of ENS neural responses. The additional spike waveforms associated with larger variability recognized by fastDTW provide new insights to understand

the ENS. The enhanced accuracy and efficiency as well as the detailed waveform reports in fastDTW offer a useful tool to study the not-well understood and highly variable ENS from EAP recording.

The experimental data processed by PCA + k-means clustering can be found in the Supplementary Materials.

4. Discussion

To illustrate the new analyses enabled by fastDTW, we further studied the correlation between the various non-stationary effects in the mouse ENS experiments.

When the captured spikes are sorted by their firing rate, for both mechanical and chemical stimulation, the BP spikes have a slightly longer half-width in the 1st phase than in the 2nd phase (Fig. 15). An increase of the BP spike half-width at firing rates above 10 Hz was observed for mechanical stimulation, while the BP spike half-width shows no correlation with the firing rate for chemical stimulation. The magnitude of the BP spikes increases with the firing rate for both the mechanical and chemical stimulation. This may result from the occurrence of compound action potentials, when the neuron colony is highly excited. Another possibility is that spikes with higher magnitude and hence SNR are easier to sort out. The MP spikes showed no significant change in the half-width, and a slight increase of magnitude with the firing rate for mechanical stimulation.

The correlation coefficient between the spike half-width and the corresponding magnitude, and the significant level of the correlation (p value) are listed in Table 3. The correlation coefficients are marked in green for significant correlation, and red otherwise. For mechanically stimulated BP spikes, the spike half-width is positively related to the corresponding peak magnitude, which is possibly due to compound action potentials at high firing rate larger than 10 Hz. The BP spikes induced by chemical stimulation showed a slight decrease in the width with increasing magnitude at higher firing rate, indicated by the negative correlation coefficient, which has been observed in different neuron systems (Stratton et al. (2012)). For MP spikes induced mechanically, no significant correlation between the half-width and magnitude was found by the large p value, while the half-width of chemically induced MP spikes is negatively correlated to the magnitude.

5. Conclusion

We have introduced fastDTW as an automatic spike classification method specially suited for real-time EAP recording with high waveform variability in time and magnitude. FastDTW provides improvements in accuracy and computational cost in comparison with CCTM and PCA + k-means clustering without time warping. We apply fastDTW to the mouse ENS neurons with high cellular motility and frequent vesicle release, using the CMOS sensor with stabilized biasing in response to various stimuli. Biphasic and monophasic spikes are successfully recognized when the variability is as large as 1.2 ms in width and a few milli-volt in magnitude. We then illustrate how the captured waveform features can be used for variation correlation analyses. As fastDTW offers improved

efficiency, accuracy and variability feature extraction, we believe it would benefit the ENS research community when large variability is expected in the EAP recording.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- An online spike classification method based on dynamic time warping for extracellular enteric neural recording with large waveform variability.
- Automatic classification for pixel-array operations without user intervention.
- Improved classification accuracy and computational cost over conventional cross-correlation based template-matching method and PCA+k-means clustering.
- Demonstrated on mouse enteric neural recordings in response to mechanical and chemical stimuli.

Figure 1.

Similarity calculation using direct DTW. (a)(b): Template (blue solid lines) and candidate spike (blue dash dot lines) with the matched points connected by red dashed lines. (c)(d): Similarity matrices with the minimum-distance warping paths (red) for the spikes in (a) and (b).

Figure 2.

FastDTW expedites direct DTW to linear time complexity. At each resolution stage, the optimal warping path is found in a window (colored square) containing the lower resolution warping path (red solid line).

Figure 3.

Flow chart of fastDTW with automatic threshold decision. (a) Band-passed data of EAP. (b) Low-passed (100Hz) waveforms (blue) on top of the raw signal (grey) with the local maxima (red) detected as candidate spikes. (c) Magnitude distribution of candidate spikes, with a threshold determined as the local minimum to the right of the largest maximum. (d) Spike template used in classification. With no available templates (middle left), the estimated spike templates (red) are obtained by averaging the candidate spikes (grey) above the threshold in the first few time bins during initialization. (e) The similarities between the candidate spikes and the template are calculated by fastDTW. (f) The automatic similarity threshold is determined in the same way as in (c). Candidate spikes with larger similarity than the threshold are assigned to the template group. (g) A new averaged spike waveform is calculated from the classified spikes in the time bin, which can modify the previous spike template adaptively to account for the continuously slow change.

Figure 4.
EAP waveforms of (a) the biphasic and (b) the monophasic spikes

Figure 5.

(a) Morphology of the neurons in mouse ENS. (b) A two-compartment circuit model of the neuron and CMOS sensor interface. (c) A picture of the CMOS sensor (the small chip on glass), and the electrode chip (the large chip with the fluidic well) used for the EAP recording.

Figure 6.

Comparison of fastDTW and CCTM in similarity calculation: (a) Similarity calculated by fastDTW with spike misalignment (top) and duration variation (bottom). (b) Similarity calculated by CCTM with spike misalignment (top) and duration variation (bottom). BP stands for biphasic, and MP stands for monophasic. The grey lines represent the minimal similarity threshold that ensures no misclassification in the ideal case without noise.

Figure 7.

R_{TP} (a) and FP + FN (b) as a function of SNR by different methods. For fastDTW and CCTM, the similarity threshold is chosen to minimize the FP + FN. Spike duration α norm(1,0.3) (Gaussian distribution) in the synthesized recording.

Figure 8.

Classified spikes projected to the plane of the first two principle components, by PCA + k-means clustering with and without TW, at SNR=10 (a) and SNR=3 (b). Spike duration α norm(1,0.6) in the synthesized recording.

Figure 9.

R_{TP} (red) and $FP + FN$ (blue), between biphasic and monophasic spikes, by different methods. Spike duration $\alpha \text{ norm}(1,0.3)$ in the synthesized recording.

Figure 10.

R_{TP} (a) and FP + FN (b) as a function of relative similarity hard threshold by fastDTW and CCTM. Spike duration α norm(1,0.3) in the synthesized recording.

Figure 11.

Trade-off between the FP+FN rates (averaged over all SNRs) and CFOM for different methods. Spike duration α norm(1,0.3) in the synthesized recording.

Figure 12.

Bandpassed experimental recording (1st row), firing rate of spikes classified by fastDTW (2nd row) and by CCTM (3rd row), averaged spike magnitude (4th row) over time for (a) mechanical stimulation, (b) chemical stimulation, and (c) TTX inhibition. (d) A zoom-in view of the 0.5-second segment under the mechanical stimulation, showing the spike train. Notice that the waveform features with magnitude larger than 3mV under mechanical stimulation around 40 s were not recognized as action potentials, but more likely due to movement between tissues and electrodes. Due to the large variability in ENS EAP recording, the robust classification algorithm is very critical, because user inspection can be misleading.

Figure 13.

(a) The Box-and-whisker plot for the half-widths of the mechanically induced spikes; The aligned waveforms of (b) MP and (c) BP spikes before, during and after the mechanical stimulation. The red lines represent the medium waveforms. The nonstationary waveform features can be clearly observed.

Figure 14.

(a) The Box-and-Whisker plot for the half-width of the chemically induced spikes. The averaged waveforms over time, for the MP spikes (b) and BP spikes (c).

Figure 15.

The Box-and-whisker plots of spike half-width and magnitude vs. the firing rate, (a) for mechanically induced spikes, and (b) for chemically induced spikes.

Table 1

Complexity per spike of different methods.

Method	Conditions	Additions	Multiplications	Divisions	Right/left shifts	Square root
PCA+k-means w/o TW	kir	$2n^2 + 2n + kpir$	$4n^2 + 2n + kpir$	pir	-	kir
PCA+k-means w/ TW	$kir+8kn$	$2n^2+(8k+2)n+kpir$	$4n^2 + 2n + kpir$	pir	$8kn$	kir
FastDTW	$8kn$	$8kn$	-	-	$8kn$	-
CCTM	-	knm	knm	-	-	-

Table 2

Variables to estimate the computational complexity.

Variable Symbol	Description	Value
n	number of samples in each spike	64
k	number of templates (clusters)	2
p	numebr of features per spike, $p \quad n$	3
i	number of iterations to find the local optimum for k-means clustering	10
r	number of replicates so that the local optimum equals to the global optimum for k-means clustering	3
m	number of stretched (shrunk) and shifted segments for each candidate spike for CCTM	3×10

Table 3

Correlation between the spike magnitude and half-width for mechanical and chemical stimulation.

Correlation coefficient (p value)	Biphasic spikes half-width		Monophasic spikes Half-width	Number of biphasic, monophasic spikes
	1st phase	2nd phase		
Magnitude, mechanical stimulation	0.3564 (<.00001)	0.2673 (0.0002)	0.1091 (0.1461)	n = 190, 179
Magnitude, chemical stimulation	-0.4681 (<.00001)	-0.2695 (0.0003)	-0.2232 (0.0043)	n=174, 162