Separation of Action Potentials in Multiunit Intrafascicular Recordings

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***Abstract*—Classification of action potentials in multiunit recordings was based on the use of various types of features to uniquely characterize action potentials from different cells. We compared classification results obtained using three types of descriptive features: digitized data points, amplitude and duration (time domain) parameters, and fast Fourier transform (FFT) coefficients. Digitized data points used as descriptive features provided good classification success and required minimal computation. Time-domain features gave comparable results but required more computation. FFT coefficients were less effective than the other features. As the signal-to-noise ratio of the recordings increased, smaller differences in feature values could be discriminated.**

INTRODUCTION

WORK in our lab has focused on the development of long-term implantable intrafascicular electrodes for recording activity from peripheral nerves. Recordings made with these electrodes include activity from multiple cells. In order to extract more useful information from these recordings, we have developed a computer-based method for classifying single-unit activity in these multiunit recordings.

Methods for discriminating single-unit activity in multiunit signals typically take advantage of the fact that action potentials produced by an individual cell have a characteristic shape which is determined by factors such as the size and conduction velocity of the axon and the distance of the cell from the recording electrode [9]. Action potentials produced by different cells may have different characteristic shapes, and once the features which uniquely specify these shapes have been identified, it is possible to classify action potential based on the evaluation of those features. In the present study, we were especially interested in action potential classification methods which can be employed continuously on-line and in real time, and which are effective for neural recordings with relatively low signal-to-noise ratios, as in the case in recordings from intrafascicular electrodes.

Action potential waveforms have been characterized by various amplitude or duration features [1], [3], [7], [11]-[13], [23], or by simply using some or all of the digitized points as a "template" [2], [4], [14], [17], [18], [22]. However, it is difficult to draw conclusions about the relative usefulness of the different approaches since standard data sets have not generally been used to compare the classification algorithms [15], [16], [21].

In the present study, we compared the efficacy of using different action potential features for unit identification, employing a fixed classification algorithm and standard set of test data to determine which features provide the most useful information for discriminating between action potentials recorded from different cells with intrafascicular electrodes.

METHODS

Recordings of multicellular neural activity were made from feline sensory nerves with intrafascicular electrodes. In addition, sequences of simulated neural data with different signal-to-noise ratios were used.

Nerve Recordings

Recordings were made with ten intrafascicular electrodes chronically implanted in the radial nerves of five cats as described elsewhere [5], [8]. Data from recordings made at 1 and 6 mo post-implant were used. The electrodes were connected to a differential amplifier with a gain of 1.5×10^4 and half-power points at 450 and 2500 Hz. This bandwidth was chosen to maximize the signal-to-noise ratio of the recordings [10]. The signals were viewed on an oscilloscope and recorded on magnetic tape for later replay. Sample oscilloscope traces of action potentials from two different units (recorded with the same electrode) are shown in Fig. 1.

The large myelinated fibers in the feline superficial radial nerve innervate cutaneous mechanoreceptors. Each peripheral sensory nerve fiber innervates one or more sensory receptors, but a given fiber innervates only one type of receptor [6], [19]. The area of skin containing mechanoreceptor(s) innervated by a single nerve fiber is called the "receptive field" for that neuron. The neuron and the mechanoreceptor(s) which it innervates are commonly referred to as "unit."

The area of skin innervated by the implanted fascicle was determined by brushing the paw and forearm with a small paintbrush, a general stimulus which activated many units. Single units were selectively activated, and the re-

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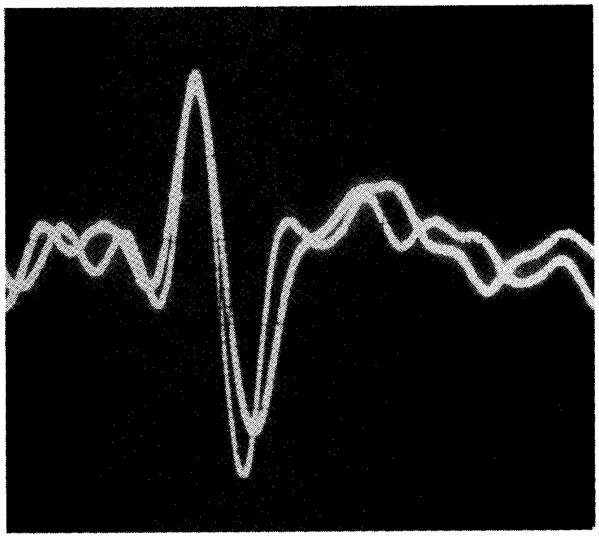

Fig. 1. Oscilloscope traces of action potentials produced by two different units, recorded with a single intrafascicular electrode. Scale: 20 μV/div by 0.5 ms/div.

ceptive field location and receptor type for each unit with a signal-to-noise ratio ≥ 1.4 was determined by its response to various mechanical stimuli [5], [6]. Recordings from a single electrode typically contained activity from ten different units, with an average signal-to-noise ratio of about 3 [8].

Simulated Neural Data

Simulated neural signals were generated on an IBM-PC/AT compatible, 386-based microcomputer. Action potential shape, amplitude, and firing frequency were specified by the user. The waveform shapes which we specified were patterned after action potentials digitized from neural recordings. In some cases we modified certain points to produce a second waveform which differed from the first in a selected feature. Computer-generated random noise was digitally filtered to produce background noise with a spectral composition matching that found in real intrafascicular electrode recordings [10]. Noise data points were generated to simulate a 33 kHz sampling rate and action potential points were then added to this signal. The values were stored in RAM and sent out on a D/A converter in real time.

Data Digitization

A 12-bit A-to-D converter was used for sampling data. Neural signals were digitized continuously at a rate of 28.6 kHz (35 μs interval) and stored in a circular buffer. Action potential occurrences were detected by setting a threshold trigger level to detect action potentials with a signal-to-noise ratio ≥ 1.4. The use of the circular buffer made it possible to retain points which were digitized before and after the trigger level was surpassed. Once an action potential was detected, the values in the circular buffer were scanned to find the positive peak of the waveform. A total of 32 data points per action potential were then saved, consisting of the 15 points preceding the positive peak of the action potential, the positive peak (which was always stored as the 16th point), and the 16 points following the positive peak. Thus, each digitized action potential was centered within the data window. The duration of the data window was long enough that given the variability in action potential duration present in our data, all action potentials would be stored in their entirety.

Template Construction

Three types of features were compared. Point features (PNT) consisted of the set of 32 digitized points of the action potential waveform. Time-domain features (TDF) included the maximum, minimum, and peak-to-peak amplitude values, and the rise and fall times of the action potential. Frequency domain features (FFT) consisted of 16 sine and 16 cosine coefficients, obtained by performing a fast Fourier transform on the digitized action potentials. Because the rectangular data window for each action potential was long enough that the signal was at the baseline level at the beginning and end of the window, it was not necessary to perform additional windowing to eliminate truncation errors.

Each unit was represented by a "template" consisting of expected values for the features which characterized the action potentials produced by the unit. Only features that provided information which was useful for discriminating between action potentials produced by different units were included. The templates for all units in a given recording utilized the same set of features; however, the specific features used varied from one recording session to the next. Separate template sets were constructed for each of the three types of features.

In a given recording session, sets of 50 action potentials were collected from each mechanoreceptor unit by selectively activating the unit with sinusoidal mechnical stimuli [5]. Feature values were determined for each of the action potentials produced by a given unit and the mean (μ) and standard deviation (σ) were calculated. The range of values expected for a given feature was then computed as

$$\text{range} = \mu \pm K \cdot \sigma.$$

The value of K, which is specified by the user, determined the size of the tails of the data distribution which fell outside the range. For example, selection of $K = 1.65$ gave 5% tails and, because both upper and lower tails were used, resulted in 10% of the action potentials being excluded from the parameter value window. A binominal test was used to verify that the actual numbers of action potentials excluded from the ranges did not differ from the predicted values (95% confidence interval).

Once ranges were determined for each feature of the action potentials from each unit in the recording, the features were ranked according to their power to discriminate between activity from different units. If, for two different

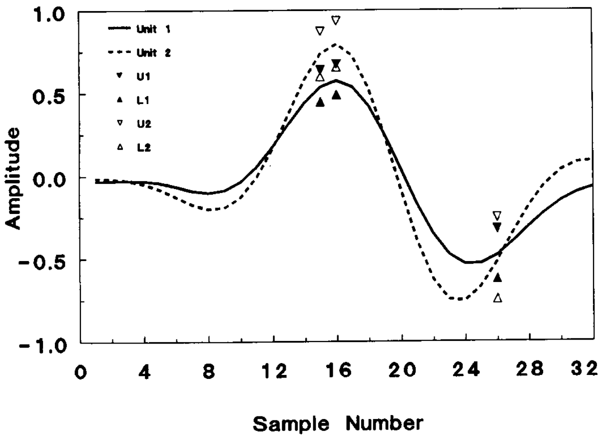

Fig. 2. Mean action potential waveforms and template values for the two units shown in Fig. 1. The action potentials were digitized at an interval of 35 μs between samples. Fifty digitized action potentials were averaged to obtain the mean values plotted here. The templates consist of the 15th, 16th and 26th points of the digitized action potentials. Upper (U1, U2) and lower (L1, L2) limits of the templates are indicated for each unit.

nerve fibers, the ranges of expected values for a particular feature did not overlap, that feature was said to discriminate between action potentials from the two units. The feature which could discriminate between the largest number of pairs, according to this criterion, was identified first. Next, only those pairs which were not separated by the first feature were considered, and the feature which discriminated between the largest number of these remaining pairs was found. This process was repeated and features were added to the list until all pairs were discriminated or no further pairs could be discriminated by using additional features.

Fig. 2 shows the mean digitized action potential waveforms for the two units which produced the action potentials shown in Fig. 1. Also shown in Fig. 2 are the template values for the units. In this particular example, point features 15, 16, and 26 were selected to make up the templates. It can be seen that points 15 and 16 provide information which makes it possible to discriminate between activity from the two units. The recording from which these action potentials were obtained contained activity from a total of 14 units. Point 26 is not useful for discriminating between the action potentials shown here, but was useful for discriminating between the potentials produced by other combinations of units.

Action Potential Classification

We used a windowing algorithm for classifying action potentials. Feature values for a given action potential were compared to the corresponding feature ranges for each template. If all the selected features were within the corresponding feature ranges for a template, the action potential was said to have fit that template. Three possible classification outcomes were obtained: 1) a single unit was identified as the source of the action potential, 2) the action potential matched templates for more than one unit, so classification results were ambiguous, or 3) the action potential did not match any of the templates. We were thus able to identify units that produced action potentials which were easily classified and units that produced action potentials which were confused with those from other units. The same algorithm was applied separately and in combination to templates generated using the three different types of features (PNT, TDF, and FFT).

RESULTS

To test whether the templates constructed as described above correctly classified action potentials, sets of 50 action potentials from single units were run through the classification algorithm and compared to templates for all units in the recording. The classification results for each recording were summarized in a "confusion matrix," such as that shown in Table I.

For a given unit, two possible types of classification error could occur. 1) An action potential from the unit may not match the template for the unit: this is an "exclusion error" (Exc). 2) An action potential from the unit could be incorrectly matched to the template(s) for one or more other units: this is an "inclusion error" (Inc). Error rates were calculated for each unit (i) as:

$$\text{Exc}_i = 1 - \frac{\#\text{APs from unit } i \text{ matched to template } i}{\text{total } \#\text{AP's from unit } i}$$

$$\text{Inc}_i = \frac{\Sigma\ \#\text{AP's from unit } j \text{ matched to template } i}{\Sigma \text{ total } \#\text{AP's from unit } j}$$

where

#AP's = number of action potentials

and

Σ is the summation over values of j ranging from 1 to the total number of units in the recording (for $j \neq i$).

The true inclusion error rate depends on the firing rates of the various cells in the recording. Since we could not determine this *a priori*, we assumed that all cells had equal firing rates in order to obtain a general estimate of the frequency of this type of error. In some cases the failure of this assumption can produce errors in firing rate estimation. For example, if two units produce action potentials which are frequently confused and the firing rate of one unit is lower than the assumed rate and the firing rate of the other is higher, the number of action potentials incorrectly matched to the less active unit will be higher than expected. Conversely, the number of action potentials incorrectly matched to the more active unit will be lower than expected.

We found that inclusion error rates determined on the basis of this assumption nevertheless give a useful indication of how well action potentials from a unit can be discriminated from action potentials produced by other

TABLE I

			Template Matched					
		N	1	2	3	4	5	6
	1	50	42	11	7	0	0	0
U	2	44	2	33	35	0	0	13
n	3	46	4	29	42	0	0	17
i	4	49	0	0	0	41	0	0
t	5	45	0	0	0	0	39	0
	6	47	0	7	20	0	0	40

Example of a "confusion matrix." Each row represents action potentials which originated from a single unit; the unit which produced the action potentials is indicated by the row label. Templates were created for all the units in the recording; column labels across the top of the matrix indicate the template to which the action potentials were matched. Thus the value in row i and column j is the number of action potentials from unit i which fit the template for unit j. When $i \neq j$ an incorrect classification occurred. The total number of action potentials in each set (N) is indicated in the first column. The numbers across a row may sum to a value different than the total number of potentials in the set, either because individual action potentials matched more than one template (e.g., unit 1), or not all the action potentials from that unit fit a template (e.g., unit 5).

units. Setting a low inclusion error criterion eliminates units which produce action potentials which overlap significantly with potentials produced by other units.

Tail Size

The size of the tails used in the template for a unit influenced both the exclusion and inclusion error rates. The exclusion error rate increases with larger tail sizes and as more features are used in the template. On the other hand, as the tail size is increased, the number of inclusion errors tends to decrease because fewer action potentials from other cells fit into the narrowed template. Exclusion and inclusion error rates at different tail sizes are shown for one unit in Fig. 3.

We felt that a criterion which required an inclusion error rate <0.15 would allow identification of those units whose activity could be readily distinguished from that of other units. In addition, we wished to limit exclusion error rates to ≤ 0.25 to ensure that not too many action potentials from the unit would be missed. If both error rates for a unit met the above criteria, we felt that reliable estimates of unit activity could be obtained, and that the unit could be considered "separable."

We were interested in optimizing template tail sizes to minimize both inclusion and exclusion error rates. Templates were constructed with 2.5, 5, 7.5, 10, and 12% tails for all the recordings. Separate template sets were constructed using each type of feature. In general, templates with 5% tails appeared to provide the best unit separation (Fig. 4). However, closer inspection of the data suggested that optimizing the tail size for individual recordings or even individual units might give better results.

Thus, we determined the number of units per recording which could be separated if the tail size was optimized for each recording and also the number of units which could be separated if the best tail size was selected on a unit-by-unit basis. As shown in Fig. 5, optimizing the tail size to individual recordings gives an improvement over using the same tail size for all recordings, and optimizing the tail size on a unit-by-unit basis provides an even greater improvement in unit separation.

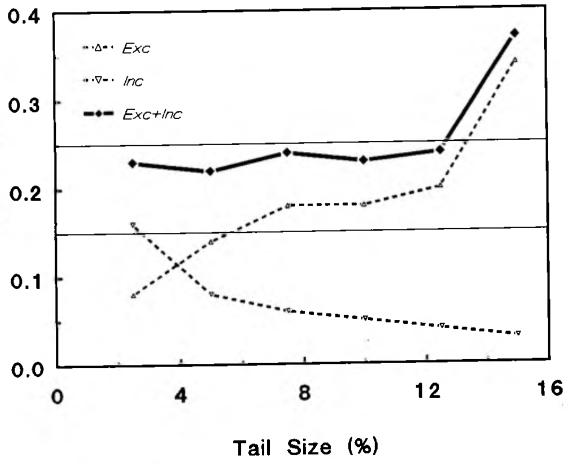

Fig. 3. Error rates as a function of tail size used in generating templates. This example is from a single unit but illustrates the typical influence of tail size on error rates. As tail size is increased, template range is narrowed, and a larger exclusion error rate results, but action potentials from other units are less likely to match the narrower template, so the inclusion error rate decreases. Also shown on the plot are limits for allowable exclusion (0.25) and inclusion (0.15) error rates. Exc = exclusion errors, Inc = inclusion errors.

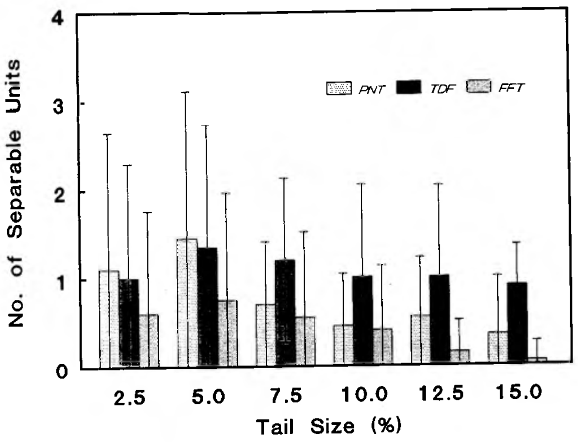

Fig. 4. Influence of template tail size of unit separation. The average number of separable units per recording is plotted as a function of tail size for templates made up of point (PNT), time domain (TDF), and frequency domain (FFT) features. The optimum global tail size was 5%, irrespective of feature type used. Data were obtained from a total of 20 recordings. Error bars show standard deviation.

Signal-to-Noise Ratio

As was expected, we found that identifying action potentials was typically easier in recordings with higher signal-to-noise ratios. Therefore, we attempted to quantify

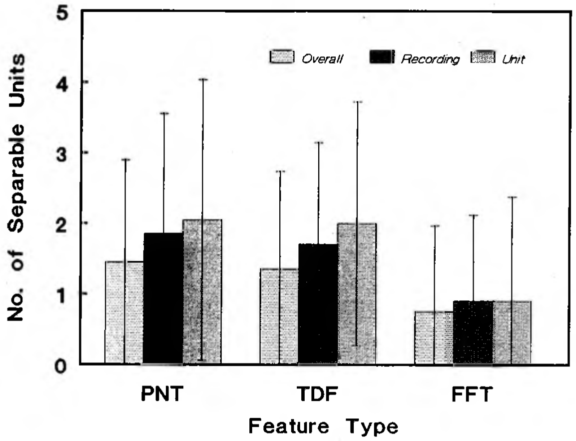

Fig. 5. Template tail size optimization. Three optimization methods are compared. 1) A tail size of 5% was used in generating the templates for all 20 recordings (overall). 2) The tail size which gave the largest number of separable units was determined and used in generating templates on a recording by recording basis (recording). 3) The optimal tail size was determined and used for making templates for each unit (unit). The average number of units per recording which could be separated using each of these methods is plotted with error bars to show standard deviation.

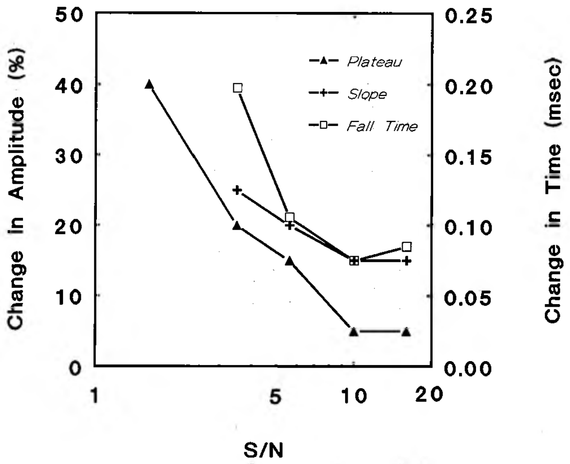

Fig. 6. Influence of signal-to-noise ratio on detection of differences in time domain feature values. A smaller relative difference in amplitude could be detected at the maximum point on the action potential (plateau) than could be detected in an area of steep slope (slope). Amplitude differences are expressed as percent of peak-to-peak amplitude of the action potential. The smallest detectable difference in fall time is given in absolute time. Note that the signal-to-noise ratio is plotted on a logarithmic scale.

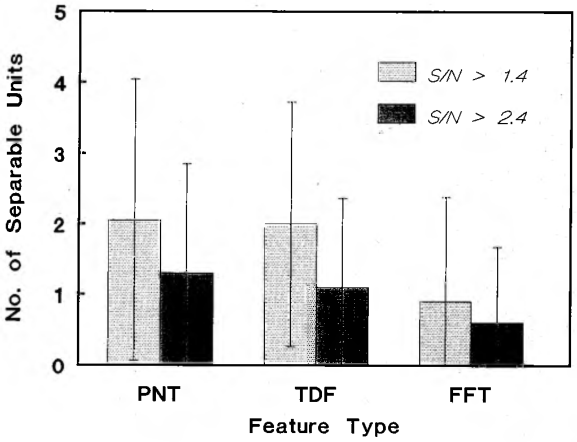

Fig. 7. Influence of action potential signal-to-noise ratio criterion on unit separation. Action potentials had to have amplitudes of at least 1.4 times the amplitude of the background noise in order to be detected by the threshold level-crossing method we have used. To test whether using a higher threshold for action potential detection would retain action potentials which could be separated easily while reducing the number of potentials which could not be separated, we conducted our unit separation procedures on data sets containing only units with signal-to-noise ratios of 2.4 or greater. The average number of separable units per recording is shown (with standard deviation error bars) for S/N cutoffs of 1.4 and 2.4. For all three feature types, the latter produced fewer identifiable units.

the influence of signal-to-noise ratio on the separability of action potential waveforms.

Signals containing action potentials which differed by a known amount in a single parameter were generated using the simulator. Three parameters were tested: the maximum amplitude, a point on the falling phase of the waveform, and the fall time. The smallest difference which could be distinguished (that is, for which nonoverlapping templates with 5% tails could be constructed) was determined at different signal/noise levels. The results are shown in Fig. 6. As signal-to-noise ratio increases, smaller feature differences can be discriminated.

We hypothesized that by using a signal-to-noise cutoff greater than 1.4, we might retain activity from units which could be readily separated, while eliminating smaller, less easily classified action potentials. Therefore, we constructed a separate set of templates for those units which had signal-to-noise levels of at least 2.4. The number of units which could be separated at the two signal-to-noise ratio cutoffs are shown in Fig. 7. Using a higher signal-to-noise ratio cutoff eliminates units which could be separated, as well as units whose action potentials could not be classified, reducing the total amount of information available from the recordings.

Feature Types

Using units with a $S/N \geq 1.4$, and using templates with tail size optimized for individual units, we compared classification results obtained when different feature types were used. The number of separable units obtained with templates made up of a single type of feature and with combinations of two or three templates of different types is shown in Fig. 8. Point (PNT) and the time domain features (TDF) produced similar results; FFT coefficients were less effective than either. Only a slight increase in the number of separable units was obtained when results from two or three templates of different feature types were combined.

An average of 2.31 features were needed in PNT templates, 2.13 in TDF templates, and 1.5 in FFT feature templates. Template sets for recordings which contained activity from many units typically made use of more features. The most commonly used PNT feature was the 16th

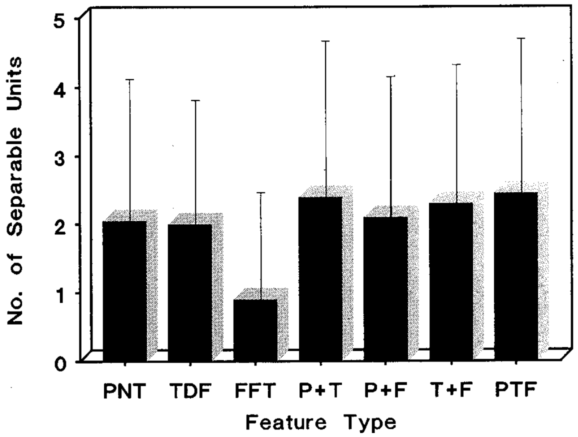

Fig. 8. Efficacy of unit separation as a function of feature type. The first three bars represent the average number of units per recording which could be separated using point (PNT), time domain (TDF), and frequency domain (FFT) features alone. The next three bars represent the number of units separated by combining separation results obtained with two template sets made up of different classes of features, and the last shows the number separated when results from all three types of features were combined. Error bars show standard deviation. Data from a total of 20 recordings.

point, which corresponded to the maximum amplitude. Points near the second minimum were also commonly used in PNT templates. In TDF templates, the most commonly used feature was the maximum amplitude of the action potential. Other commonly used TDF features were the difference between the maximum and the second minimum, and the second minimum. These results indicate that information about maximum and minimum values was useful for discriminating between action potentials from different cells, and could be obtained from either PNT or TDF data. Coefficients corresponding to frequencies of 2678, 3571, and 1785 Hz were the most frequently used FFT features.

DISCUSSION

Identifying activity from individual nerve fibers in multiunit neural recordings is important in cases where it is desirable to record activity from several cells at once in order to examine the functional relationships between the cells, or where limited choices of feasible recording techniques necessitate the use of multi-unit recordings. The goal of this study was to determine which of three different types of features would be most useful for uniquely characterizing and classifying action potentials in multiunit recordings made with intrafascicular electrodes. In order to make comparisons, we used the same test data, template construction, and classification algorithms in all cases. This also allowed us to investigate the influence of several other variables on classification success.

Feature Types

Our template construction algorithm selected only those features which provided information useful for discriminating between action potentials produced by different nerve fibers. The validity of this algorithm was confirmed by constructing templates which included all features of a given type (e.g., all 32 digitized points for the PNT sets, or all coefficients in the FFT sets). It was found that features which had not been selected by the template construction algorithm did not provide any additional information which improved classification (MacNaughton, unpublished observations).

Our results indicate that the use of single digitized points as descriptive features is an effective approach for classifying action potentials. The point features appear to provide the same information as do the TDF features, except that PNT features require minimal computation. This finding was somewhat unexpected since the TDF parameters incorporate the relations between digitized points, as well as the values of the points themselves. The computationally most complex features, FFT, proved to be the least powerful for discrimination of action potentials.

The usefulness of features which contain information about action potential maximum and minimum amplitudes has been noted by others. Using principle component analysis on eight amplitude and time parameters, Vibert and Costa found that the maximum, the second minimum and the fall time provided the best information for describing action potentials from different cells [20].

Tail Size

Tail size must be chosen carefully during the template-construction process. Best classification results are obtained if the tail size is optimized for each unit. For a given application, the optimal choice of tail size will depend on the relative costs of inclusion and exclusion errors. Although these results are of immediate importance in classification methods which use a windowing approach, they also have implications for methods which make use of distance measures for making action potential classifications. How close an action potential must be in order to be assigned a particular cluster should depend on the individual unit.

Signal/Noise

Increasing apparent signal-to-noise ratio by increasing the trigger level for action potential detection was not particularly effective for eliminating units which could not be classified, since units with readily classified action potentials were excluded as well. However, both the results obtained with simulated neural signals and qualitative evaluation of neural signals recorded with intrafascicular electrodes indicate that, as would be expected, unit separation is improved when signal-to-noise ratio for the recording is increased. Improved recording techniques which increase signal-to-noise ratios will certainly make it possible to obtain more information from multiunit recordings.

Summary

In addition to providing useful guidelines for further development and improvement of action potential classification techniques, our results also show that good classification can be obtained through the use of a small number of easily calculated features, allowing the development of an on-line real-time classification technique in either hardware or software.

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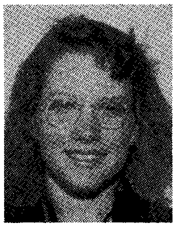

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