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A method to measure the strength of multi-unit bursts of action potentials

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Abstract

Both the numbers of neurons that are active during multi-unit bursts of spikes and the frequencies with which individual neurons fire in these bursts can vary in response to changes in excitation. Here is a digital-filtering method that measures the strength of a burst of spikes by calculating the area of a polygon derived from the squared voltages that record the burst, and dividing this area by the burst's duration. The method was developed in the SigmaPlot© environment, and makes use of the Fast-Fourier Transform functions provided in the SigmaPlot© transform language. To test the method's performance, I constructed multi-unit bursts of spikes with known structure and calculated the strengths of these known bursts. To demonstrate the method's usefulness, I applied it to a train of 23 bursts of spikes in motor axons recorded during a spontaneous bout of patterned motor output. The measured strengths of these bursts varied 30-fold, and were well-correlated with the differences in the original recording. The results demonstrate that the method effectively measures burst strength independent of burst duration.

Keywords: Burst strength; Firing frequency; Excitation; Motor pattern

1. Introduction

Recordings from many neurophysiological experiments include simultaneous bursts of action potentials in many neurons. During an experiment, the numbers of neurons firing and the frequencies with which they fire can vary spontaneously and in response to experimental manipulation. Temporal features of these bursts like duration or period are straightforward to measure from recordings of voltages and times, but quantitative changes in numbers of units recruited and their firing frequencies are often more difficult to measure. In the laboratory, we often speak of strong and weak bursts because visual inspection of the recordings and aural monitoring of the recording during the experiment lead to the intuitive sense that bursts can vary in "strength". How can we measure this intuitive parameter when the numbers of active units and their individual firing frequencies are unknown?

The amplitude of spikes recorded with an extracellular electrode in a restricted extracellular space is proportional to the diameter of the axon, whether the electrode configuration is monophasic or triphasic (Pearson et al., 1970). Units with larger diameter axons have larger spikes, a feature that is apparent in many motor systems where recruitment of motor units proceeds in order of size. I sought a method that would sum the activity of all the neurons that fired during a burst, taking into account both spike frequency and spike amplitude. In the past, analog rectification and integration of bursts has been useful under some circumstances (e.g. Mulloney et al., 1987; McClellan and Hagevik, 1997), but these methods tended to confound firing frequency and burst duration. I have developed a digital-filtering method that takes a recorded train of bursts, isolates each burst, and calculates an area proportional to the numbers and sizes of spikes and to the burst's duration. If the burst's duration is known independently, this area can be divided by this duration to create a measure of burst strength, that is, the numbers and sizes of spikes that occurred during the burst.

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I describe the method and the logic behind it, demonstrate its performance on synthetic bursts of spikes with known composition, and apply it to an experimental recording of bursts that varied in intensity but whose detailed composition was unknown.

2. Methods

2.1. Physiological data

We isolated the abdominal ventral nerve cord from crayfish, *Pacifastacus leniusculus*, using procedures that are described in Tschuluun et al. (2001). Action potentials were recorded extracellularly from either the first segmental nerve (N1) of abdominal ganglion A3 or A4 (Mulloney and Hall, 2000), or from the superficial branch of the third segmental nerve (N3) of these ganglia (Kennedy and Takeda, 1965). We used pin electrodes in a triphasic configuration for N1 recordings and suction electrodes for N3 recordings (Pearson et al., 1970; Stein and Pearson, 1971). High-gain amplification and band-pass filtering (0.3–5 kHz) of these recordings was conventional. Recordings were saved as computer files in abf format by digitizing the amplifiers' output at 100 μs sampling rate using an Axon Instruments Digidata 1200B board and Axoscope software (Axon Instruments, Foster City, CA). The selected recordings were imported into SigmaPlot© using the DataAccess plug-in (Bruxton Corp., Seattle, WA).

2.2. Test data

To construct bursts of spikes with known composition, I used a single spike recorded from N3, duplicated it repeatedly, and scaled these duplicates to create trains of spikes with specified frequencies and specified sizes. In bursts with five "units", the largest unit was five times the size of the smallest unit. Spike frequencies in these bursts conformed to the size principle known to govern the orderly recruitment of motor units in crayfish and other animals (Davis, 1971); the smallest unit fired at the highest frequency, the largest at the lowest frequency. The numbers of spikes per second for each unit was varied systematically to create bursts with low (range 17–5 Hz), medium (range 61–17 Hz), and high (range 97–57 Hz) levels of firing; these corresponded to different levels of excitation in a real motor pool.

These bursts were constructed by first creating a list of time–voltage pairs the length of one cycle period that matched the sample-frequency of the original spike. All voltage values in this list were initially zero. For each unit, the scaled list of voltages derived from the single spike was then added periodically to the voltages in the new list, starting at times specified by the firing frequency of that unit. This method allowed units whose spikes overlapped in time to sum algebraically, as do real spikes in multi-unit recordings.

3. Description of the method for measuring areas of burst envelopes

This method for measuring the intensity of bursts begins with paired lists of times and voltages (t, V) that describe a recording, organized in two columns. These lists are then processed in four steps:

1. The list of voltages (V) is squared. This operation makes all values positive or zero.
2. The list of squared voltages (V^2) is then smoothed to create a continuous envelope whose height is proportional to local values of V^2.
3. Values of this smoothed envelope smaller than a threshold value are clipped out by setting them to zero; other values are unchanged. Given an appropriate choice of threshold, this isolates a polygon corresponding to each burst.
4. The area of each polygon is calculated. This area is a measure of intensity times duration.
5. If the duration of each burst is known independently, dividing the area of each burst by its duration gives a measure of burst intensity, or strength, independent of burst duration.

The different features of this procedure are discussed next.

3.1. Smoothing

V^2 is highly irregular, and the boundaries of each burst are not well-enough defined to permit algorithmic identification. In order to define bursts as opposed to individual spikes, it is necessary to smooth V^2. The choice of a smoothing procedure is a crucial feature of this method. I tested several different low-pass smoothing filters, including both time-domain boxcar filters and frequency-domain filters, and found the most effective to be one provided with SigmaPlot©, the "Smoothing transform". This is a procedure that transforms V^2 to the frequency domain, filters it there using a triangular smoothing kernel, and restores the result to the time domain. Hamming (1977) describes the logic behind these FFT filters and discusses the choice of filtering kernels. This procedure is by comparison very fast, and does not generate bogus humps in the output that would confound the calculation of areas associated with each burst.

This smoothing procedure makes use of several functions provided in the SigmaPlot© transform language, and requires seven steps:

1. The list of V^2 values is transformed into the frequency domain using the FFT function.
2. A "triangular smoothing kernel" is constructed. The width of this kernel determines how many neighboring points will be considered in calculating the value that will replace each raw data point in the smoothed list, and weights these neighboring points by their distance from the data point.
3. The "triangular smoothing kernel" is transformed to the frequency domain using the FFT.