Sympathetic activity and the underlying action potentials in sympathetic nerves: a simulation

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Tang, Xiaorui, Ankit R. Chander, and Lawrence P. Schramm. Sympathetic activity and the underlying action potentials in sympathetic nerves: a simulation. Am J Physiol Regul Integr Comp Physiol 285: R1504–R1513, 2003. First published August 14, 2003; 10.1152/ajpregu.00339.2003.—Understanding the relationship between activity recorded in sympathetic nerves and the action potentials of the axons that contribute to that activity is important for understanding the processing of sympathetic activity by the central nervous system. Because this relationship cannot be determined experimentally and is difficult to predict analytically, we simulated the summed action potentials of 300 axons. This simulation closely resembled actual sympathetic activity and permitted us to know how many action potentials contributed to each burst of simulated sympathetic activity and the durations and amplitudes of each burst. We used these simulated data to examine a statistical method (cluster analysis) that has been used to identify and quantify bursts of sympathetic activity. Simulation indicated that the integrals of bursts, whether determined directly from the simulation or by integrating bursts detected by cluster analysis, were linearly correlated to the number of action potentials contributing to bursts. The variances of samples of the simulated signal were also linearly correlated to the number of action potentials. The amplitudes of bursts of sympathetic activity were less well correlated to the number of underlying action potentials. A linear relationship existed between the average number of action potentials contributing to simulated bursts and the integral of the amplitude spectra obtained by Fourier transform of the simulated activity. Finally, simulated experiments indicated that relatively brief recordings might be sufficient to detect statistically significant changes in sympathetic activity.

multunit nerve recordings; summating action potentials; cluster analysis

POSTGANGLIONIC sympathetic nerve activity in several species occurs in synchronized discharges (bursts) that may reflect the complex manner in which the central nervous system (CNS) regulates cardiovascular functions through the autonomic nervous system. The frequency, duration, and amplitude of these bursts have been used to estimate sympathetic activity, and these properties have been extensively investigated, both in the basal state and during cardiovascular reflexes, under both normal and pathophysiological conditions, and in a variety of species (5–7, 13, 16). For instance, in baroreceptor-innervated cats, Barman et al. (2) showed that patterns of sympathetic nerve discharge might include either a “pure” cardiac-related rhythm or mixtures of the cardiac-related rhythm with a 10-Hz rhythm. In baroreceptor-denervated cats, a pure 10-Hz rhythm or variable mixtures of the 10-Hz rhythm with either irregular 2- to 6-Hz oscillations or strongly rhythmic activity near 4 Hz appear in sympathetic nerve discharges (1–3, 22). Malpas and Ninomiya (13) showed that the frequency and amplitudes of renal sympathetic bursts appeared to be independent and differentially affected by baroreceptor input. Several laboratories have either shown or assumed that changes in burst amplitudes are correlated to changes in the number of active axons contributing to bursts (see, for instance, Refs. 5–7, 16). However, other relationships between recorded activity and the underlying action potentials have been proposed (4, 9, 15). Because the relationship between bursts of activity recorded from sympathetic nerves and the action potentials of individual axons contributing to those bursts cannot be determined experimentally, estimating this relationship has been a classic problem in neurophysiology.

In the present study, our simulation permitted us to know, precisely, the time of onset and offset of bursts of sympathetic activity (and, therefore, their duration) and the number of action potentials contributing to each burst. Using these data, we could calculate the exact relationships between the numbers of action potentials contributing to bursts and the integrals and amplitudes of bursts. These relationships, then, provided a reference for examining similar relationships using a statistical method, called cluster analysis, to detect the onsets, offsets, and maximum amplitudes of bursts of sympathetic activity. The simulation also permitted us to determine the validity of using the fast Fourier transform to estimate changes in the numbers of action potentials. Finally, we used the simulation to conduct virtual experiments in which we computed the duration of physiological recordings that would be neces-

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necessary to detect statistically significant changes of different magnitudes in sympathetic activity.

METHODS

Simulation of nerve activity. Three hundred independent axons were available to generate bursts of sympathetic activity. Each axon was modeled with a probability function that determined its likelihood of exhibiting an action potential at any instant within bursts. The temporal resolution of the simulation was 1 ms. The probability function was a triangular waveform with a repetition rate of 3 Hz to which we added uniformly distributed random noise. The triangular waveform, which simulated the modulation by arterial blood pressure of sympathetic activity, was the same for all axons. For each burst, a threshold value was produced from an inverse Rayleigh distribution (10), generated as the difference between 1 and values of the Rayleigh distribution, which was scaled between 0.0 and 0.05. Therefore, the thresholds generated by this process ranged from 1.00 to 0.95. The maximum length of time within which axons could discharge, called a "window," was inversely related to the value of the threshold for that burst. Therefore, when the threshold was at its minimum value, 0.95, the maximum window length was 166.6 ms (one-half the period for the 3-Hz modulation signal), and when the threshold was at its maximum value, 1, the maximum window length was 0 ms (permitting no action potentials). During a window, an axon was eligible to discharge only if it was not in a refractory period. The refractory period was the same for all axons and was fixed at 166.6 ms (one-half the period of the waveform). Therefore, no axon could discharge more than once during the same burst (Fig. 1A). When an axon discharged, its action potential was added to the summed sympathetic activity (Fig. 1B).

Analysis of raw data from simulations. The number of action potentials contributing to bursts and the durations of bursts were obtained directly from the unfiltered output of the simulation (Fig. 1B). In addition, three calculations were made from this output. The first was the magnitude of each 1-ms sample of the summed action potentials contributing to a burst. These magnitudes were calculated as

$$X_i = |x_i - \mu|$$

(1)

where $x_i$ was each sample, $\mu$ was average of all values of $x_i$ within a burst, and $X_i$ was the absolute value of each sample, offset-corrected by the average of all values of $x_i$ within a burst.

Throughout the following, $X_i$ will be referred to as "samples."

The second calculation was the variance of the samples within a burst. This variance was calculated as

$$\sigma^2_j = \frac{1}{w} \sum_{i=1}^{w} X_i^2$$

(2)

where $w$ was the number of samples in a burst. Throughout the following, $j$ is the index for bursts within a simulation.

To facilitate graphical comparisons of data from different simulations, the variances of samples within bursts were normalized by the average of the variances of samples within all bursts. These normalized variances were calculated as

$$\sigma^2_{j,norm} = \frac{1}{N} \sum_{j=1}^{N} \sigma^2_j$$

(3)

where $N$ was the number of bursts in a simulation.

The third calculation was the integral of each burst, calculated as

$$I_j = \sum_{i=1}^{w} X_i$$

(4)

where, again, $w$ was the number of 1-ms samples in each burst.

As with variances, we graphed the normalized integrals of bursts. These normalized integrals were calculated as

$$I_{j,norm} = \frac{I_j}{\bar{I}}$$

where $\bar{I}$ was the average of all integrals.

Fig. 1. Steps involved in simulating sympathetic nerve activity. A: 300 independent axons were available to generate bursts of sympathetic activity. Inset: shape and values (at 1-ms intervals in arbitrary units) of the action potentials. B: bursts of summed action potentials. C: rectified, band-pass filtered, and downsampled (200 Hz) simulated bursts used as data for analyzing the cluster analysis technique. Inset defines the amplitude of the processed burst (Amp) and the magnitudes of the samples of the bursts ($X_i$).
Cluster analysis of data from simulations. The amplitudes of bursts (Amp; Fig. 1C, inset) and the durations of bursts were direct outputs of a statistical technique originally developed by Veldhuis and Johnson (19) for use with endocrinological data. They called this method “cluster analysis,” and Malpas and Ninomiya (12, 13) were the first to apply it to the analysis of sympathetic activity. The technique searches for significant increases and decreases between small clusters of samples to detect the presence of a significant burst and then determines the amplitude and duration of that burst.

Cluster analysis determined the onset of a burst in sympathetic activity by searching for a significant increase in activity by comparing two contiguous sequences of samples (called “clusters”) by Veldhuis and Johnson using a t-test. The experimenter chooses the number of samples included in these clusters, the critical value of the t-statistic, and a threshold criterion for the minimum amplitude of a burst. The first cluster of samples was defined as a possible nadir and the second cluster as a possible peak. All significant increases in value throughout the data were detected by shifting the “nadir cluster” by one sample and retesting against a corresponding potential “peak cluster,” also shifted by one sample. After all significant increases were identified, the signal was rescanned to search for significant decreases. A burst in sympathetic activity was defined as a significant increase followed by a significant decrease between two nadirs.

The signal that was analyzed by cluster analysis (Fig. 1C) was generated by subjecting simulated activity such as that shown in Fig. 1B to rectification, band-pass filtering (−3 dB at 0.7 and 40 Hz), and downsampling at 200. We used parameters similar to those used by Malpas and Ninomiya (13), five and four samples for nadir and peak detection, respectively, and a t-statistic of 4.1 for both significant increases and decreases. For the downsampled 200-Hz signal, samples of five and four corresponded to 25 and 20 ms of sympathetic activity, respectively. For an increase in simulated activity to be considered a “burst,” we required that its peak amplitude be larger than the average of the peak amplitudes minus 1 SD of those peak amplitudes. Malpas and Ninomiya (13) required that peak amplitudes exceed 25% of the largest peak amplitude observed in a recording for activity to be considered a “burst.” In practice, these criteria were similar. Compared with the incidence of known bursts, cluster analysis failed to detect actual bursts at a rate of <4% of the total number of bursts in simulated recordings, and it incorrectly “detected” bursts in the absence of actual bursts at a rate of <4%. These error rates are comparable to those reported by both Malpas and Ninomiya (13) and Veldhuis and Johnson (19).

The integrals of the bursts that were detected by cluster analysis were calculated by summing the magnitudes of the 5-ms samples between the onset and offset of each burst. Because these samples were analogous to the samples of the original simulation described above (Eq. 1), we also denoted them as \( I_j \) (see Fig. 1C). As with values calculated directly from the simulation, we found it useful to normalize the integrals of bursts detected by cluster analysis using the method described by Eq. 5. Because the signals that were analyzed by cluster analysis had been rectified and smoothed, we did not calculate the variance of the samples within bursts as we did in Eq. 2 for the analysis of data taken directly from the model. Instead, we investigated the amplitudes of detected bursts (Fig. 1C, inset). Here again, normalized amplitudes proved most useful. These normalized amplitudes were calculated as

\[
\text{Amp}_{j\text{norm}} = \frac{\text{Amp}_j}{\frac{1}{N} \sum_{j=1}^{N} \text{Amp}_j}
\]

where \( N \) was the number of bursts in a simulation.

Amplitude spectra of simulated sympathetic nerve activity. The spectra of simulated signals were estimated using Welch’s averaged periodogram method (20) with a resolution of 0.024 Hz. The rectified, smoothed, and downsampled simulated signal was divided into overlapping sections, each of which was detrended and windowed by a Hanning window. The magnitudes of the squared values of the 8,192 points of the discrete Fourier transform of these overlapping sections were averaged to compute the power spectrum of the signal. The amplitude spectrum was the square root of the power spectrum. The integral of the amplitude spectrum was calculated by summing all values of the amplitude spectrum.

Necessary recording length. To estimate the duration (or the number of bursts) necessary to detect statistically significant changes in sympathetic nerve activity, we simulated experimental situations in which the average number of action potentials contributing to bursts increased by 10, 20, and 40%. Using the integrals of bursts as our measure, Wilcoxon’s rank sum tests (21) with a critical value of \( P < 0.05 \) were used to estimate the corresponding recording durations necessary to detect those known changes in simulated sympathetic activity. Results are presented as means ± 1 SE.
We also compared the integrals and amplitudes of bursts determined by cluster analysis with the number of action potentials contributing to those bursts. The integrals of bursts were linearly related to the number of contributing action potentials (Fig. 4A). The amplitudes of bursts determined by cluster analysis and the number of action potentials contributing to those bursts also appeared to be linearly related (Fig. 4B). However, the correlation coefficient for the latter relationship was much smaller than that for the relationship between the numbers of action potentials and integrals of bursts. The threshold that was set for the detection of bursts by the cluster analysis technique caused the offset of the amplitude relationship on the ordinate shown in Fig. 4B.

Relationship between average number of action potentials contributing to bursts and integral of amplitude spectrum was linear. Some investigators, instead of measuring rectified and filtered sympathetic activity, the integrals of bursts, or the variance of amplitudes of bursts, measure the integral of the amplitude spectrum of sympathetic activity as determined by a Fourier transform. We examined the relationship between the known average number of action potentials in bursts and the integrals of the amplitude spectra. Integrals of amplitude spectra were linearly related to the number of action potentials (Fig. 5).

Brief recordings detected statistically significant changes in sympathetic nerve activity. We simulated experiments in which the number of action potentials...
contributing to bursts in an experimental condition was increased by 10, 20, and 40% with respect to the number of action potentials under a control condition (Fig. 6A). We then tested for significant differences between these distributions using a Wilcoxon rank sum test with a critical value of $P < 0.05$. The numbers of bursts necessary to detect a significant difference in the numbers of action potentials between the control and experimental conditions when known differences were 10, 20, and 40% were $129 \pm 5$, $89 \pm 11$, and $16 \pm 3$ bursts, respectively, corresponding to recordings of approximately 43, 30, and 5 s, respectively, at a burst rate of 3 Hz. Further examination of this relationship for repeated “experiments” over a wide range of experimental effects confirmed that, under the conditions of this simulation, and with an average rate of bursts of 3 Hz, relatively brief recordings would be necessary for detecting statistically significant changes in sympathetic activity (Fig. 6B).

**DISCUSSION**

To our knowledge, this study represents the first attempt to simulate sympathetic activity in bursts that were similar to those recorded in many mammals. For the results of this simulation to be useful for interpreting in vivo experiments, it was important that simulated sympathetic nerve activity correspond closely to actual recordings of sympathetic nerve activity. Nevertheless, the design of the simulation required several simplifications. First, although the modulation of bursts of sympathetic activity by a single source at a constant frequency underestimated the complexity of the factors that normally modulate this activity, we do not feel that this simplification detracted significantly from the simulation’s veracity. Second, estimation of the number of axons contributing to bursts of sympathetic activity would be confounded if sympathetic postganglionic axons discharged more than once in a burst. Our focus was on the relationships between measures of the magnitudes of bursts (integrals, amplitudes, and variances of samples of bursts). By fixing the frequency of bursts and permitting each axon only one action potential in each burst, we precluded investigation of the relationship between the frequency of action potentials and the frequency of the summed sympathetic activity (12).
We feel justified in assuming that axons discharged only once during a burst of simulated activity for the following reasons. Although short interspike intervals have been observed in single sympathetic postganglionic axons in humans, these are rare events, and most axons discharge at average frequencies between 0.3 and 0.4 Hz (11). The low discharge rate of sympathetic postganglionic neurons is not a property of the postganglionic neurons themselves. Indeed, sympathetic postganglionic neurons can be artificially driven to discharge at relatively high frequencies. However, their discharge rates are usually limited by the long refractory periods of their synaptic antecedents, the sympathetic preganglionic neurons (17). The average discharge rate of single renal sympathetic postganglionic neurons in chloralose-urethane- or althesin-anesthetized rabbits is between 2 and 2.5 Hz (8), and 1.2 Hz for splenic nerves in the cat (14). Therefore, the likelihood of multiple discharges within a burst at a burst rate of 3 Hz would have been unlikely.

We were successful in achieving a realistic simulation of sympathetic activity. The greatest difference between simulated and actual sympathetic activity was seen in the distribution of values of amplitudes of bursts (Fig. 2). Simulated data exhibited a larger number of smaller amplitudes and a smaller number of larger amplitudes than actual renal sympathetic activity. This difference, however, should not have affected the subsequent processing and analysis of the simulated data.

**Integrals of bursts and variances of samples of bursts were linearly related to number of action potentials contributing to bursts.** Our simulations indicated that, if we could assume that individual axons have similar action potentials and that coincident discharges added linearly, the integrals of bursts and the variances of samples of bursts were linearly related to the number of active axons contributing to bursts. Others have noted a similarly linear relationship in activity that was not simulated as discrete bursts. In multiunit neural activity studies, Dulli et al. (9) demonstrated that, over a wide range of discharge frequencies, the integral of rectified and summated activities was proportional to the number of discharging axons. With 300 available axons capable of discharging only once every 166.6 ms, our simulation falls within the range investigated by Dulli et al. (9) and our conclusions are consistent with theirs.
Measurement of integrals of bursts is closely related to the most commonly used method of measuring sympathetic activity, calculation of the time average of that activity. However, instead of integrating over fixed periods of several seconds or averaging activity at time constants of several seconds, calculating the area of bursts integrates over the duration of each burst. Because in many mammals most sympathetic activity is organized in bursts, the rationale for the two methods is roughly equivalent. However, two differences are worth noting. First, integration of bursts may permit greater temporal resolution than averaging techniques. Second, the assumption that most sympathetic activity is manifested in the form of synchronized bursts may not be justified in some species or after some experimental procedures (18).

The linear relationship we observed between numbers of action potentials and the variances of samples is consistent with conclusions from several studies. In an analysis of recordings of multiple action potentials, Biro and Partridge (4) demonstrated analytically that when action potentials with identical probability summed, the variance of the amplitudes of the resulting activity was linearly proportional to the number of contributing units.

Dulli et al. (9) used both analytic and simulation methods to compare three techniques used for measurement of multiunit neural activity in sympathetic nerves. Using amplitudes of activity (rather than integrals), they concluded that, for an unrestricted range of activity, only the variances of amplitudes were linearly related to the activity of single neurons contributing to that activity. In a Monte Carlo simulation of arbitrary and identical waves, Moore (15) found that the root-mean-square (rms) voltage of the signal was proportional to the square root of the number of waves summed. For a signal with zero mean, the rms value and SD are identical to the square root of the variance. Using a different simulation and a different method of analysis, our observation of a linear relationship between the number of action potentials contributing to bursts and the variance of the samples of the bursts supports this conclusion.

The theoretical basis for this linear relationship is the well-known fact that, when several sets of similarly distributed data are added, the variance of the sum is equal to the sum of the variances of the individual sets. The most stringent restriction on the use of variances to estimate underlying neuronal activity is the uniformity of amplitude distributions of the contributing unitary activities. Recruitment of axonal populations with different amplitude distributions during an experiment would limit the accuracy of estimates of underlying neuronal activity. However, this restriction applies equally to estimates of changes in numbers of action potentials based on changes in integrals.

Differences between direct analysis of simulated data and analysis based on cluster analysis technique. From the simulation, we were able to determine the exact onset, offset, and integral of each burst of simulated activity and the number of axonal discharges that contributed to each burst. These data could then be used as a reference for evaluating the cluster analysis of the same data. Detection and quantification of bursts by cluster analysis resulted in a linear relationship between integrals of bursts and numbers of action potentials contributing to bursts that was very similar to that obtained when analysis was based on the raw simulated data (Figs. 3A and 4A). Therefore, we conclude that integrating bursts detected by cluster anal-
Fig. 6. A, left: representative segments of simulated recordings in which the number of action potentials contributing to bursts in the "experimental" signal of each pair was greater than the number of action potentials in the "control" signal by the noted percentages (note differences in time scales). A, right: distributions of integrals of bursts for each of the "control" and "experimental" signals. B: number of bursts in experimental records required to detect changes of different magnitudes at $P < 0.05$. 
ysis provides a satisfactory option for analyzing sympathetic activity.

Because simulated nerve activity was rectified before cluster analysis (much as actual nerve activity would be), the relationship between the number of action potentials contributing to bursts and the normalized variances of the amplitudes of bursts was nonlinear (data not shown). Therefore, we investigated the simpler relationship between the number of action potentials contributing bursts and the normalized amplitudes of bursts as detected by cluster analysis. This relationship appeared to be linear but not strongly correlated (Fig. 4A). In addition to this relatively poor correlation, our simulation raised another caution against the use of signal amplitudes, especially amplitudes determined by cluster analysis, as measures of the number of action potential contributing to sympathetic activity. The normalized amplitudes from the simulations were not linearly related but parabolically related to the number of contributing action potentials (Fig. 7).

The apparent linearity of the relationship between the number of action potentials and amplitudes of bursts determined by cluster analysis was artifactual. In using cluster analysis, the experimenter assigns a threshold to preclude small, questionable bursts. In our simulation this threshold eliminated a steeply nonlinear portion of the relationship at small numbers of action potentials (Fig. 4B). Therefore, the remaining relationship appeared to be linear. The quasilinear portion of this relationship might be used to estimate changes in the number of action potentials contributing to bursts. Indeed, DiBona and Jones (6) described experimental conditions under which a close relationship between integrated RSNA and the amplitudes of bursts of RSNA tracked one another closely during experimental procedures. However, under other conditions the correlation between these measures may be limited.

**Relationship between average number of action potentials contributing to bursts and the integral of the amplitude spectrum was linear.** Investigators who study sympathetic activity in the frequency domain often are interested in changes in the total sympathetic activity reflected by their recordings. Because the variance of samples of the simulated sympathetic activity equals the integral of the activity's amplitude spectrum and the variance of samples is linearly related to the number of action potentials contributing to bursts (Fig. 3B), it would seem appropriate to measure changes in sympathetic activity by measuring the integral of the amplitude spectrum of the signal. Our simulation provided an opportunity to test this prediction directly by measuring the relationship between the integral of the amplitude spectra of multiple trials of simulated sympathetic activity and the number of action potentials contributing to that activity. The strong linear relationship between the average number of action potentials contributing to bursts and the integrals of the amplitude spectra suggests that changes in these integrals faithfully represent changes in numbers of action potentials.

**Brief recordings detected statistically significant changes in sympathetic nerve activity.** The temporal resolution of measurements during in vivo experiments is often limited by the duration of recordings that are predicted to be necessary to establish that an experimental manipulation has, or has not, caused a significant change in sympathetic activity. Experimenters are usually conservative, sometimes averaging many minutes of data for both control and experimental levels of activity, thereby limiting the number of procedures that can be accomplished during an experiment. Furthermore, during experiments in which steady states are difficult or impossible to achieve, experimenters need to make measurements of short duration to ensure that they were made during a quasi-steady state. Although the statistical parameters of our simulated activity were unique to this program, we felt that it would be useful to determine the duration of recordings that would be necessary to detect experimentally produced changes in activity of different mag-

![Fig. 7. Relationship between amplitudes of bursts of simulation-generated sympathetic activity and the numbers of action potentials contributing to those samples.](image-url)
nitudes. For this analysis, we used changes in the integrals of bursts because this measure exhibited the best correlation with the numbers of action potentials contributing to bursts (Figs. 3A and 4A). Our results indicated that detection of changes of the sizes usually considered important physiologically required relatively small numbers of bursts. These results indicate that under experimental conditions which produce data that are statistically similar to the data simulated in this study, relatively brief recordings may be required to achieve statistically significant results. Longer recordings would be necessary if sympathetic nerve activity were measured as either variances of samples of bursts or as amplitudes determined by cluster analysis.

In summary, using sympathetic activity simulated as physiologically realistic, discrete bursts, we have confirmed that both integrals and variances of activity are linearly related to the number of action potentials contributing to that activity. Integrals, however, provide a somewhat better prediction of underlying activity than variances. Under some conditions, amplitudes of activity may provide a measure of underlying action potentials, but this measure is substantially less reliable than either integrals or variances. For experimenters who routinely work in the frequency domain, the integral of the amplitude spectrum of sympathetic activity provides a good measure of underlying action potentials. Finally, we predict that, using integrated sympathetic activity, statistically significant, experimentally produced changes in sympathetic activity may be detectable with relatively brief recordings.

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DISCLOSURES

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