Detection of Spontaneous Synaptic Events with an Optimally Scaled Template

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ABSTRACT Spontaneous synaptic events can be difficult to detect when their amplitudes are close to the background noise level. Here we report a sensitive new technique for automatic detection of small asynchronous events. A waveform with the time course of a typical synaptic event (a template) is slid along the current or voltage trace and optimally scaled to fit the data at each position. A detection criterion is calculated based on the optimum scaling factor and the quality of the fit. An event is detected when this criterion crosses a threshold level. The algorithm automatically compensates for changes in recording noise. The sensitivity and selectivity of the method were tested using real and simulated data, and the influence of the template parameter settings was investigated. Its performance was comparable to that obtained by visual event detection, and it was more sensitive than previously described threshold detection techniques. Under typical recording conditions, all fast synaptic events with amplitudes of at least three times the noise standard deviation (3σ) could be detected, as could 75% of events with amplitudes of 2σ. The scaled template technique is implemented within a commercial data analysis application and can be applied to many standard electrophysiological data file formats.

INTRODUCTION

Spontaneous synaptic events can be recorded in almost any neuron and may be divided into two classes: those resulting from a spontaneous action potential in a presynaptic neuron (spontaneous postsynaptic current, sPSC), and those resulting from the release of a single transmitter vesicle in the absence of presynaptic activity (miniature postsynaptic current, mPSC). Miniature events are of particular interest because they report synaptic function at the level of a single terminal. They have been used to investigate the site of expression of synaptic modulation. A presynaptic modulation will generally alter the frequency of mPSCs, and a postsynaptic modulation will alter their average amplitude (discussed in Clements, 1993). They are sometimes termed "quantal" events, reflecting the all-or-none nature of transmitter release. Information about the distribution of mPSC amplitudes is important for various forms of quantal analysis (reviewed in Redman, 1990).

Miniature synaptic events are invariably embedded in background recording noise, which may obscure small events and bias the sample toward larger events. An increase in average mPSC amplitude or a reduction in the recording noise may increase the detection rate for small events and produce an artifactual increase in measured event frequency. Quantitative analysis of mPSCs is only possible when systematic errors are monitored and, where necessary, corrected over the full range of experimental conditions.

The eye is an excellent feature detector, and visual event detection provides a standard of performance against which other techniques can be judged. However, it is arduous and time consuming, and introduces subjective selection criteria that make it impossible to reproduce results accurately. Clearly there is a need for a reliable automatic detection algorithm that is at least as sensitive as visual detection, and whose performance can be carefully characterized under a variety of signal-to-noise conditions.

Automatic algorithms for detecting events in electrophysiological signals can be separated into three categories: those that compare a fixed template to a recorded transient and search for an accurate match (Salganicoff et al., 1988; Yang and Shamma, 1988; Yamada et al., 1992; Oghalai et al., 1994), a recently described technique that searches for local maxima in the cross-correlation between a template and the data set (Abdul-Ghani et al., 1996), and algorithms that search for an event that crosses an amplitude or first-derivative threshold (Liu and Kim, 1983; Morales et al., 1985; Cocatre Zilgien and Delcomyn, 1990; Bergman and DeLong, 1992; Cochran, 1993; Ankri et al., 1994; Carlson and Krieger, 1996). Fixed-template techniques have been used to detect action potential waveforms in extracellular or optical recordings. They are highly selective for the events of interest. Artifact and noise transients are automatically rejected because they do not match the template time course. However, these techniques use a template with only one or at most a few fixed amplitudes, whereas spontaneous synaptic events exhibit a broad and continuous range of amplitudes. For this reason, fixed-template techniques have not been used to detect spontaneous synaptic events. An alternative approach based on the cross-correlation between a template and the data is not selective for events of a

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particular amplitude, and has been used to detect spontaneous synaptic events. However, the description of the method is limited to a figure legend, its performance has not been characterized, and no guidelines are provided for selecting the correlation period and detection threshold parameters (Abdul-Ghani et al., 1996). Automated detection of spontaneous synaptic events generally has relied on threshold techniques. An event is detected when the amplitude or first derivative of the recorded postsynaptic signal exceeds a selected threshold level (Liu and Kim, 1983; Morales et al., 1985; Cocatre Zilgien and Delcomyn, 1990; Bergman and DeLong, 1992; Cochran, 1993; Ankri et al., 1994; Carlson and Krieger, 1996). These techniques have the advantage of being relatively fast and easy to implement. An added advantage of the first-derivative approach is that it is not sensitive to drift or low-frequency noise in the membrane potential or holding current (Morales et al., 1985; Cocatre Zilgien and Delcomyn, 1990; Ankri et al., 1994). In contrast, amplitude threshold algorithms must correct for drift by using high-pass filtered recordings (AC-coupled), or by measuring event amplitude relative to a preevent baseline (Liu and Kim, 1983; Bergman and DeLong, 1992; Cochran, 1993; Carlson and Krieger, 1996). However, these techniques are relatively nonselective. Any noise or artifact transient that is sufficiently large or fast will be detected, regardless of its shape. Some of the threshold techniques address this problem by applying additional selection criteria (Bergman and DeLong, 1992; Cochran, 1993; Ankri et al., 1994). They compare the time course of each detected event to the expected synaptic time course and reject events that do not match, using a variety of approaches. An interesting approach based on a scaled template has been used to reject corrupt or noisy evoked EPSCs (Liao et al., 1992), but it has not been applied to the selection of spontaneous synaptic events. Detection techniques that retrospectively apply time course criteria can achieve the selectivity of the fixed amplitude template technique. However, these post-processing techniques may suffer from a lack of sensitivity at the initial threshold detection step. The performance of any threshold detection technique is critically dependent on the recording conditions and the threshold setting. These techniques are compromised by high-frequency noise, and the data generally must be smoothed by additional offline filtering before a threshold technique can be applied. Optimal filter and threshold settings must be determined by a trial-and-error approach for each data file.

In the present study, a simpler and more sensitive method for detecting spontaneous synaptic events is described. It is based on a synaptic template with a variable amplitude and is very selective for the synaptic events of interest. It can be applied directly to raw data with no need for additional filtering and incorporates automatic threshold adjustment. The performance of the technique was systematically investigated under a variety of conditions, and optimum parameter settings were determined.

METHODS

Experimental recordings

Hippocampal slices (400 μm thick) were prepared from 2–3-week-old Wistar rats, as described previously (Bekkers et al., 1996). Slices were perfused with extracellular solution containing 110 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 2.5 mM $MgCl_2$, 4 mM $SrCl_2$, 26 mM $NaHCO_3$, 2.5 mM NaH_2PO_4, and 10 mM glucose bubbled with 95% O_2/5% CO_2. Bicuculline (10 μM) was added to block inhibitory synaptic currents. Patch electrodes contained 125 mM Cs-gluconate, 5 mM CsCl, 10 mM EGTA, 2 mM Na_2ATP, and 2 mM $MgCl_2$. Miniature EPSCs were recorded from dentate gyrus granule neurons in the whole-cell recording configuration. Data were collected with an AxoPatch-1D, filtered at 2 kHz, and sampled at 5 kHz.

The template function

The template function used to search for spontaneous synaptic events has a flat baseline region followed by an idealized synaptic time course consisting of an exponential rise and decay:

$$\text{TEMPLATE}(t) = 0 \quad (t \leq 0)$$

$$\text{TEMPLATE}(t) = \text{NORM}(1 - \exp(-t/\text{RISE}))\exp(-t/\text{DECAY}) \quad (t > 0)$$

where t is the time from onset of the idealized synaptic event, NORM is the scaling factor used to normalize the peak amplitude, RISE is the time constant of the rising phase of the template, and DECAY is the time constant of the falling phase of the template. The detection algorithm is not dependent on, or limited to, a template of this form. Any other waveform could be substituted.

Selecting the template parameters

The precise time course of the template function is not critical. An event with a time course that deviates from the template can still be detected, although with slightly reduced sensitivity (see Results). A reasonable strategy is to set the rise and decay time constant in the middle of the range expected for the synaptic events. A two-pass approach was used in this study. The template time course parameters were estimated from a few large synaptic events that were detected manually. This template was used for the first pass of the automatic detection procedure through a portion of the data file. The detected events were captured and aligned at their onset, and the average time course of the captured events was calculated. The rise and decay time constants were adjusted to give the best fit between the template and the averaged synaptic event. This optimally fitted template was used for the second pass of the detection procedure through the full data set.

Fitting the template to the data

The template was slid along the data trace, one point at a time, and was optimally scaled and offset to fit the data at each position:

$$\text{FITTED\_TEMPLATE} = \text{TEMPLATE} * \text{SCALE} + \text{OFFSET}$$

where SCALE is the template scaling factor, and OFFSET is added offset. The SCALE and OFFSET parameters are calculated to minimize the sum of squared errors (SSE) between the fitted template and the data region:

$$\text{SCALE} = \frac{\Sigma(\text{TEMPLATE} * \text{DATA}) - \Sigma\,\text{TEMPLATE} * \Sigma\,\text{DATA}/N}{\Sigma\,\text{TEMPLATE}^2 - \Sigma\,\text{TEMPLATE} * \Sigma\,\text{TEMPLATE}/N}$$

$$\text{OFFSET} = (\Sigma\,\text{DATA} - \text{SCALE} * \Sigma\,\text{TEMPLATE})/N$$

where N is the number of points in the template, Σ is the sum from 1 to N, and DATA is the data points to fit. The derivations of the above formulas are given in Appendix 1.

Event detection criterion

The detection criterion is calculated from the template scaling factor, SCALE, and from the goodness of fit between the scaled template and the data (STANDARD_ERROR). The STANDARD_ERROR is derived from the SSE between the data points and fitted template:

$$\text{STANDARD\_ERROR} = (\text{SSE}/(N-1))^{1/2}$$

where

$$\begin{aligned}\text{SSE} &= \Sigma(\text{DATA} - \text{FITTED TEMPLATE})^2 \\ &= \Sigma\,\text{DATA}^2 + \text{SCALE}^2 * \Sigma\,\text{TEMPLATE}^2 + N * \text{OFFSET}^2 \\ &\quad - 2(\text{SCALE} * \Sigma(\text{TEMPLATE} * \text{DATA}) \\ &\quad + \text{OFFSET} * \Sigma\,\text{DATA} - \text{SCALE} * \text{OFFSET} * \Sigma\,\text{TEMPLATE})\end{aligned}$$

The derivation of the formula for SSE is given in Appendix 1.

The detection criterion is

$$\text{DETECTION\_CRITERION} = \text{SCALE/STANDARD ERROR}$$

A strategy for fast computation of DETECTION_CRITERION is given in Appendix 2.

This criterion was selected for the following reason. When the template is aligned with and accurately fitted to a synaptic event in the data, the numerator and denominator of the above equation have clear meanings. The numerator, SCALE, approximates the peak amplitude of the event, and the denominator, STANDARD_ERROR, approximates the noise standard deviation. Thus the detection criterion is closely related to the signal-to-noise ratio for the detected event. The background noise signal will very rarely exceed 4 times the standard deviation of the noise, so the absolute value of the detection criterion is unlikely to exceed 4 in the absence of an underlying event. Thus a threshold setting of 4 provides close to optimum detection sensitivity while ensuring a low rate of spurious events (false positives). The same threshold setting can be used on all data sets, independent of the background noise level. To put this another way, the detection algorithm automatically adjusts to compensate for changes in recording noise and to maintain optimum performance.

An event is detected when the detection criterion crosses the selected threshold level, reaches a peak, and then drops back below the threshold as the template is slid past the event (Fig. 1,*A* and *B*). The detection criterion typically exhibits a sharp, unambiguous peak when the template is optimally aligned with a synaptic event (Fig. 1 *B*). The peak in the detection criterion gives an accurate indication of the time at which the event occurred.

More selective event detection

The scaled template technique developed above is intrinsically selective for events that conform to the template time course. However, it will still detect events that deviate from this time course, especially if they are large. This can be an advantage, because the mEPSC time course may vary from event to event. However, detection and measurement problems could arise if the spontaneous events are contaminated by extraneous events with a different time course. Deviations from the template time course may indicate a nonsynaptic or a contaminated synaptic event. When uncontaminated events are plentiful, a good strategy is to automatically eliminate events suspected of being corrupt. For example, events may be rejected where the standard error between the fitted template and the data exceeds a certain threshold. This criterion will become more stringent for larger events.

To obtain a pure population of synaptic events, additional selection criteria may be applied to the detected events, based on shape parameters (Bergman and DeLong, 1992; Cochran, 1993; Ankri et al., 1994). The computer program developed for this study permits optional rejection of events with large standard errors, excessively fast or slow rise times, or large baseline standard errors or offsets. The influence of these optional features on the performance of the analysis program was not systematically investigated in this study.

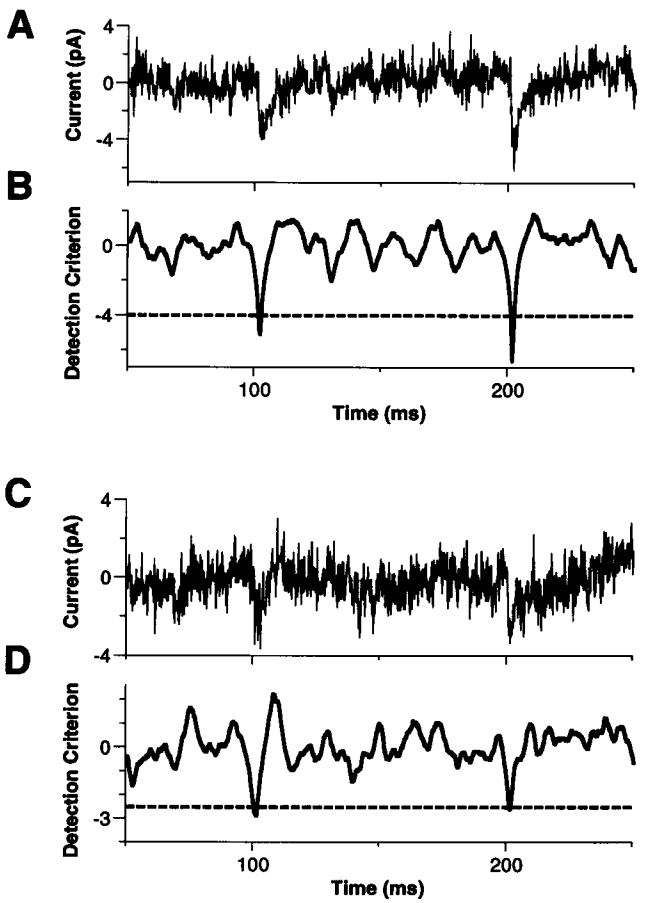

FIGURE 1 A detection criterion, calculated from the optimally scaled template, was used to detect small synaptic events. (*A*) A simulated data trace containing two synaptic events superimposed on recording noise (σ = 1 pA). The EPSCs are both 4 pA in amplitude and occur at 100 ms and 200 ms. (*B*) A template with the same time course as the synaptic events (1-ms rise, 4-ms decay time constant), was stepped along the record shown in *A*, and the detection criterion was calculated at each position (see Methods for details). The detection criterion exhibits a sharp negative-going peak when the template is optimally aligned with a synaptic event. With the detection threshold set to −4 (*dashed line*), both synaptic events will be detected. (*C*) Another simulated data trace containing two synaptic events at 100 ms and 200 ms. The EPSCs are both 2 pA in amplitude, half the size of the events in *A*. (*D*) The detection criterion exhibits a peak when the template is optimally aligned with a synaptic event, but the threshold had to be lowered to −2.5 (*dashed line*) to detect both synaptic events.

Simulations

The detection algorithm was tested using simulated synaptic events. These events had the same shape (exponential rise and decay) as the product-of-exponentials template function described above. Two types of simulated data set were generated. In the first, all events had the same amplitude and occurred regularly, one every 100 ms, to simplify the counting of correctly and incorrectly identified events. In the second, simulated events were generated in closely spaced pairs to investigate the sensitivity of the detection algorithm to event overlap and collision (see Results).

The simulated events were superimposed on simulated electrophysiological noise that was generated as follows. Two arrays, representing real and imaginary components of the complex noise spectrum, were filled with random numbers between −1 and 1. Each point of the real and imaginary spectrum was scaled by (1 + 300/frequency), and then the total power of the spectrum was normalized back to 1. A complex inverse fast Fourier transform was performed on the spectrum, and the resulting real array was scaled by

$$\text{Noise\_SD}/(N_d/\pi)^{1/2}$$

where Noise_SD is the desired standard deviation of simulated noise and N_d is the number of data points in the array. The resulting noise spectrum is approximately $1/f$ at low frequencies, then rolls off to white noise around 300 Hz. The value of 300 Hz is typical for whole-cell recording from cultured hippocampal neurons, but may vary with different preparations and recording conditions. The simulated recording noise had a standard deviation of 1 pA and a peak-to-peak range of approximately ±5 pA.

Typical values for the rise and decay of a fast excitatory synaptic current in a cultured hippocampal neuron at 23°C were used in the simulations (RISE = 1 ms, DECAY = 4 ms). The performance of the detection algorithm was measured by counting the number of events correctly or incorrectly identified. A detected event was counted as correctly identified if its onset time was within 1 ms of a known simulated event onset.

The software package for simulation, detection, and analysis of spontaneous synaptic events was developed on a Macintosh computer using AxoGraph 3.5 (Axon Instruments, 1101 Chess Drive, Foster City, CA 94404, USA; http://www.axonet.com/pgraph3.htm). The programs and supported data file formats are described in Appendix 3.

RESULTS

Analysis of electrophysiological data

Evoked and spontaneous miniature excitatory postsynaptic currents (mEPSCs) were recorded from dentate gyrus granule cells in the whole-cell voltage-clamp configuration. Recordings were made in the presence of strontium (4 mM), which depresses the synchronous component of evoked transmitter release and enhances the asynchronous component. Minimal stimulation was applied to the perforant pathway at 0.5 Hz. Each stimulus produced a small evoked response followed by a shower of asynchronous mEPSCs that lasted several hundred milliseconds. Recordings were made from three neurons, a total of 2366 traces were searched by eye, and 3417 synaptic events (evoked and spontaneous) were visually identified. These manually detected events were compared with the events detected in the same data set with the scaled template technique.

A different template time course was used for each neuron, based on the average mEPSC time course (see Methods). For the first neuron it had a 0.8-ms rise and a 5-ms decay time constant. To test the false-positive rate in this neuron, the threshold was first set to +2.5, and the detection procedure was applied to the baseline region of the data before the stimulus. A positive threshold selects for events with a positive amplitude, and so should not pick up the occasional negative-going mEPSC that occurred in the baseline period (50 ms per episode). The results gave a false-positive rate of 0.01 s^{-1}, corresponding to <10 false-positive events in the entire data set for this threshold setting. Next, scaled template analysis was applied to the time period after the stimulus (150 ms), with the threshold set to −2.5 (Fig. 2 *A*). The automated procedure detected 1970 spontaneous synaptic events, compared with 1360 detected by the visual approach. The amplitude of detected events was measured by subtracting the average current over a 2-ms period preceding the event, from the average over a 1-ms period centered on the peak of each response. The amplitude distributions of the events obtained with these two detection techniques were compared (Fig. 2 *B*). The visual and automated approaches gave virtually identical results for large, well-resolved synaptic events (>5 pA), but the automated algorithm picked up many more smaller mEPSCs with amplitudes just out of the baseline noise (σ = 1.1 pA). It found twice as many events in the range of 0–5 pA as the visual technique (1056 versus 509). Most of the "extra" events must be of synaptic origin,

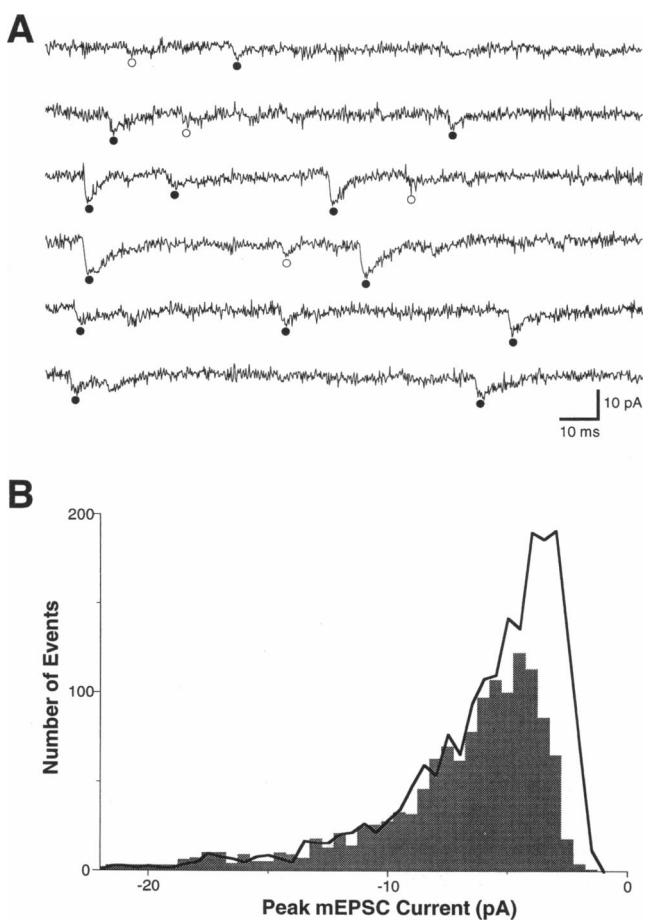

FIGURE 2 A comparison between the amplitude distributions of synaptic events identified by the scaled template and visual detection techniques in a recording from a dentate gyrus granule cell. (*A*) Selected traces are shown that contain only small spontaneous events (2–10 pA). Events that were detected by the scaled template technique are identified by filled circles (threshold = 2.5) or open circles (threshold = 2). (*B*) A total of 1360 events were visually detected; their amplitude distribution is plotted as a shaded histogram. The scaled template technique (threshold set to 2.5) detected 1970 synaptic events; their amplitude distribution is shown as a solid line. The visual and scaled template approaches gave virtually identical results for large, well-resolved synaptic events (>5 pA).

because the automated technique has a very low false-positive rate. However, they failed to satisfy the subjective detection criterion during the visual search. A similar divergence between automated and visual techniques was seen for small events in the results from the two remaining cells (not shown). This does not mean that the automated technique was intrinsically more sensitive than the visual technique. It merely reveals that, in this case, visual detection was more selective and/or the detection threshold was higher than for the automated approach. Two major advantages of the automated technique over the manual approach are that its results can be easily verified and reproduced, and its false-positive rate can be estimated quickly and reliably. In summary, the automated template detection technique was at least as sensitive and reliable as visual detection.

Analysis of simulated data

Two measures were used to analyze the performance of the automated detection technique. These were the percentage of events in the data that were correctly identified (detection sensitivity) and the false-positive rate. Several of the factors that influence performance were investigated, including the detection threshold setting, the template length and time course, and the frequency and time course of spontaneous events. The factors affecting the false-positive rate will be considered first.

Factors affecting the false-positive rate

The detection procedure was applied to 100 traces 2.1 s long sampled at 5 kHz, and containing only simulated electrophysiological noise (no synaptic events). The template function had a 1-ms rise and a 4-ms decay. The threshold setting was adjusted and its effect on the false-positive rate was explored. When the threshold was set to 4, no false positives were detected, but as it was reduced, the false positive rate rose steeply (Fig. 3 *A*). For example, at a threshold setting of 2.5 and a template length of 16 ms (8-ms baseline and 8-ms template function), the false-positive rate was 0.81 s^{-1}.

The other adjustable parameter in the detection procedure is the length of template. A long template should be less sensitive to spurious events, because they are more likely to deviate from the ideal synaptic time course for longer regions. To confirm this, the effect of template length on the false-positive rate was investigated. Several templates were constructed using the same time course (1-ms rise, and 4-ms decay), but with lengths from 8 to 32 ms. The false-positive rate decreased significantly as the length of the template increased (Fig. 3 *A*). At a threshold of 2.5, the false-positive rate decreased 10-fold from 2.5 s^{-1} for a template length of 8 ms, to 0.24 s^{-1} for a 32 ms long template.

The false-positive rate also depended on the time course of the template function. For a given threshold setting and template length, a template function with a slower rise and decay produced fewer false positives (Fig. 3 *B*). For a 16-ms-long template with a threshold of 2.5, slowing the template time course parameters fourfold (rise/decay slowed from 0.5/2 ms to 2/8 ms) decreased the false-positive rate from 2.0 s^{-1} to 0.32 s^{-1}.

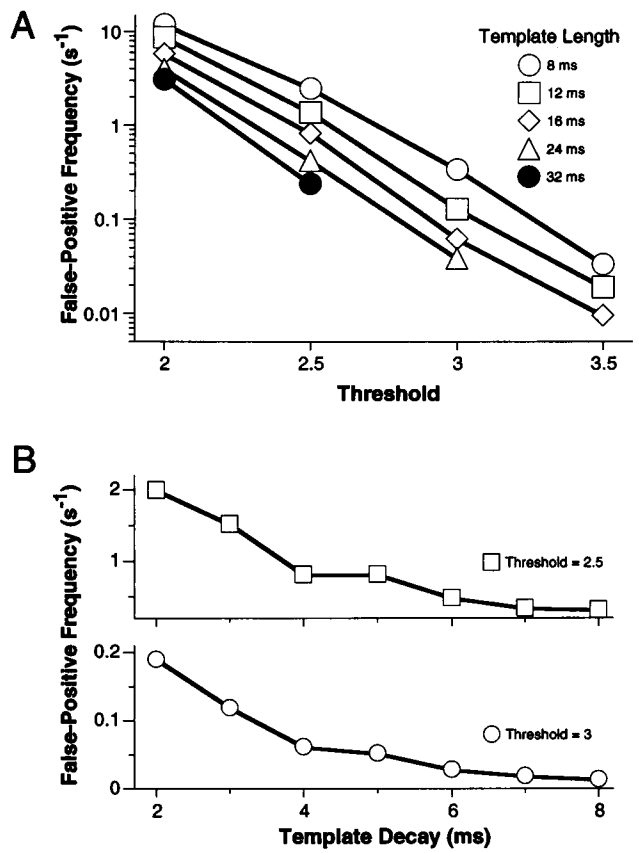

FIGURE 3 Factors affecting the false-positive rate of the scaled template technique. A data set containing only simulated recording noise (σ = 1 pA) was analyzed, and the number of false-positive events was counted. (*A*) As the detection threshold was increased, the false-positive rate dropped steeply. The false-positive rate also dropped as the template length was increased. (*B*) The false-positive rate dropped as the rise and decay time constants of the template function were increased. This trend was independent of the detection threshold, and results are shown for a threshold setting of 2.5 (*top*) and 3.0 (*bottom*).

Factors affecting sensitivity

The automatic detection algorithm was applied to five groups of 100 traces containing simulated synaptic events. Each trace was 2.1 s long, sampled at 5 kHz, and contained 20 simulated synaptic events superimposed on simulated electrophysiological noise. So each group of traces contained 2000 events of the same amplitude and time course. The amplitudes were 1, 2, 3, 4, and 5 pA for groups 1 to 5, respectively. The template function had a 1-ms rise and a 4-ms decay, identical to the simulated synaptic events. The template length and detection threshold settings were adjusted and their effect on the detection sensitivity was explored.

Events greater than or equal to 4 pA were reliably detected for all threshold settings tested (≥98% detected; template length 12 ms). Fig. 1 *A* shows two typical 4-pA events with onsets at 100 and 200 ms. Fig. 1 *B* shows the corresponding detection criterion. This trace contains two sharp peaks that easily exceed the detection threshold of −4 (*dashed line*). However, only ~10% of 2-pA events were detected at this threshold setting. Lowering the threshold increased the number of smaller events that were detected (Fig. 4 *A*). At a threshold setting of 2.5, 78% of 2 pA events were detected. This is an excellent result, considering that the 2 pA events were much smaller than the peak-to-peak noise (±5 pA) and were scarcely visible by eye (Fig. 1, *C* and *D*). The price paid for this sensitivity was a relatively high false-positive rate (1.4 s^{-1}; Fig. 3 *A*). In summary, the detection threshold setting determines the trade-off between sensitivity and the false-positive rate.

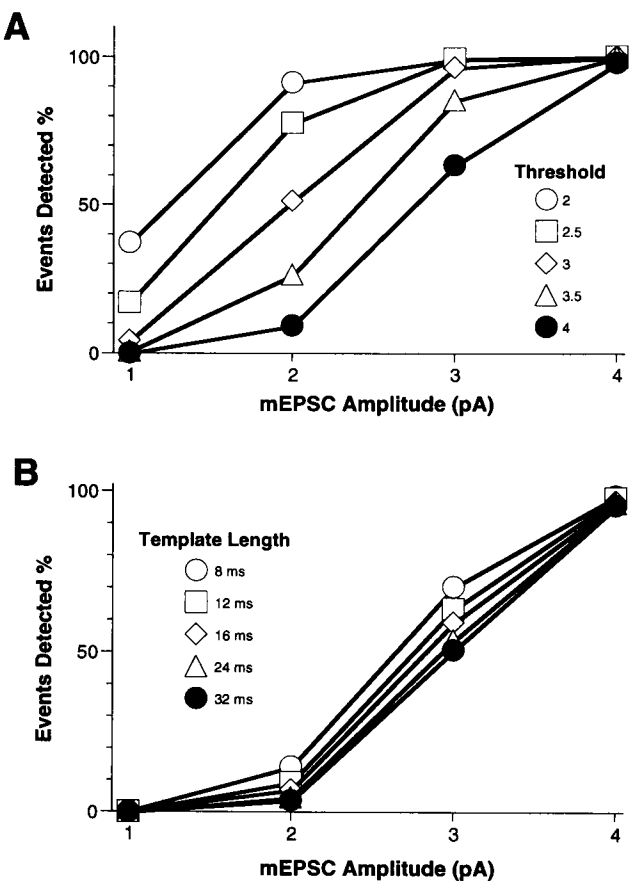

FIGURE 4 Factors affecting the detection sensitivity of the scaled template technique. Simulated data sets, each containing 2000 synaptic events of various amplitudes and superimposed on recording noise (σ = 1 pA), were analyzed, and the number of detected events was counted. (*A*) Detection sensitivity increased for larger event amplitudes, and almost all 4-pA events were detected. Up to 78% of 2-pA events could be detected, even though these were very close to the recording noise level. Increasing the detection threshold decreased the number of events that were detected. The template length was 12 ms for all measurements. (*B*) The percentage of events that were detected decreased slightly as the template length was increased, but this effect was relatively small. The detection threshold was 4 for all measurements.

The effect of template length on detection sensitivity was investigated. Increasing template length decreased sensitivity (Fig. 4 *B*). For example, with a threshold of 3, increasing the template length from 8 to 32 ms decreased the detection success from 70% to 50% (Fig. 4 *B*). This small decrease in sensitivity was due to an increase in selectivity for events with a time course that matched the template. The increased selectivity is also reflected in the lower rate of false positives with longer templates (Fig. 3 *A*). Longer templates may suffer further loss of sensitivity if the spontaneous event frequency is high (see below). In summary, the template length setting affects the trade-off between sensitivity and selectivity, but performance is not very sensitive to this parameter.

Variable synaptic time course

The time course of mEPSCs typically varies from event to event (Bekkers and Stevens, 1989). This variation will affect the reliability of the automated detection procedure, because the detection template will not precisely match all of the synaptic events. The loss of sensitivity produced by a mismatch between the template and the synaptic time course was investigated. The time course of simulated synaptic events was systematically varied, and the automatic detection procedure was applied, using a fixed time course template. Sixteen data sets were analyzed, each consisting of 100 simulated traces containing 2000 identical synaptic events. The fixed template function had a 1-ms rise and a 4-ms decay time constant. The simulated events had rise times that ranged from 0.5 to 2.5 ms, and decay time constants that ranged from 2 to 10 ms. Slower events are intrinsically easier to detect, because it is possible to average over a longer interval around the peak of the event and so reduce the effective background noise. Noise standard deviation is inversely proportional to the square root of this interval, so the amplitudes of the simulated events were made proportional to the square root of their decay time constant. As expected, detection sensitivity was best for events with a time course that exactly matched the template. However, moderate time course deviations produced little loss of sensitivity. It was reduced by only 2% for events that were 25% faster than the template, and by 7% for events that were 20% slower than the template (Fig. 5 *A*). Larger time course deviations produced more dramatic reduction in sensitivity, which reflects the selectivity of the scaled template method.

Collisions between synaptic events

When two spontaneous synaptic events are separated by less than the decay time of the first event, the second event will be superimposed on the tail of the first (a "collision"). This will create problems for the detection and measurement of both events. These problems are unavoidable and affect all visual and automated detection techniques. It is possible to

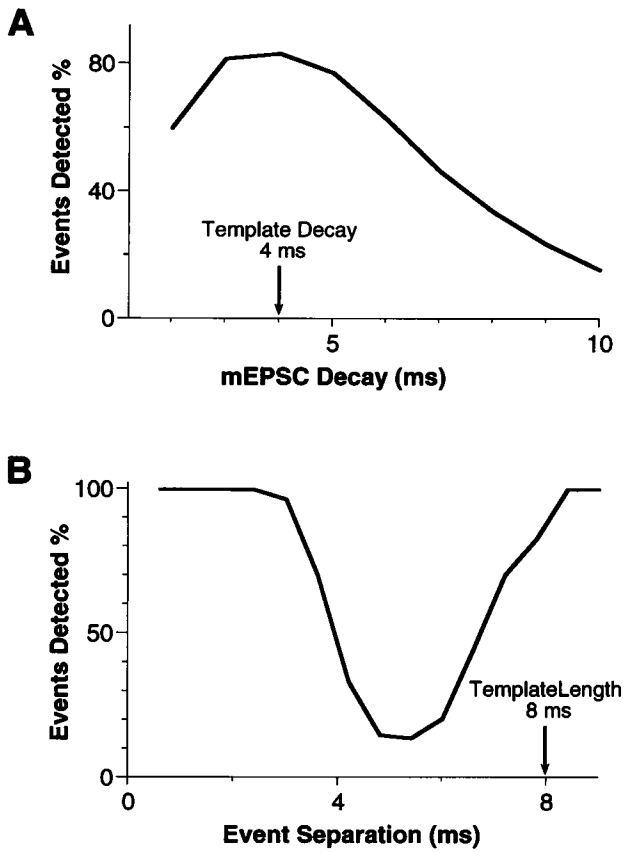

FIGURE 5 The influence of variations in synaptic time course and collisions between spontaneous events on the detection sensitivity of the scaled template technique. (*A*) Simulated data sets, each containing 2000 synaptic events with various rise and decay time constants, were analyzed, and the number of detected events was counted. Detection sensitivity was lower for events that had a time course different from that of the template. This loss of sensitivity was small for a time course difference up to about 25%, but fell away steeply for larger differences. (*B*) Detection sensitivity was reduced when two events occurred within a few milliseconds of each other. Events separated by less than the template rise time were incorrectly detected as a single event, but with twice the normal amplitude. As the separation increased, the detection sensitivity dropped steeply. Sensitivity recovered when the event separation approached the template length (8 ms).

reduce problems arising from collisions by carefully post-processing the detected events (Ankri et al., 1994; Carlson and Krieger, 1996). However, some measurement artifacts will persist. For example, when two events are separated by less than the rise time of the first event, postprocessing will not separate the events reliably.

The sensitivity of the scaled template technique is reduced by collisions. In most cases, the effect of a collision is to increase the standard error due to the mismatch between the template and the compound synaptic time course, and so reduce the peak amplitude of the detection criterion. Thus one or both events involved in a collision may escape detection. In general, it is possible to correct for missed events caused by collisions. When spontaneous synaptic events occur at random times, the interval between pairs of events will have a Poisson distribution. An interval histogram can be constructed and a Poisson function fitted over an interval range not affected by collisions. Comparison between the extrapolated Poisson function and the interval histogram at shorter intervals will reveal how many events have been missed due to collisions.

To characterize the loss of sensitivity associated with event overlap and collision, the performance of the scaled template detection algorithm was measured as a function of event separation. Sixteen data sets were analyzed, each consisting of 100 simulated traces containing 1000 closely spaced pairs of identical synaptic events. Each simulated data set had a different separation between pairs of events. The template function had a 1-ms rise and a 4-ms decay time constant. It was relatively short, with a 2-ms baseline and a total length of 8 ms. The simulated events had amplitudes of 3 pA, with a 1-ms rise and a 4-ms decay time constant. Events separated by up to 2.4 ms were reliably detected, but were counted as a single event (Fig. 5 *B*). Sensitivity dropped dramatically as event separation was increased and reached a minimum at 5.4 ms. Sensitivity recovered as event separation increased toward the length of the template (Fig. 5 *B*).

Comparison with first-derivative threshold technique

The performance of the scaled template algorithm was compared with a previously described automatic synaptic event detection technique. This approach detects an event when the first derivative of the membrane potential or current time course crosses a threshold (Morales et al., 1985; Cocatre Zilgien and Delcomyn, 1990; Ankri et al., 1994). The scaled template and the first-derivative techniques were both applied to the same simulated data sets containing 2 or 3 pA events (1-ms rise and 4-ms decay time constants).

Typically, data are smoothed with an offline filter before the first-derivative threshold technique is applied. The smoothed first-derivative approach is mathematically very similar to the amplitude threshold technique, in which amplitude is measured relative to a preevent baseline. This is because the amplitude is proportional to the slope of the signal (first derivative) from the end of the baseline to the measurement point at the peak of the event. Thus the optimized performance of the smoothed first-derivative technique will be very similar to the optimized performance of the amplitude threshold technique.

The first-derivative threshold technique has two adjustable parameters, the detection threshold and the low-pass frequency for the smoothing filter. It is not clear from previously published work how to determine an optimum filter setting for a given data set. In the present study, a range of offline filter settings from 1 kHz to 50 Hz were applied to the simulated data. For each filter setting, a first-derivative threshold was chosen by trial and error to

give a false-positive rate of ~0.25 s^{-1}. The optimal sensitivity was obtained with a filter setting of 100 Hz (Table 1). However, the scaled template technique, with a threshold of 2.5, detected many more events (Table 1; Fig. 6), while producing the same false-positive rate as the first derivative threshold technique (0.25 s^{-1}).

DISCUSSION

A scaled template technique for detection of spontaneous synaptic events was developed and tested. It was at least as sensitive as visual event detection when applied to real electrophysiological data. It exhibited greater sensitivity and selectivity than previously described threshold techniques. The scaled template approach also has two practical advantages over other automated techniques. The data do not have to be additionally filtered before they are analyzed, and the detection algorithm automatically compensates for changes in the background noise level, so the same threshold setting can be used on all data sets. Reliable event detection can be achieved by using a simple systematic procedure to select the template parameters and threshold level, as described below. To extract optimal performance, some additional adjustment of these parameters may be required. The two main disadvantages of the scaled template approach are its relatively slow performance due to the large number of numerical calculations involved, and its failure to detect pairs of events that are separated in time by less than the length of the template. Both of these disadvantages can be minimized by selecting a short template. Speed is not a serious concern, because most data files can be analyzed offline in a few minutes with this technique.

The sensitivity of the scaled template technique is dependent on two parameters, the length of the template, and the threshold setting for the detection criterion. Sensitivity increases as the threshold is reduced, but if it is set too low, then the false-positive rate will become unacceptably high. A threshold setting of 4 will produce virtually no false positives and will detect at least 95% of well-resolved synaptic events (amplitude > 4σ). This is a good default setting when the spontaneous events of interest are large relative to the recording noise. For optimum sensitivity to smaller events, the threshold should be set as small as possible while producing a manageable number of false positives. This setting depends in a complex way on the characteristics of the noise and the shape of the template. To determine the optimal setting, a section of data containing no synaptic events (e.g., recorded in the presence of antagonist) is analyzed several times. At each pass the detection level is lowered until spurious detected events begin to appear. The detection criterion is set at the lowest level that gives an acceptable false-positive rate (for example, 1% of the spontaneous event rate). The optimum threshold setting may also be determined from a data set containing spontaneous events. For example, if the events are all negative-going, then testing a positive threshold should provide a useful estimate of the false-positive rate associated with a given setting. As a third alternative, simulated electrophysiological noise with the same standard deviation as in the recorded data could be used. A program for simulating electrophysiological data, with or without spontaneous events, is included in the AxoGraph event detection package (see Appendix 3). The optimum detection threshold will generally fall in the range

$$2.5 < \text{abs(OPTIMUM\_THRESHOLD)} < 3.5$$

where abs is the absolute value function. The other parameter that influences the sensitivity and selectivity of the

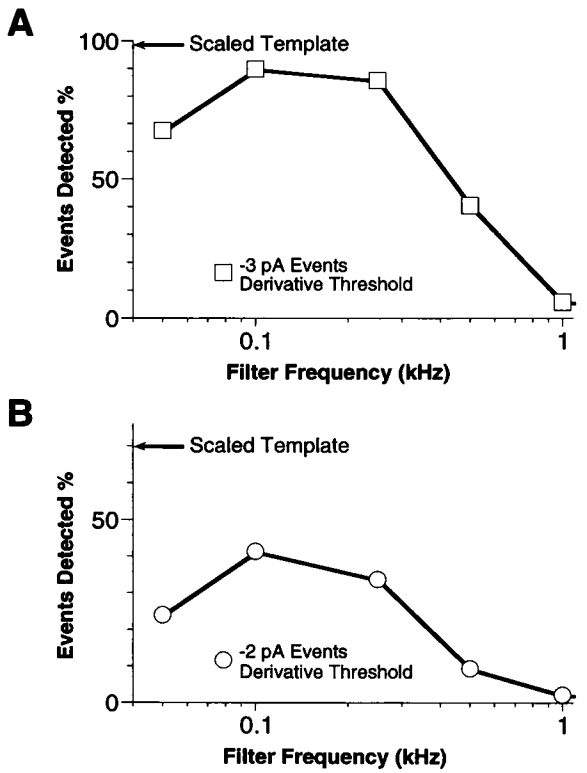

FIGURE 6 A comparison between the sensitivity of the scaled template technique and first-derivative threshold technique. (*A*) A simulated data set containing 2000 synaptic events was analyzed. The events were all −3 pA in amplitude and were superimposed on recording noise (σ = 1 pA). The scaled template technique was applied directly to the unfiltered data and detected 99% of the events (*dashed line*). The data were then smoothed with a low-pass filter set at various cutoff frequencies and analyzed using the first-derivative threshold technique. The detection threshold was adjusted to give the same false positive rate as the scaled template technique. Detection sensitivity was maximized with a filter setting of 100 Hz, but at a lower level than obtained with the scaled template technique. (*B*) Same analysis as in *A*, but all simulated events were −2 pA in amplitude.

TABLE 1 Performance of the first-derivative and scaled template techniques

	2 pA events (% detected)	3 pA events (% detected)	False positives (s^{-1})
Scaled template	70	99	0.25
First derivative	40	90	0.25

scaled template technique is the length of the template; however, this parameter has much less effect on performance than the threshold setting. A reasonable default is to set both the template baseline length and the template synaptic waveform length equal to twice the synaptic decay time constant. This gives a total template length of 4 time constants. Again, a trial-and-error approach can be used for choosing the optimal setting. Increasing template length increases the selectivity for synaptic events, but at a cost of slightly decreased sensitivity. Longer templates suffer further loss of sensitivity if the spontaneous event frequency is high, because two events that are separated by less than the template length may not be detected. Another practical disadvantage of using a long template is that it requires more computations, so the analysis procedure takes longer.

It is inevitable that some spontaneous synaptic events will be missed by any detection algorithm (including visual detection). With a little effort, it may be possible to correct for these lost events. For example, if a simulation study predicts that 50% of events in the amplitude range from 2 to 2.5 pA will be missed, then the number of counts in the corresponding bin(s) of the synaptic amplitude histogram could be doubled. A similar strategy can correct the amplitude distribution for contamination with false-positive events. The scaled template analysis is applied to data containing no synaptic events (preferably from the same cell that generated the spontaneous synaptic event data). The resulting false-positive events are binned and their amplitude distribution is subtracted from the synaptic amplitude distribution. This strategy permits the detection threshold to be set to a low level, which maximizes sensitivity for small events. Postprocessing then minimizes the distortion of the amplitude histogram due to false-positive events. However, this approach will not work if parameters other than synaptic amplitude are to be measured from the detected events.

A peak in the scaled template detection criterion gives an accurate indication of the time at which the underlying synaptic event occurred. The detection program optionally aligns and captures the detected events into a new episodic data file for postprocessing. It uses all of the information in the event to estimate onset time, including the best resolved data at the peak of the response. In contrast, threshold methods typically estimate event onset from the point at which it first exceeds the detection threshold. This approach only uses information from the rising phase of the event when it is not well resolved from the noise. Accurate alignment of the events detected with threshold techniques may require postprocessing.

A scaled template technique was previously developed to automate the elimination of corrupt evoked EPSCs (Liao et al., 1992); however, the template was fixed at a single location, not slid along the data trace. The sliding scaled template technique described above could be adapted to analyze evoked events. With the appropriate parameter settings, it could eliminate corrupt events and align the remaining events at their onset to reduce latency jitter in the evoked responses.

In summary, the performance of the scaled template event detection technique has been carefully characterized under a variety of recording conditions. It is at least as sensitive as visual event detection, and more sensitive than threshold techniques. Default template and threshold settings will produce good results, and it is relatively easy to optimize these settings. The technique is implemented within a commercial data analysis application, and so can be applied to most standard electrophysiological data file formats.

APPENDIX 1: DERIVATION OF THE TEMPLATE SCALE AND OFFSET PARAMETERS

Let N be the number of points in the template, e the normalized template points, f the optimally scaled and offset template, and y the data points to be fitted. The parameters to optimize are S, the template scale factor, and C, the added offset. These parameters are used to generate the scaled and offset template:

$$f = Se + C$$

Expression to minimize:

$$\begin{aligned}\text{SSE} &= \text{Sum of Squared Errors}\\ &= \Sigma(y - f)^2\\ &= \Sigma(y - Se - C)^2\\ &= \Sigma y^2 + S^2\Sigma e^2 + NC^2 - 2(S\,\Sigma ey + C\,\Sigma y - SC\,\Sigma e)\end{aligned}$$

At the SSE minimum

$$\partial\text{SSE}/\partial S = 0$$

$$\partial\text{SSE}/\partial C = 0$$

$\Rightarrow$

$$2S\,\Sigma e^2 - 2(\Sigma ey - C\,\Sigma e) = 0$$

$$2C\,N - 2(\Sigma y - S\,\Sigma e) = 0$$

$\Rightarrow$

$$S = (\Sigma ey - C\,\Sigma e)/\Sigma e^2$$

$$C = (\Sigma y - S\,\Sigma e)/N$$

Substitute for C

$$S = (\Sigma ey - \Sigma e(\Sigma y - S\,\Sigma e)/N)/\Sigma e^2$$

$$\Rightarrow S = (\Sigma ey/\Sigma e^2 - \Sigma e \cdot \Sigma y/(N\,\Sigma e^2))/(1 - \Sigma e \cdot \Sigma e/(N\,\Sigma e^2))$$

Multiply top and bottom by Σe^2

$$S = (\Sigma ey - \Sigma e \cdot \Sigma y/N)/(\Sigma e^2 - \Sigma e \cdot \Sigma e/N)$$

and

$$C = (\Sigma y - S\,\Sigma e)/N$$

APPENDIX 2: OPTIMIZING THE COMPUTATIONS

When the template is stepped along the data trace, the template scale factor, S, the added offset, C, and the standard error between the scaled template and the data must be calculated at each position. Large performance improvements can be made by eliminating redundant computations. The two sums calculated over the template do not change as the template is moved, so Σe and Σe^2 only have to be calculated once, and can be reused at each position. The sums over the data, Σy and Σy^2, must be calculated in full for the first template position, but a faster calculation can be made for subsequent positions. When the template is stepped one point to the right along the data trace, the leftmost data point (y_{old}) drops out of the data section, and a new point (y_{new}) is added at the right. Thus the sums over the new section, $\Sigma y'$ and $\Sigma y^{2\prime}$, are

$$\Sigma y' = \Sigma y + y_{new} - y_{old}$$

$$\Sigma y^{2\prime} = \Sigma y^2 + y_{new}^2 - y_{old}^2$$

The sum, Σey, must be calculated in full at each position along the data trace.

APPENDIX 3: THE SYNAPTIC EVENT DETECTION SOFTWARE PACKAGE AND SUPPORTED DATA FILE FORMATS

The software package for simulation, detection, and analysis of spontaneous synaptic events was developed on a Macintosh computer using AxoGraph 3.5 (Axon Instruments). The event detection package is distributed with the latest version of AxoGraph (v3.5.5). (A demonstration version of AxoGraph, including the event detection programs, can be obtained at ⟨http://www.axonet.com/pgraph3.htm⟩.) AxoGraph incorporates a multi-language development environment. The simulation and analysis programs were written in C, using only the simplest features of the language. They make extensive use of built-in AxoGraph commands for implementing the graphical user interface and displaying the results, but the core code for sliding the template along the data trace and computing the SCALE, OFFSET, and DETECTION parameters is written in standard C and could be ported to another development environment.

The import of many binary digitized data file formats is supported, including pClamp, AxoTape, AxoData, AxoScope, MacLab, Chart, CED, and Igor binary files. In addition, any binary digitized file with data organized as a continuous block of 16-bit integers, recorded on a PC or Macintosh computer, can be read in via an import dialog. Other digitized data file formats could be handled via a custom import program.

The AxoGraph event detection package consists of three programs. The first implements the scaled template detection algorithm and can be applied to continuous or episodic data. The amplitudes, rise times, and other parameters describing each event are calculated and plotted against event number in a summary results window. Detected events are optionally captured, aligned at onset, and saved in a new file. The second program in the package simulates electrophysiological noise and spontaneous synaptic events. The third program can be used to eliminate corrupt events that have been manually identified in the captured events.

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