

TAC

Single-channel analysis of patch clamp recordings.

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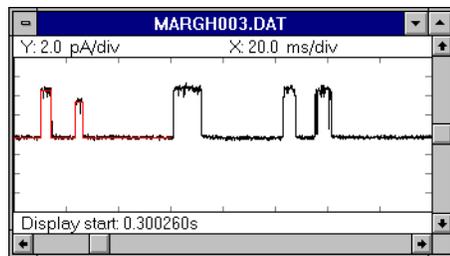
Overview

The TAC package performs single-channel analysis of patch clamp recordings. The software uses threshold event detection to idealize your recorded data. The event detection algorithm is robust and can process poorly resolved events. A built-in digital filter allows you to improve the signal-to-noise ratio of your recordings. A powerful leak subtraction facility allows you to remove stimulation artifacts.

For quick analysis without event detection, you can directly fit acquired data histograms.

TAC allows you to analyze event tables created by TAC. You can create and fit duration and amplitude histograms. All fits use stable maximum-likelihood methods. The package also provides scatter plots and stationarity plots. A powerful event filtering facility is provided. For example, you can select only short openings that follow long closings.

The software provides extensive graphics and data export to simplify presentation, publication, and further analysis of your data.



Description

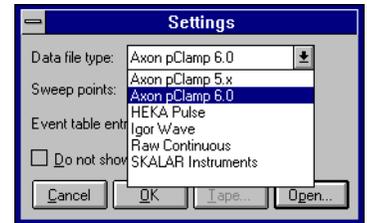
What it does

The TAC package performs two functions: event detection (idealization), and event analysis. Event detection builds a table of channel state transitions, including transition amplitude and transition time. Event analysis builds duration (dwell time) histograms and amplitude histograms, and fits these histograms to theoretical curves.

TAC also provides raw data (all-points) histograms, amplitude/duration scatter plots, stationarity plots (P_o), and other useful graphs.

During event analysis, events can be filtered by duration, amplitude, and preceding and following events. For example, only those events might be selected for analysis that are channel openings following long closed intervals.

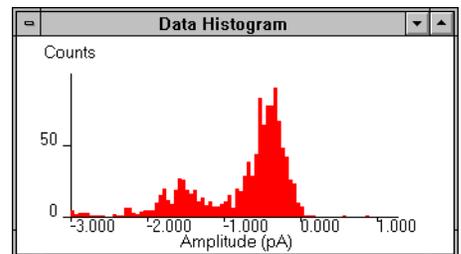
TAC is compatible with most popular electrophysiological data acquisition packages. TAC exports data and graphics in formats that are easily accessible to other programs.



How it works

The TAC package consists of two programs: TAC, which performs event detection, and TACFit, which performs event analysis.

To analyze a data file, first open the file in TAC. TAC detects events as threshold transitions. Set the threshold to use, and begin event



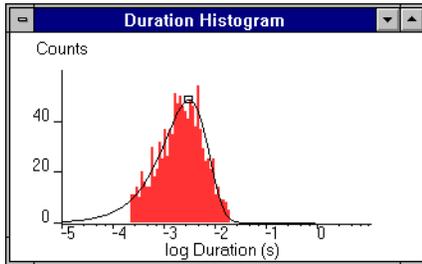
detection. For many data files, you can have TAC set the threshold and baseline automatically from the data (all points) histogram.

TAC provides both automatic and manually-assisted event detection. The threshold-detection technique requires that channel events be easily distinguished from recording noise. TAC provides

a built-in digital gaussian filter to improve the signal-to-noise ratio on your recordings. This allows you to record data with a wide bandwidth, then reduce the bandwidth during analysis to minimize the noise.

Files acquired with Clampex typically require leak subtraction before event detection. TAC allows you to create a leak template as an average of sweeps with no channel transitions. Segments of the leak template may be fit to a theoretical curve to reduce noise and to eliminate gaps due to missing data. Fits may be to a level, an exponential, or a ramp. This template is then automatically subtracted from each sweep.

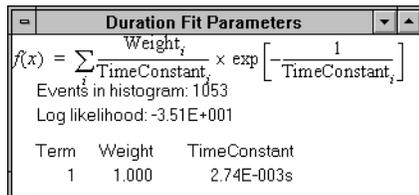
TACFit reads event tables generated by TAC. TACFit creates amplitude histograms, duration histograms, stationarity plots (P_o v.s. time), amplitude/duration



scatter plots, graphs of amplitude vs. independent variable, and graphs of duration vs. independent variable.

TACFit can fit a duration histogram to a theoretical curve described as a sum of exponential terms, and an amplitude histogram to a theoretical curve described as a sum of gaussian terms. To fit a graph to a theoretical curve, you select the number of components in the theoretical curve, and graphically adjust the starting values for the fit. TACFit then performs an automatic maximum likelihood fit.

TACFit displays a log likelihood value, so you can compare the quality of this fit with other fits.



You can compare fits with various numbers of terms, judging the improvement of adding a single term by the change in log likelihood.

When you process events in TACFit, you can select the event to analyze using powerful event filters. You can select events to process by level (e.g. open or closed), duration,

amplitude, or tag value supplied separately. You can also filter events by position in a sweep. For example, you can process only

first events in a sweep. TACFit also allows you to select events by the preceding or following event. For example, you can select only channel openings that follow long closed intervals. In addition, TACFit allows you to filter events by sweep.

Both TAC and TACFit provide an extensive set of data and graphic export facilities. Data can be exported as text in spreadsheet format or IGOR wave format. Data can also be exported in binary form, either as a raw binary file, or an IGOR binary wave. Graphics export is supported in metafile format (PICT on the Macintosh), Encapsulated PostScript, or Adobe Illustrator format.

Application Note: Tag Values

TAC supports a tag value associated with each event and sweep. You can use this tag value in your analysis.

For example, suppose you have a concentration time course. You can export the TAC event table, then merge the ion concentration information into the column for the tag value using a spreadsheet. Read the table into TACFit, and you can filter by tag value, or plot amplitude or duration against tag value.

Application Note: Sampling Rate

You may be undersampling your data.

If you have been using computer-based data acquisition for many years, you are probably used to older computers, software, and disk drives that cannot acquire and store large amounts of data quickly. Therefore you may be used to undersampling your data because of the constraints of your computer.

Newer computers are fast and have large disk drives. Newer software is efficient at processing large amounts of data quickly. Increase your recording bandwidth and your sampling rate. You can filter and decimate in your analysis programs if necessary.

For example, perhaps your older system was limited to sampling at 25kHz, so you set your amplifier for 5kHz cutoff and sampled at 25kHz, 5 times the highest frequency. You may obtain more information, and you will certainly obtain better time resolution, by increasing your amplifier cutoff to 10kHz and your sampling rate to 100kHz.

For single-channel data, if you need to decrease the bandwidth to increase the signal-to-noise ratio, you can set the digital filter in TAC.

Data you discard during acquisition by sampling too slowly can never be recovered.

Features

This section describes the features of TAC, in approximately the sequence they would be used for analyzing a recording. You perform event detection (idealization) first, then event analysis.

File Support

TAC reads common file formats, including Axon Instruments ABF, Bruxton Acquire, and HEKA Pulse. ABF is the file format used by pClamp 6 and later programs from Axon Instruments.

When you open a file, you can select the data to analyze. You may want to analyze only data acquired during a given segment or epoch. If so, you can specify this when you open the file. You can also specify that you want a value from the file, such as the stimulus amplitude, recorded as an independent variable for analysis.

HEKA Pulse:

Channel: Channel 0

Y Segment: Relevant 1

H Segment: Relevant 1

Relevant Value: None

Cancel OK

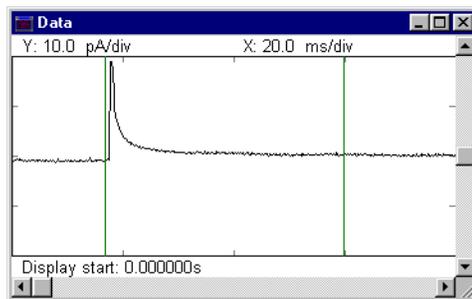
TAC reads several additional file formats useful for compatibility with user-written programs. For example, TAC reads raw files containing data as 16-bit integer or 32-bit IEEE 754 floating point values.

Data Preparation

For those working with voltage-gated channels, the first step to analyzing your data is to remove the passive current induced by the stimulus. If you use P/N subtraction in your data acquisition program, this has already been performed, at the expense of increased noise.

TAC provides a powerful leak subtraction facility that allows you to build a leak template representing the passive current, and subtract it from your acquired data. To eliminate the added noise, you can fit your leak template piecewise to a level, ramp, or exponential.

The next step is to filter your data for analysis. Threshold detection requires a good signal-to-noise ratio. Rather than achiev-



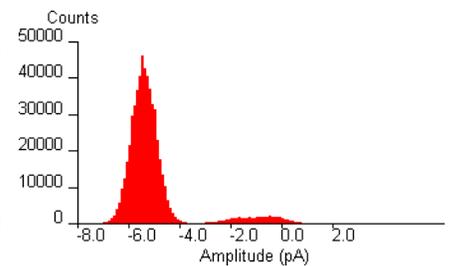
ing this by recording your data with limited bandwidth, you can use the low-pass filter in TAC to remove high-frequency noise.

The final step is to select data for analysis. If you have long continuous recordings, you are likely to want to analyze only specific sections of interest. TAC allows you to make this selection manually. For data files recorded with episodic stimulation, TAC also allows you to select a specific epoch or segment for analysis. Using this method, you can bypass leak subtraction, by analyzing only data following an exponential recovery from a stimulus step.

Notice that you do not have to perform a correction for baseline drift. The threshold detection algorithm used by TAC is insensitive to slow baseline drift.

Data Histogram

You can calculate a histogram on your data. Assuming negligible baseline drift over the selected data, the histogram shows the channel current in each distinguishable state.



TAC can fit your amplitude histogram to a sum of gaussian functions, providing you direct information about channel current and noise. You can have TAC automatically set event detection parameters from the amplitude histogram fit.

Event Detection

TAC performs event detection based on threshold transitions. You can perform automatic or semi-automatic event detection. For semi-automatic event detection, TAC presents you with each detected event. You can accept the event, reject it, or adjust it. For either automatic or semi-automatic event detection, you can review the detected transitions, and remove those caused by artifacts.

To use event detection, you must set the threshold and the risetime. TAC can set the threshold automatically from the fit to the data histogram. You can measure the risetime easily using the Data window.

The event detection algorithm in TAC can properly detect substate transitions, given a sufficient signal-to-noise ratio. However, TAC provides no analysis appropriate to substate transitions.

TACFit

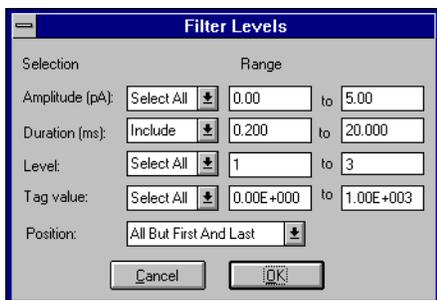
When you have completed event detection, you can read the resulting event tables into the TACFit program, provided as part of the TAC package. TACFit can process multiple event tables simul-

taneously. This allows you to analyze the results of a series of experiments at one time.

Event Filtering

You can select events to analyze from the event tables loaded into TACFit.

You can filter events by characteristic. For example, you can select only open intervals or only closed intervals. You can filter events by duration, amplitude, level number (e.g. open or closed), and tag value. The tag value can be supplied by another program. You can also filter events by position within a sweep. The duration of a closed interval at the beginning of a sweep provides the first latency.



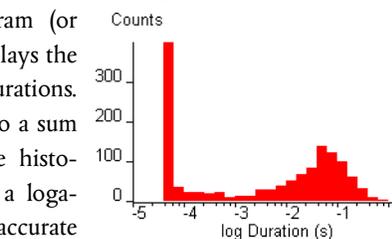
You can filter events by characteristic of the preceding or following event. For example, you can accept only openings that follow closed intervals at the beginning of a sweep. You can also filter events by characteristic of the sweep. For example, you can accept only events in sweeps with a relevant value (stimulus value) of 50mV or less.

TACFit allows you to filter bursts, collapsing them to single events for the purpose of analysis.

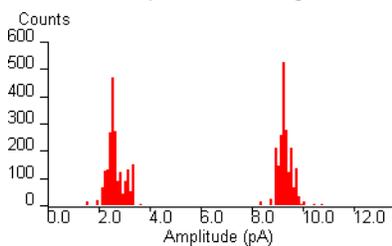
Graphs

TACFit provides a variety of analysis graphs.

The duration histogram (or dwell-time histogram) displays the distribution of event durations. The histogram can be fit to a sum of exponential terms. The histogram is constructed with a logarithmic x axis to provide accurate results, even when the exponentials represent time constants that differ by orders of magnitude.



The amplitude histogram displays the distribution of event amplitudes. The histogram can be fit to a sum of gaussian terms.



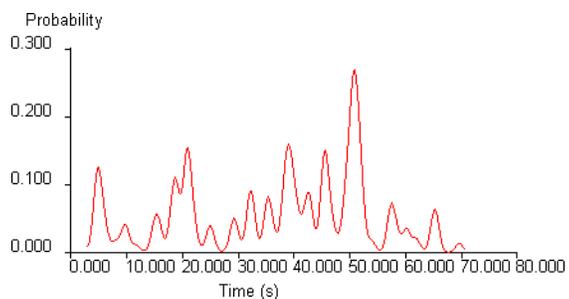
All fitting is maximum-likelihood. TACFit

displays the log likelihood result, so you can determine the relationship between the number of terms in the fit and the log likelihood. Adding terms to the fit should always increase the log likelihood, but the amount of increase may be insufficient to justify the additional term.

Histograms provide both linear and square-root y axis scaling. Square-root y axis scaling allows you to easily judge the quality of the fit of a theoretical curve to a histogram. With square root scaling, the vertical displacement between a histogram and a theoretical curve represents the number of standard deviations of difference, independent of the value in the histogram bin.

TACFit also provides scatter plots of duration or amplitude against independent variable, or amplitude against duration.

For analysis of long-term trends, TACFit provides the stationarity plot. The stationarity plot measures the average value of a parameter over a sweep.



Graphics Export

The TAC package allows you to export all graphs for further analysis, presentation, or publication.

For further analysis, TAC provides text export of graphs in spreadsheet or IGOR Pro format.

For presentation, TAC provides quick graphical export to the clipboard, or as a file in metafile format (Windows) or PICT format (Macintosh). Such export is quick and convenient, but the graphs have limited resolution, so they appear 'jagged' when enlarged.

For publication, TAC provides graphical export in Adobe Illustrator or encapsulated Postscript format. Adobe Illustrator is an infinite-resolution, editable format that can be read by most professional illustration programs. In the exported file, graphical objects are properly segmented. This allows you to easily select items (such as labels) and remove them. Encapsulated Postscript is an infinite resolution format that can be edited by only a few programs.

For graphics windows, TAC allows you to specify the color of most items for convenient viewing. You can also control the display of items such as axes and labels.

Specifications

Systems Microsoft Windows NT, Windows 95, Apple Macintosh system 7 or later. A math coprocessor is required.

Data file formats Axon Instruments pClamp 5.x, Axon Instruments ABF, Bruxton Acquire, HEKA Pulse, WaveMetrics IGOR Pro binary wave, raw continuous

Data export formats Spreadsheet, WaveMetrics IGOR Pro wave.

Graphics export formats Adobe Illustrator, Encapsulated Post-Script (with PICT header on the Macintosh, no header under Windows), metafile (under Windows), and PICT (on the Macintosh).

Event detection User-selectable threshold with transition time determined as 50% point. Gaussian digital filter to improve signal-to-noise ratio. Automatic and manually-assisted mode.

Fits Maximum likelihood fitting for duration histogram (sum of exponentials) amplitude histogram (sum of gaussians), and raw-data (all-points) histogram (sum of gaussians).

Graphs TAC provides the following graphs:

Graphs

Graph	Notes
data	Includes idealized trace and leak template
data histogram	Fit to a sum of gaussians
duration histogram	Fit to a sum of exponentials
amplitude histogram	Fit to a sum of gaussians
scatter plot	amplitude vs. duration
amplitude	v.s independent variable
duration	v.s. independent variable
stationarity	P_o vs. t, amplitude vs. t, duration vs. t

Notes

Automatic event detection must be manually reviewed for reliable results.

The amount of memory required depends on the data file being read and the number of detected events. Generally 8MB to 16MB available memory is required.

Ordering

When ordering TAC, specify either TAC for Windows or TAC for Macintosh. You will receive the TAC software on CD, a hardware dongle, and a printed Quick Start guide. The manual is included on the distribution in Adobe Acrobat format.

Dongle

The dongle must be attached to your computer in order to run the software. The Windows dongle attaches to the parallel port of your computer. The Macintosh dongle attaches to your ADB bus, that is, to the cable attached to your keyboard.

Disclaimers

This datasheet applies to TAC V3.0.

All specifications subject to change without notice. Not responsible for errors and omissions.