

# Single-Channel Analysis: An Introduction

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This is an informal introduction to analysis of patch-clamp recordings from single ion channels. This document provides you with an intuitive understanding of the TAC single-channel analysis program. For more rigorous introductions to the subject, see the bibliography.

## Overview

The principles behind single-channel analysis are simple. Single-channel analysis is complex, because of various factors inherent in the underlying biology, the patch-clamp technique, and current recording technology.

This introduction describes the principles of single-channel analysis, then describes some of the complications that occur in practice.

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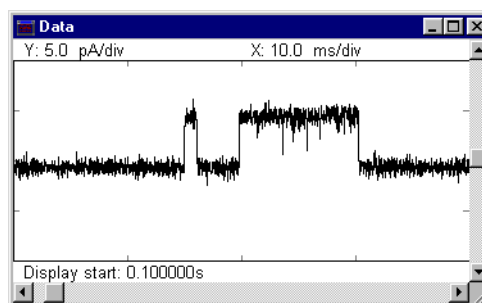
The introduction is designed to provide you with an understanding of the problems solved by the TAC single-channel analysis program.

## Background

Patch-clamp recordings of single-channel activity are measurements of the current flowing through an ion channel over time. These recordings are distinctive, because they generally show sudden transitions between two well-defined levels of current flow.

The two levels of current flow are normally interpreted as representing closed and open states of an ion channel. The transitions appear to be sudden, because they occur far

more quickly than can be resolved by the recording system.



**Figure 1** *Single-channel data. Data courtesy of Dr. Margherita Milhone, laboratory of Dr. Andrew Engel, Mayo Foundation.*

Analysis of single-channel patch clamp recordings would be uninteresting if a channel simply opened in response to a stimulus and closed in its absence. However, ion channels fluctuate between open and closed states. Stimuli affect the transition rates of these fluctuations. Analysis of openings and closings allows inferences to be made about the ion channel and its biological activity.

## Analysis

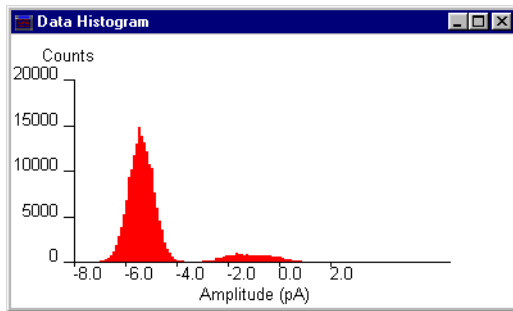
Several strategies are applied to analyze single-channel recordings. The following sections describes them, in increasing order of complexity.

### Analysis: Po

The investigator is often content to know only the fraction of time that an ion channel is open during a recording. This is represented symbolically as  $P_o$ , the probability that a channel is open.

$P_o$  can be inferred from a histogram of an ion channel

recording as the fraction of time the recorded current represents an open channel.



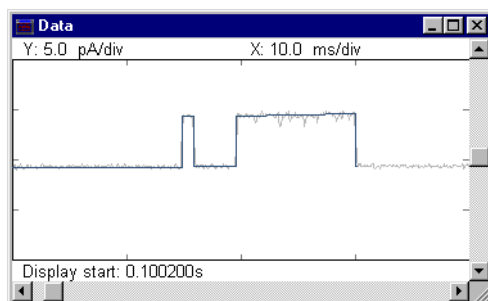
**Figure 2** Raw data ('all-points') histogram from a single-channel recording.  $P_o$  can be calculated as the fraction of data samples that fall into the open-channel current distribution.

Direct  $P_o$  measurement provides limited information, since it ignores channel kinetics, however, it is extremely useful as a quick indicator of channel activity.

The value of  $P_o$  has a biological interpretation. For cells that demonstrate an action potential, the action potential is the sum of the single-channel current through many ion channels. That is, the action potential is proportional to  $P_o(t)$ , measured over a large number of channels.

### Analysis: Idealization

Although ion channel state transitions are sudden, the dwell time of an ion channel at a given conductance level is frequently long enough to be observed with common recording techniques. The investigator can identify channel transitions (openings and closings) in the recorded data. The result is an 'event table' describing each transition of an idealized data trace. This process is also called 'event detection'.



**Figure 3** Idealization of the data shown in Figure 1.

Idealization of a single-channel recording allows the investigator to infer kinetic parameters of the ion channel. For example, the analysis program can fit the distribution

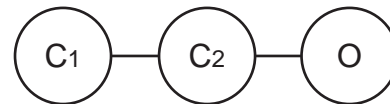
of channel open or closed times to a sum of exponential terms. The time constant of each exponential is related to the rate of kinetic state transitions of the ion channel.

Idealization is most commonly performed using threshold detection. Events are identified as transitions if the transition amplitude exceeds a threshold. A simplification that some programs employ is to identify transitions based on the absolute current flow.

### Analysis: Kinetic Modeling

New techniques have become available that allow a kinetic model to be fit to patch clamp recordings of single ion channels. This analysis is interesting, not only in understanding the structure of ion channels, but also in understanding how ion channel behavior changes based on the electrical or chemical environment.

For this type of analysis, the investigator builds a kinetic model of ion channel behavior, showing states and transitions. A computer program then fits this model to recorded data, adjusting the model parameters to provide the best fit to the data.



**Figure 4** Simple three-state kinetic model

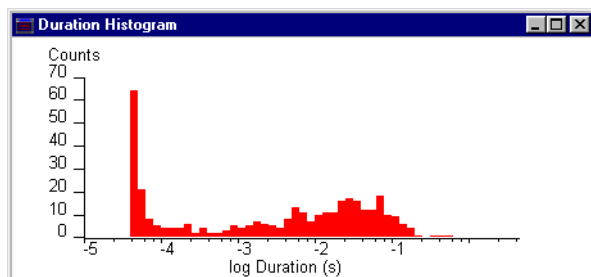
### Complications: Biology

The behavior of ion channels themselves complicates single-channel analysis.

#### Complication: Indistinguishable States

If an ion channel has a single open state and a single closed state, the distribution of open time durations fits a single exponential, as does the distribution of closed time durations. In many cases, multiple exponential terms are needed to properly fit these distributions, suggesting that

channels have multiple open and multiple closed states.



**Figure 5** *Closed-time duration histogram showing a distribution that implies multiple indistinguishable states.*

The patch-clamp recording technique observes the current flow through an ion channel, and is capable of resolving open and closed states of a channel. However, it is not capable of directly resolving distinct open or closed states. For example, if a channel makes a transition from one closed state to another, this will not be directly observable from the recorded data.

#### Indistinguishable States: Idealization

When idealization is performed, kinetic information is extracted by fitting the distribution of dwell times. Given a simple two-state model of the kinetics of an ion channel, the distribution of open times should fit a single exponential.

Given a more complex model with multiple open and closed states, the distribution of dwell times will fit a sum of exponential terms. Because distinct open and closed states can be distinguished only by their dwell time distribution, accurate fitting of multiple exponential terms is required. These terms might have time constants that differ by orders of magnitude.

To accurately represent a wide distribution of durations, TAC constructs duration histograms using logarithmic bin widths on the time axis. For example, one bin might represent durations from 1.0 to 1.2 milliseconds, while another might represent durations from 10 to 12 milliseconds.

To accurately fit histograms with sums of exponential terms, TAC employs a maximum-likelihood fit rather than the more common least-squares fit.

For a more complete discussion of fitting, see [Colquhoun and Sigworth, 1995], P. 543.

#### Indistinguishable States: Kinetic Modeling

Without hidden states, kinetic modeling would be sim-

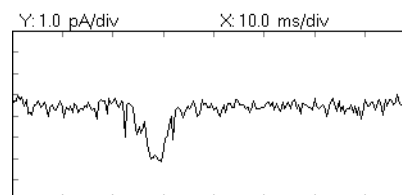
ple. The state of an ion channel would be directly observable from the recorded current trace, and transition rates could be determined from the dwell time distribution in each state. However, since the state of an ion channel is hidden from the investigator, more complex techniques must be employed.

A technique that has generated considerable interest is to view an ion channel as fitting a Hidden Markov Model, or HMM. Ion channel kinetics fit a Markov model, since the channel makes essentially instantaneous transitions between a small set of states. The Markov model is hidden, since the kinetic state of an ion channel cannot be observed directly.

Eventually, kinetic modeling using a Hidden Markov Model may become the analysis method of choice for many single-channel recordings.

#### Complication: Sublevels

The rest of this discussion deals with ion channels that display two current levels, corresponding to a channel being open and closed. However, recordings sometimes display several open channel current levels from a single ion channel.



**Figure 6** *Downward opening with sublevel transition.*

Typically these sublevels or substates appear during a the transition of a channel. For example, a channel might open first to a sublevel, then to full amplitude.

#### Sublevels: Idealization

Given a sufficient signal-to-noise ratio, TAC can properly detect sublevel transitions using threshold detection. To do this, fix the event detection threshold in TAC low enough to catch the sublevel transitions. TAC cannot properly fit the resulting duration histograms.

#### Sublevels: Kinetic Modeling

Kinetic modeling can deal with substates directly. In some sense substates assist in direct kinetic modeling, since a substate represents one or more open states that are distinguished from full open states, providing more informa-

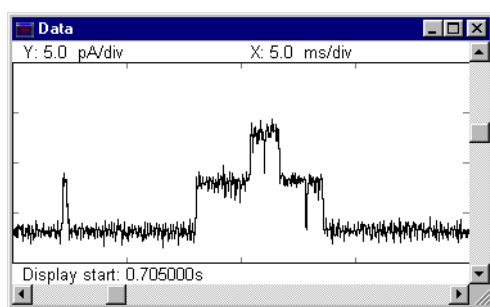
tion to the algorithm that fits the model.

## Complications: Technique

This section discusses complications that arise from the patch-clamp technique itself.

### Complication: Multiple Channels

Patches of cell membrane do not always conveniently contain a single ion channel. If more than one ion channel is active in a patch from which a recording is made, the resulting data may be difficult to analyze.



**Figure 7** Patch-clamp recording showing multiple channels. Data courtesy of Dr. Margherita Milhore, laboratory of Dr. Andrew Engel, Mayo Foundation.

### Multiple Channels: Idealization

TAC can use threshold detection to find transitions in data from a patch containing multiple active ion channels, but it cannot properly analyze the resulting event list. When a channel opens or closes TAC is unable to determine to which channel the transition should be assigned.

A traditional solution to this problem is to assign the closing randomly to one of the channels. This avoids distorting channel statistics.

Meyer Jackson has worked out a more sophisticated technique that can be used for analysis of an event list resulting from a patch containing multiple channels [Jackson, 1985].

### Multiple Channels: Kinetic Modeling

Kinetic modeling can in principle handle recordings from patches containing multiple active channels. A simple method is to build a combined model with all channels. Unfortunately, this leads to an explosion in the number of states. Creation of the models is complex if the ion channels are distinct. In practice, it appears that kinetic model-

ing may be useful when analyzing recordings from patches containing two identical channels, if the kinetic model for each channel is kept relatively simple.

### Complication: Baseline Drift

The baseline current level, that is, the current level corresponding to a closed channel, may drift slowly over the course of an experiment.

#### Baseline drift: $P_o$

Baseline drift must be taken into account, if the baseline drift amplitude is significant in comparison to the open channel current.

#### Baseline drift: Idealization

TAC uses a threshold-detection technique that is insensitive to slow baseline variations. It measures the absolute current level prior to and following each transition. The difference between the two levels is the amplitude of the transition. The absolute current level following the transition is used as the starting point for detecting subsequent transitions. This technique does not depend on baseline stability over long periods.

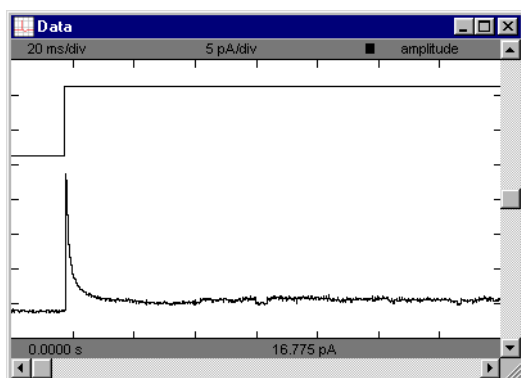
#### Baseline drift: Kinetic modeling

Baseline drift must be taken into account, otherwise the model may not be able to fit the data.

### Complication: Passive Currents

A common patch-clamp recording technique for voltage-gated channels is to stimulate the patch using a voltage pulse, and measure the ion channel current that flows as a result. However, the stimulus itself induces a passive current in the patch. This passive current charges membrane capacitance, and has a characteristic exponential time course. The current can easily exceed the magnitude of the ion channel

currents by two orders of magnitude.



**Figure 8** *Stimulus pulse and associated passive current. Data courtesy Dr. Jean-Pierre Benitah, laboratory of Dr. Christèle Horeau, INRA, Montpellier.*

### Passive current: Recording

The passive current can be eliminated during recording using techniques such as P/N subtraction. The recording program issues one or more attenuated stimulus pulses, and measures the response. If the stimulus is sufficiently attenuated, it will not cause the ion channel to open. The response is then subtracted from the actual recording.

P/N subtraction is convenient and automatic, but it increases the recording noise, and requires additional recording time.

### Passive current: analysis

The passive current can be eliminated during recording by constructing an 'idealized' passive response, then subtracting it.

The standard technique for eliminating the passive response is to average portions of response currents that show no single-channel activity. The average can then be subtracted from each sweep. This method has the disadvantage that it increases the noise in the resulting traces.

The noise in the passive current trace can be eliminated by fitting the passive response piecewise to appropriate functions (e.g. exponential, ramp).

Forming an 'idealized' passive response is complicated by the variation in stimulus amplitude. Typically a sequence of stimuli are applied, with varying amplitude. The passive current varies linearly with the amplitude of the stimulus. Therefore, when the 'idealized' passive current is subtracted from each trace, the 'idealized' trace must be scaled to correct for the stimulus amplitude.

## Complications: Recording

The limitations of current recording technology cause complications in data analysis.

### Complication: Recording Bandwidth

Ion channel openings and closings are transitions made by large molecules. These transitions occur in far less time than can be resolved by the patch-clamp recording apparatus. Channel openings and closings are recorded as having the risetime of the measuring apparatus.

### Recording bandwidth: Idealization

The transition risetime introduces a delay into the measurement of each transition. For accurate interval measurements it is important that the delay be the same for channel openings and closings.

TAC always calculates the 50% point of each transition, and uses this as the time of the transition. This 50% time is not necessarily the same as the threshold. For example, suppose the threshold is set at 2pA and a 6pA transition is encountered. TAC will determine that the 50% point is 3pA, and will calculate the time at which the transition crossed 3pA.

The 50% point is chosen because it results in the same delay for both openings and closings. This eliminates error due to bandwidth limitations in the measuring apparatus.

### Complication: Missed Events

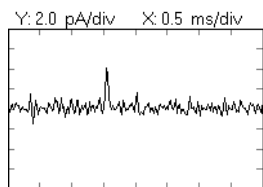
The bandwidth limitations of the recording apparatus can cause events to be missed or their timecourse to be distorted.

### Missed events: Idealization

A preceding section explained how bandwidth limitations do not affect data analysis for idealization. However, this is not true for short intervals. If an ion channel makes two transitions within less than a few risetimes of the measuring apparatus, the two transitions will blend in the recorded data. Short openings and short closings can be distorted.

If the transitions are spaced far enough apart to be resolved, but still not far enough to reach full amplitude, the duration of the resulting transitions will be reduced. If the transitions are closely enough spaced, they will appear as noise rather than as a pair of transitions. They will be

“missed transitions”.



**Figure 9** Short event is poorly resolved. If the interval between opening and closing were much shorter, the event would appear to be merely noise.

These effects are discussed in [Colquhoun and Sigworth, 1995], P. 500.

If the processing of short openings and closings can be predicted analytically, it is possible to correct for missed events. It is also possible to correct the duration of events that do not reach full amplitude.

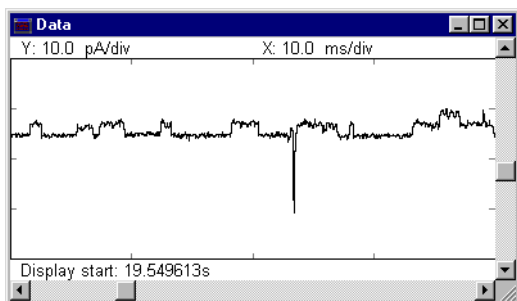
The threshold-detection technique used by TAC is inherently amenable to analysis. Therefore missed event corrections can be applied, although TAC itself does not perform missed event corrections to the duration histograms. TAC does include a correction to the duration of short events.

#### Missed events: Kinetic modeling

Kinetic modeling can inherently take the recording bandwidth into account when predicting the likelihood that a transition is observed.

#### Complication: Artifacts

The patch-clamp technique measures very small electrical currents, and is susceptible to artifacts during recording.



**Figure 10** Artifact during recording. Data courtesy of Libby Sunderman, laboratory of Dr. William Zagotta, University of Washington.

Ultimately, there is no replacement for manual inspec-

tion of data and especially of results. See [Magleby, 1992], P. 768 for a discussion of the effects of artifacts.

#### Artifacts: Idealization

Artifacts detected as transitions must be removed manually from the event table. This requires visual inspection of the data. TAC allows the user to review the detected transitions, removing those that are the result of artifacts.

#### Artifacts: Kinetic Modeling

For kinetic modeling, the best solution is to select artifact-free data for analysis. This means that data to be analyzed must be first reviewed manually.

TAC allows the user to review a recording, selecting only specific data for analysis.

#### Complication: Noise

The threshold detection technique requires a good signal-to-noise ratio. Patch-clamp recordings can contain substantial background noise. The amplitude of the noise increases with frequency, so the noise is generally reduced using a low-pass filter. See [Colquhoun and Sigworth, 1995], P. 484 for a discussion of filtering.

TAC provides a low-pass Gaussian digital filter that can be applied to the recorded data during event detection. Applying such a filter in TAC is useful, because it allows data to be recorded with as wide a bandwidth as desired, without considering the signal-to-noise requirements of the event detection. The signal-to-noise ratio can then be improved during single-channel analysis.

#### Complication: Sampling

Periodic sampling reduces a continuous analog signal to a sequence of measurements taken at fixed intervals. If a transition occurs in the sampled data, it may cross the 50% point between two samples. This can lead to errors in the calculated duration of an opening or closing. These errors cause a “binning” of data in duration histograms with an interval equal to the sample interval.

TAC reduces this error substantially by calculating the time of crossing of the 50% point, interpolating between the two nearest sampled data points. This interpolation extends the time resolution of the duration histogram, and allows better fits of theoretical curves to the measured data.

## Bibliography

*Enzymology, Volume 207, Ion Channels*, B. Rudy and L. E. Iverson, eds., Pp. 763-791.

### Books

The field of single-channel recording is blessed with several excellent volumes describing the technique and its application. Useful references for the technique are: *Single-Channel Recording*, Second Edition, Bert Sakmann and Erwin Neher, eds., 1995, and *Methods in Enzymology, Volume 207, Ion Channels*, B. Rudy and L. E. Iverson, eds., 1992.

For a discussion of ion channels themselves, see *Ionic Channels of Excitable Membranes*, Second Edition, by Bertil Hille, 1992.

### Web Pages

Dr. Joe Patlak of the University of Vermont has developed a single-channel analysis technique called 'Mean-Variance Analysis', not covered in this introduction. You can obtain a program that implements the technique at his Web site, <http://salus.med.uvm.edu/~patlak/patlak.html>

Drs. Qin, Auerbach, and Sachs of SUNY Buffalo have developed a single-channel analysis program based on a Hidden Markov Model. This program is available on their Web site, <http://www.qub.buffalo.edu/>.

### References

[Colquhoun and Hawkes, 1995] David Colquhoun and Alan G. Hawkes, The Principles of the Stochastic Interpretation of Ion-Channel Mechanisms in *Single-Channel Recording*, Second Edition, Bert Sakmann and Erwin Neher, eds., Pp. 397-482.

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