Measurement and Analysis of Postsynaptic Potentials Using a Novel Voltage-Deconvolution Method

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Richardson MJ, Silberberg G. Measurement and analysis of postsynaptic potentials using a novel voltage-deconvolution method. J Neurophysiol 99: 1020–1031, 2008. First published November 28, 2007; doi:10.1152/jn.00942.2007. Accurate measurement of postsynaptic potential amplitudes is a central requirement for the quantification of synaptic strength, dynamics of short-term and long-term plasticity, and vesicle-release statistics. However, the intracellular voltage is a filtered version of the underlying synaptic signal and so a method of accounting for the distortion caused by overlapping postsynaptic potentials must be used. Here a voltage-deconvolution technique is demonstrated that defilters the entire voltage trace to reveal an underlying signal of well-separated synaptic events. These isolated events can be cropped out and reconvolved to yield a set of isolated postsynaptic potentials from which voltage amplitudes may be measured directly—greatly simplifying this common task. The method also has the significant advantage of providing a higher temporal resolution of the dynamics of the underlying synaptic signal. The versatility of the method is demonstrated by a variety of experimental examples, including excitatory and inhibitory connections to the neuron. Here it will be demonstrated that an elementary deconvolution technique is applicable to cases of high variability and to non-passive membrane dynamics such as the sag-rebound characteristic of the presence of the I$_h$ voltage-activated current. The approach also reveals the synaptic signal at considerably higher temporal detail, allowing for the resolution of apparently unitary PSPs into component release events.

Deconvolution methods have a long history in signal analysis and have been introduced into the neurosciences on a number of occasions: in the analysis of synaptic amplitude histograms (Jack et al. 1981; Wong and Redman 1980; for a review see Dityatev et al. 2003), in the analysis of postsynaptic currents measured in voltage-clamp mode (Dempster 1986; for a review see also Neher and Sakaba 2003) to the inference of the somatic current from the spike rate of neurons with adaptation (Ahmed et al. 1998), and in the analysis of fast changes in functional MRI data (Hinrichs et al. 2000).

The principal effect of deconvolution on a signal is to sharpen it in time, by reversing the smoothing effect of some biophysical filtering process. In the context of intracellular voltage traces, it is the combined capacitive and conductive effects of the cell membrane that filter the synaptic drive (Rall 1967).

Here we demonstrate the considerable advantage of using this simple technique to measure the amplitudes and dynamics of synaptic events. The method, illustrated using both basic neuron models and multicompartment reconstructions, will be applied to a broad variety of experimentally measured connections. Although a biological interpretation of voltage deconvolution can be found, the deconvolution approach is a basic application of linear filter theory and as such does not strictly require a biological interpretation for its successful application (resolving closely spaced events). However, it will be seen that the underlying deconvolved signal shares many features of voltage-clamp current measurements, such as synaptic events with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and γ-aminobutyric acid type A (GABA$_A$) kinetics clearly visible. The similarities and differences between the deconvolved voltage and voltage-clamp current will be examined in the DISCUSSION.

INTRODUCTION

The extraction of synaptic amplitudes and waveforms from intracellular voltage traces is a basic component of electrophysiological analysis. However, the measurement of postsynaptic potential (PSP) amplitudes is complicated by the intrinsic filtering properties of the membrane: PSPs that are separated by timescales of the order of the membrane time constant overlap, leading to a distortion of the ongoing synaptic events. This is a common scenario for the types of presynaptic firing patterns used to probe the timescales of synaptic dynamics (Abbott et al. 1997; Thomson and Deuchars 1994; Tsodyks and Markram 1997). Many different methods have been used to account for preceding pulses, such as the fitting of an exponential decay and subtraction of the preceding pulses or the fitting of templates of averaged PSP shapes. These methods do not reveal the dynamics of the underlying signal and can become prohibitively laborious for voltage traces with large numbers of overlapping PSPs.

Here it will be demonstrated that an elementary deconvolution method can be used to significantly reduce the filtering of the synaptic drive in intracellular voltage traces measured away from the synapse and can be conveniently applied to the entire voltage trace in one step. The aim of the method is not to obtain the full dendritic filter, but rather to provide a simple procedure for the analysis and quantification of closely spaced PSPs. The method is applicable to cases of high variability and to non-passive membrane dynamics such as the sag-rebound characteristic of the presence of the I$_h$ voltage-activated current. The approach also reveals the synaptic signal at considerably higher temporal detail, allowing for the resolution of apparently unitary PSPs into component release events.

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METHODS

Experiments

Synaptic connections were recorded between neurons in rat somatosensory cortical slices, by using simultaneous whole cell patch clamp mode or postsynaptic current (in voltage-clamp mode) on somatosensory cortical slices, by using simultaneous whole cell patch clamp mode (or postsynaptic current (in voltage-clamp mode) averaged over ≈30 repeated sweeps. The cell pairs presented here are composed of layer 5 pyramidal-to-pyramidal and Martinotti cell-to-pyramidal connections measured in current-clamp mode, and pyramidal-to-pyramidal and pyramidal-to-basket cell connections measured in both voltage-clamp and current-clamp modes. Further experimental details are provided in Silberberg et al. (2004).

EPSP pair model

For the model neuron receiving two closely spaced excitation postsynaptic potentials (EPSPs; used for Fig. 1) each EPSP $\mathcal{E}(t - t_0)$, with onset at time $t_0$, was modeled as a sum of three exponentials of amplitude $a_i$ and time constant $\tau_i$

$$\mathcal{E}(t - t_0) = \theta(t - t_0) \sum_{i=1}^{3} a_i \exp[-(t - t_0)/\tau_i]$$  

where $\theta(t - t_0)$ is the Heaviside, or step, function taking the value 0 for $t < t_0$ and 1, otherwise. The constants are $a = (0.636, -2.01, 1.34)$ mV and $\tau = (1, 3, 40)$ ms. The postsynaptic voltage trace is given by $V(t) = \mathcal{E}(t) + \mathcal{E}(t - \Delta)$, where $\Delta$ is the interval between the EPSP onsets in milliseconds.

Least-squares template-fit method

This method of extracting PSP amplitudes (Richardson et al. 2005a) provides a comparison (in Fig. 3D) for the deconvolution method. The voltage response $V(t)$ is matched, using a least-squares method, to a linear model with $n_p$ PSP templates $\mathcal{E}_p(t)$

$$V(t) = \sum_{i=1}^{n_p} b_i \mathcal{E}_p(t - t_i)$$  

For this method, each of the PSP templates is identical in form and built out of the difference of two exponentials

$$\mathcal{E}_p(t) = \exp(-t/\tau_a) - \exp(-t/\tau_d)$$

with the rise time constant $\tau_a$, decay time constant $\tau_d$, and amplitudes $b_1, b_2, b_3$, and so forth providing the free parameters of the fit. These parameters are varied until the difference between the fit voltage $V_{fit}$ and the true voltage is minimized, in the least-squares sense.

Pyramidal cell model

The reconstructed layer 5 pyramidal cell shown in Fig. 6 (rat somatosensory cortex, PN day 15; see also Silberberg and Markram 2007) consisted of 102 dendritic compartments and a soma. Its passive electrophysiological properties (capacitance $C_m = 1 \mu F \cdot cm^{-2}$, conductance $g_m = 1/400,000 S \cdot cm^{-2}$, giving $\tau_m = 40$ ms, resting voltage $E_m = -65$ mV, and axial resistance $R_s = 155 \Omega \cdot cm$) were simulated using the software package NEURON (http://neuron.duke.edu; M. Hines, Yale University, New Haven, CT) with each compartment consisting of 50 segments. Six excitatory alpha synapses (reversal $E_e = 0$ mV, $\tau_e = 1$ ms, $g_s = 0.0002 \mu S$) were placed on the dendritic structure at different distances from the soma. They were activated independently and both the somatic voltage in current-clamp mode and the current in voltage-clamp mode were measured in separate simulations. The somatic voltage clamp was implemented using the SEC1amp command with a target voltage of $-65$ mV and an access resistance $r_s = 0.1$ MΩ.

Deconvolution and reconversion

The temporal derivatives used in the passive-membrane deconvolution $x + x = f$ are defined at time step $k = t/dt$ for time $t$, where $dt$ is the time unit for each step, as $\tau(x_{k+1} - x_k)/dt + x_k = f_k$ to be consistent with the reconversion $x$ of a signal $f(t)$, which can be found through integration using the forward scheme $x_{k+1} = x_k + dt(f_k - x_k)/\tau$.

RESULTS

The effect of voltage deconvolution will first be illustrated by a simple model neuron receiving two closely spaced EPSPs. This model provides a basic motivation for the deconvolution method at a level of detail sufficient for its practical application (a more detailed cable-theory justification can be found in the APPENDIX). The method will then be demonstrated on a number of experimental examples that cover excitatory and inhibitory connections, as well as passive and nonpassive membrane responses.

Model: effect of deconvolution

An electrotonically compact model neuron is considered, with membrane properties characterized by a time constant $\tau$.
and resting potential $E_m$. The neuron receives a synaptic drive $I_{syn}$ and so the voltage $V(t)$ at time $t$ obeys the equation

$$\tau \frac{dV}{dt} = E_m - V + RI_{syn} \quad (4)$$

where $R$ is the input resistance. The voltage solution to this equation would take the form of the synaptic current exponentially filtered over a timescale $\tau$. In the context of experimental voltage recordings this filtering hinders experimental access to the fine temporal detail of synaptic events. However, a simple rearrangement of Eq. 4 yields

$$E_m + RI_{syn} = \tau \frac{dV}{dt} + V \quad (5)$$

The left-hand side of this equation contains the unfiltered synaptic current and is identical to the defiltering of the voltage or, equivalently, the voltage deconvolution

$$D(t) = E_m + RI_{syn} \quad (6)$$

This deconvolution can easily be extracted from intracellular voltage traces by using the right-hand side of Eq. 5. All that is required is knowledge of the filter constant; the measured voltage is simply differentiated, multiplied by $\tau$, and then added back to itself.

To interpret this process correctly, it is important to note that this defiltering is the removal of the principal filter (longest time constant) present in the recorded intracellular trace. It is not a measure of the full dendritic filter between the point of recording to the synapse itself. However, as will be seen, so long as this defiltering increases temporal resolution and can be reversed, it has a great deal of utility in the measurement of closely spaced PSPs. Further comment on the full dendritic filtering can be found in the Discussion and Appendix.

This process is modeled in Fig. 1 using a protocol in which two EPSPs of identical synaptic strength are separated by a time $\Delta$. The aim is to see when, in the voltage EPSPs or deconvolution pulses, the second event is discernible, and to measure its relative amplitude. In this modeled connection the EPSP rise time is 3 ms and the decay (or filter) constant is 40 ms. Superimposed voltage traces for this protocol are plotted in Fig. 1A for three pulse spacings: $\Delta = 5, 15,$ and 30 ms. For the 5-ms spacing the two EPSPs are not resolvable as separate events, but appear as a single EPSP with twice the amplitude. For longer delays the second EPSP is resolved, but its amplitude (plotted as a function of $\tau$) is underestimated because it rides on the decay of the preceding EPSP. It can also be seen in this panel that the threshold for resolving the EPSPs into two separate events is at $\Delta = 10$ ms. Figure 1B shows the deconvolutions, using Eq. 5, corresponding to these three voltage traces. The deconvolution pulses are sharper because they decay with the EPSP rise time of 3 ms, and so the deconvolved EPSPs with $\Delta = 5$ are already resolved into two separate pulses, the threshold for this resolution being $\Delta = 2$ ms. Thus closely spaced PSPs measured from the voltage trace can be accurately resolved only for spacings at a scale greater than the decay time of the EPSP—the membrane filter constant. However, the amplitudes measured from the deconvolved voltage are accurate at a much finer length scale—at a scale set by the rise time of the EPSP. Therefore the finer temporal resolution of the deconvolved traces allows for the composite structure of apparently unitary EPSPs to be easily distinguished (an experimental example of this is given later in Fig. 4).

**Experiment: a single EPSP**

To perform the voltage deconvolution it is necessary to know the filter constant $\tau$, which appears in Eq. 5. A direct approach would be to fit an exponential to the tail of an EPSP. Using the postonset period 20 to 100 ms (marked with dashed lines across Fig. 2A) this yields a decay time constant of $\tau = 40.7$ ms. However, for closely spaced EPSPs, this method is not always practicable. A second, variational method for finding $\tau$ will now be described.

**MEASURING THE FILTER CONSTANT.** A robust variational method, which can be easily extended to nonpassive voltage dynamics, may be derived from the fact that when a deconvolution is performed with the correct membrane filter constant the resultant trace $D(t)$ is flat away from the synaptic pulses (see Fig. 1). This is because in these intervening periods the neuron receives no synaptic input. In Fig. 2B two examples are given of a trial deconvolution $D_0(t)$ for which the chosen value of the filter constant $\tau_0$ is incorrect. It can be seen for this...
excitatory connection that when \( \tau \) is too small the deconvolution is above the baseline and if \( \tau \) is too large the deconvolution is below the baseline. This is exactly what the simple model in Eq. 4 predicts. If a deconvolution \( D_t(t) \) is calculated with a trial decay constant \( \tau \), it is straightforward to show that

\[
D_t = \frac{dV}{dt} + V = \frac{\tau}{\tau} D + \left( \frac{\tau - \tau_t}{\tau} \right) V
\]

Thus a trial deconvolution with an incorrect time constant

\[
\text{contains in the sense that only the voltage trace itself is required.}
\]

CROP AND RECONVOLUTION. The short deconvolved pulse seen in Fig. 2C decays quickly, with the rise constant of the original EPSP. It may be cropped out of the trace, in this case 5 ms before and 15 ms after the pulse onset, by replacing the noisy baseline outside this region with its average value, and then reconvolved using the integral solution for \( V \) of Eq. 4

\[
V_c(t) = \int_0^t \frac{dx}{\tau} e^{-\frac{t-x}{\tau}} D_c(s)
\]

where \( D_c \) is the cropped deconvolution. The algorithm for calculating this integral from data is provided in METHODS. This reconvolved voltage is compared with the true voltage in Fig. 2D and seen to be in close agreement, demonstrating that almost all the information required to reconstruct the EPSP is contained in the decay constant and the underlying deconvolved pulse. This deconvolution–reconvolution exercise is unremarkable for a single EPSP, but as will now be shown, it can be used to isolate closely spaced EPSPs.

Experiment: separating trains of PSPs

The deconvolution–crop–reconvolution method is now applied to a typical experimental paradigm used for measuring synaptic dynamics: an averaged voltage trace consisting of eight EPSPs separated by 50 ms (Fig. 3A). The same pair of cells from Fig. 2 was used so the filter constant is again 40 ms (Fig. 3A, inset). However, this quantity could equally well be found from a flatness criterion, where for this case regions around all of the pulses would need to be masked out.

The voltage response and its deconvolution are plotted in Fig. 3A. It can be seen that the deconvolution \( D(t) \) is resolved into a well-spaced train of pulses, where the flat regions between each pulse signify that the filter constant was correctly estimated and, furthermore, that the assumption of a linear summation of PSPs is a good one, despite the large amplitude of this connection. In Fig. 3B the deconvolved pulses are shown in detail. Their superposition (Fig. 3B, inset) demonstrates that they retain the same shape despite the vesicle rundown in this synapse, which exhibits synaptic depression (Abbott et al. 1997; Tsodyks and Markram 1997). The relative baseline-to-peak amplitudes are plotted in Fig. 3D. It can be further noted that, although some residual filtering from the dendrites will still be present, the decay constants (2 ms) of the deconvolution pulses in Fig. 3B are consistent with that of AMPA kinetics.

The amplitudes of the separated EPSPs can be obtained by cropping and reconvolving the deconvolved pulses. The intermediate cropped traces are plotted in Fig. 3C. These can be reconvolved to yield the eight isolated EPSPs plotted in Fig. 3C (bottom set of green curves) and from which the absolute EPSP amplitudes can be easily read off (plotted in Fig. 3D). As a “checksum,” the isolated EPSPs can be summed together and compared with the original voltage waveform. This comparison is also plotted in Fig. 3C above the isolated EPSPs where it can be seen that the agreement is such that it is difficult to discern the two traces. This checksum is an important step that provides verification of the method. If the resummed PSPs are
significantly different from the original voltage trace, it signals that there are membrane filtering effects present that are not captured correctly by the passive filter model given in Eq. 4. This might be due to the activation of voltage-gated currents. For such cases a more complex model must be used; this will subsequently introduced in Nonpassive membranes.

In Fig. 3D a least-squares fit method (see METHODS) is compared with the measurement of the amplitudes (relative to the initial EPSP) from the deconvolved pulses and the reconvolved EPSPs. It can be seen that the three methods give closely similar results. However, care should be taken for cases where the successive deconvolved pulses have different shapes (this was not the case here, as shown in Fig. 3B, inset). If this is the case, then the pulses should be reconvolved and it is the amplitude of the reconvolved PSPs that must be used.

Experiment: fluctuating voltage traces

The deconvolution method can also be used to analyze traces with higher variability, such as a recording of spontaneous activity in vivo or a single sweep instead of the averaged EPSP trains that were used in Fig. 3. In Fig. 4 such a sweep is presented. Although the voltage is strongly fluctuating, the deconvolution procedure again produces a train of well-separated pulses. Its higher resolution allows fine detail, such as the double event in the second pulse, to be clearly resolved. This resolution of two closely spaced EPSPs (with separation $\Delta \approx 5$ ms) provides an experimental example of the separation effect of deconvolution that was modeled in Fig. 1—a feature that has obvious application to the resolution of synaptic timing events. This resolution of apparently unitary events is analogous to that used for vesicle release-rate analysis in voltage-clamp current traces (Dempster 1986; Neher and Sakaba 2003), and so has the potential to facilitate greatly the measurement of vesicle-release statistics from voltage traces.

Nonpassive membranes

Many neurons show the effects of subthreshold voltage-gated channels, such as the h-current sag/rebound, in their response to synaptic input or current injection. The passive filter model of Eqs. 4 and 5 is not general enough to capture the response properties of neurons that show the effects of activated voltage-gated currents. However, the model can be extended easily by considering a multivariable linear model of the voltage dynamics (Brunel et al. 2003; Cox and Griffith 2001; Hodgkin and Huxley 1952; Koch 1984; Koch and Segev 1998; Mauro et al. 1970; Richardson et al. 2003; Rinzel and Ermentrout 1989; Surkis et al. 1998) to deconvolve the voltage correctly in the presence of voltage-gated currents.

MODELING NONPASSIVE MEMBRANES. Here the passive model is generalized to two variables, consisting of the voltage $v$ (mea...
sured from the baseline \( v = V - E_m \) and a second variable \( w \).
This variable affects the voltage with a strength \( \gamma \), proportional
to the excess current flowing through the voltage-gated channels,
and itself follows the voltage with a time constant \( \tau_w \). The
two equations describing the voltage \( v \) and membrane-current
variable \( w \) can be written

\[
\tau_v \frac{dv}{dt} = -v - \gamma w + D \tag{10}
\]

\[
\tau_w \frac{dw}{dt} = v - w \tag{11}
\]

where \( D(t) \) would again be proportional to the synaptic drive
for a compact cell. The voltage-gated current variable \( w \) is
hidden from direct experimental view; its behavior, governed
by \( \gamma \) and \( \tau_w \), is inferred from the effect it has on the voltage.
Its explicit appearance in the two equations can be made implicit
by integrating Eq. 11 between 0 and \( t \) [with the assumption that
the neuron is at its resting voltage \( v(0) = w(0) = 0 \) at \( t = 0 \)]
to yield \( w \) in terms of \( v \) as

\[
w(t) = \int_0^t \frac{dx}{\tau_w} e^{-(t-s)/\tau_w} v(s) \tag{12}
\]

which can then be inserted into Eq. 10 to yield an equation for
the voltage only. It is straightforward to rearrange this to give
the deconvolved voltage \( D(t) \) (analogous to Eqs. 5 and 6) at
time \( t \)

\[
D(t) = \tau_v \frac{dv}{dt} + v + \gamma \int_0^t \frac{dx}{\tau_w} e^{-(t-s)/\tau_w} v(s) \tag{13}
\]

This is the two-variable extension of the passive deconvolution.
The first two terms on the right-hand side of this equation are
identical to the passive form (Eq. 5) with time constant \( \tau_v \),
but the equation also comprises an additional term that accounts
for the activation of the voltage-gated currents.

To perform a passive, one-variable deconvolution the only
free parameter to be extracted from experiment is the membrane
filter constant \( \tau_v \). However, Eq. 13 requires three parameters:
\( \tau_v \), \( \tau_w \), and \( \gamma \). The variational approach coupled with a
flatness criterion, as illustrated in Fig. 2 for passive cells, can
be used to obtain these unknown quantities. The method is as
follows: 1) an initial set of parameters \( \tau_v \), \( \tau_w \), and \( \gamma \) is used
to deconvolve the voltage trace using Eq. 13; 2) the flatness of
the trace is then examined away from the underlying pulses (the pulses are cut using some appropriately sized window around the identified onsets); 3) this is repeated over a range of each of \( \tau_v \), \( \tau_w \), and \( \gamma \) until the flattest trace is found; and 4) the crop and reconvolution stages are then carried out in the same way as was shown in Fig. 3, except
that for the reconvolution it is the integration of Eqs. 10 and
11 that is required.

It can be noted that, as a by-product of this procedure, the
method provides all the parameters required to generate reduced
models that treat active membranes in the linear approximation.

**EXPERIMENT: NONPASSIVE MEMBRANES.** In Fig. 5 two examples
are given [trains of EPSPs and inhibitory postsynaptic potentials
(IPSPs)] of the two-variable deconvolution method applied
to cells with sag/rebound responses characteristic of the
h-current (Silberberg and Markram 2007).

For Fig. 5, A–C, the case of an EPSP train, the deconvolved
pulses have a decay constant of 2 ms, consistent with AMPA kinetics.
Thus despite the very different membrane response, the two-variable deconvolution yields an underlying pulse that is
very similar to that seen for the deconvolution of the cell
with a passive voltage response in Fig. 3.

For the IPSP train in Fig. 5, D–F the deconvolved pulses show
a 10-ms decay constant, consistent with GABA\(_A\) kinetics.
Although the summation of the reconvolutions agrees well with the
original voltage trace, the deconvolved pulses are at the limit of
what can be considered separated. This is because the GABA\(_A\)
decay constant is of an order similar to that of the pulse separation
of 50 ms. A shorter separation would give rise to the effect
demonstrated in Fig. 1, B and C for \( \Delta < 15 \) ms (that model was of
an excitatory connection with AMPA-like kinetics) for which
subsequent deconvolved pulses are affected by the decay of those
preceding.

Before concluding this section, it should be noted that the
two-variable method easily generalizes to more complex mem-
brane responses that require three or more additional \( w \) vari-
able. Such dynamics can be accounted for by adding extra interaction terms

\[
\tau_v \frac{dv}{dt} = -v - \sum_{n} \gamma_n w_n + D \tag{14}
\]

with \( n \) equations for \( w_n \) of the form of Eq. 11. In this way a
broad range of dynamics can be handled—equations of the linear form (Eq. 14) have already been used to model the effects of: sodium and potassium spike-generating currents near threshold (Hodgkin and Huxley 1952); calcium-activated potassium adaptation currents (Fuhrmann et al. 2002); and persistent-sodium and slow-potassium currents (Richardson et al. 2003). Finally, it should be noted that the parameters $\tau_\alpha$, $\gamma$, and so forth, which were found by a variational method here, have a clear biophysical interpretation and can be systematically related to the underlying conductance-based model of the neuron (Koch 1984).

**Discussion**

A deconvolution technique was demonstrated that defilters voltage traces to leave a signal with higher temporal detail from which EPSPs may be readily extracted and their amplitudes measured. The generality of the method was established through a variety of experimental examples, including both AMPA and GABA_A synapses and neurons with passive and nonpassive membranes.

As a final part of the analysis, two aspects of the method will be examined in more detail. First, the scope of the linearity assumption, which is shared by any technique that measures PSP amplitudes from intracellular voltage traces, will be assessed. Second, the similarity between the deconvolved voltage waveform and voltage-clamp current measurements will be investigated using cable theory and further experiments.

**Strongly nonlinear voltage-gated currents**

The deconvolution–reconvolution method requires that the filter properties remain constant throughout the recording, i.e., that $\tau$ for the passive case in Eq. 5 or $\tau_\alpha$, $\gamma$, and $\tau_\nu$ for the two-variable case in Eq. 13 do not change their values during the measurement process. Two cases of neurons showing the effects of voltage-gated currents were treated in Fig. 5 and from the checksum in Fig. 5, $C$ and $F$ it can be seen that the membrane response properties do remain constant over the period of the experiment, despite the fact that the connections were strong ones. However, it is possible that for considerably stronger activation or for different classes of voltage-gated currents with sharper activation curves, the linear approximation (Hodgkin and Huxley 1952; Koch 1984) underlying the two-variable deconvolution would not be as valid. Such nonlinearities would temporarily disrupt any method that attempts to measure synaptic amplitudes from the intracellular voltage. Deconvolution methods can be augmented to deal with nonlinearities, for example the nonlinear delayed glutamate clearance at the calyx of Held (Neher and Sakaba 2001), but...
nonlinear effects are considerably richer in their dynamics and must be dealt with on a case-by-case basis (i.e., requiring knowledge of the voltage dependence of the filter constant, as was seen in Supplemental Fig. 2 of Chadderton et al. 2004). In any such case, the linear deconvolution method presented here can be used to identify when such nonlinear effects are significant via the checksum procedure illustrated in Figs. 3C and 5, C and F.

Synaptic reversal potential nonlinearities

Synaptic current is voltage dependent and thus PSP amplitudes depend on the voltage at the location of the synapse. Because preceding PSPs bring the voltage closer to the synaptic reversal potential, this will reduce the current flowing through subsequent channel openings at the same synapse. It was recently noted (Banitt et al. 2005) that this can lead to a type of synaptic depression. For excitatory connections this effect is weak due to the large difference between the rest and AMPA synapse reversal potential (see Banitt et al. 2005, in which the extended shape of the neuron is accounted for; an example of an excitatory synapse is given in which this effect is estimated at 5%). However, for very strong EPSPs, and particularly IPSPs, the relation between the measured voltage amplitude and conductance amplitude will be nonlinear, and any method that extracts synaptic amplitudes from the somatic voltage traces will suffer from this problem. For neurons that are noncompact some estimation of the voltage at the synapse itself must be used to probe the synaptic waveform. This unnecessarily requires the use of more sophisticated and involved methods such as the voltage-jump method introduced by Häusser and Roth (1997), simulations of multicomponent reconstructions of cells (Banitt et al. 2005), defiltering of voltage-clamp recordings for synapses with a measurable N-methyl-D-aspartate (NMDA) component (Kleppe and Robinson 1999), or, as has been proposed on theoretical grounds, a combination of multisite recordings (Cox 2004). Nevertheless, in two experimental comparisons (subsequently shown in Fig. 7) of amplitudes measured in voltage-clamp and current-clamp modes no synaptic nonlinearities are seen, suggesting that, at least for EPSPs, this effect is not significant.

Deconvoluted and voltage-clamp current

The deconvolved waveforms, in which the AMPA or GABAA receptor kinetics can be seen in the pulse decays, show a striking similarity to the current measured in voltage-clamp mode. This similarity will now be examined through modeling and experiment, with the mathematics underlying the modeling to be found in the Appendix.

MODEL NEURON WITH TWO DENDRITES. A basic model is first considered with a soma of surface area $A_s$ and two passive dendrites with space constant $\lambda$ and radius $a$ (see Fig. 6A and the Appendix for further details). One dendrite receives an instantaneous charge injection at time $t = 0$ either proximally $\lambda = 0.1$, or distally $\lambda = 0.5$. An example of the spatial voltage distribution is given for the current-clamp and voltage-clamp modes in Fig. 6B at three different times and for a case for which $A_s = A_d$, where $A_d$ is an electrotonic length constant’s worth of dendritic surface area. Cable theory (Tuckwell 1988) states that current flowing into the soma is proportional to the dendritic voltage gradient near the soma. The spatial voltage distributions for the current-clamp and voltage-clamp cases are different, and so the currents flowing from the activated dendrite into the soma are not the same.

Sharpness of the filtering. The synapse was modeled as being fast (delta-pulse) and so the spread of the deconvolution and voltage-clamp current waveforms at the soma directly give the filter shape. For voltage clamp, the current at the soma is independent of the somatic area; this is plotted in black for the distal and proximal synapses in Fig. 6C. The deconvolved somatic voltages (plotted with the sign inverted) are shown for proximal and distal synapses in the same panels for different ratios $\rho = A_d/A_s$ of the dendritic to somatic areas. In all cases the deconvolution waveform is sharper than that of the voltage-clamp current. This is particularly clear for distal synapses on a neuron with a relatively small soma (dot-dashed red line, Fig. 6C, right); for the case of a distal synapse on a dendrite of length $L = 0.5A$ it can be shown that the voltage-clamp filter time constant is fourfold larger than that of the deconvolution filter (see the Appendix).

Large somata. If the synaptic area of the soma is large then the input resistance will be dominated by the somatic resistance. In this limit the soma does not significantly depolarize, and thus the deconvolution waveform (Eq. 6) is identical to the voltage-clamp current waveform. This effect can be seen in Fig. 6C when the model neuron has a dendritic–somatic area ratio of 0.25.

Proximal and distal synapses. When the synapse is close to the soma, the somatic voltage initially charges up but then, after a certain time (see Appendix), this charging becomes inferior to the current lost due to charge dissipation along the dendrite (see also Roth and Häusser 2001). At this point, the deconvolution filter changes sign, resulting in a weak overshoot, as is just visible in Fig. 6C (left, dot-dashed red line). This effect becomes increasingly negligible as the synapse becomes more distal. It should be further noted that both the voltage-deconvolution and voltage-clamp filters have shapes dependent on the synapse location.

In summary, voltage-clamp measures the current flowing into the soma when the somatic voltage is clamped, whereas deconvolution measures the net current into the soma when the neuron is in its natural current-clamp state, i.e., the difference between the magnitudes of the currents flowing into and out of the soma (see Appendix). For large somata, the soma does not depolarize significantly and so the deconvolution and voltage-clamp current waveforms become identical. In all cases the deconvolved waveform is sharper than the voltage-clamp current. These results are derived mathematically in the Appendix.

MULTICOMPARTMENTAL PYRAMIDAL-CELL MODEL. Results from the analyses of the simplified model also hold for a model with a more realistic dendritic structure: a layer 5 pyramidal-cell reconstruction (see Fig. 6D). A passive membrane has been chosen so that the spatial effects may be the focus of concentration. Synapses with identical time constants and peak conductances (see Methods) were placed at various positions on the dendrites and the corresponding somatic EPSPs and excitatory postsynaptic currents recorded (see Fig. 6E), with synapse 1 the most proximal and 6 the most distal. In Fig. 6F the waveforms (normalized to the same peak) of the deconvolved voltage (red), voltage-clamp current (black), and synaptic cur-
In summary, although the voltage-deconvolution and voltage-clamp current waveforms give rather close results for PSP
amplitudes, there are a number of important differences. The voltage-clamp current has the advantage of being less corrupted by noise because the trace is not differentiated, and also gives an indication (depending on the quality of the space clamp) of the current flowing through the synapse. The deconvolved waveform has the advantage of being sharper, particularly for electronically extended neurons and, importantly, it is measured in current-clamp mode in which the neuron is in its natural state. This allows for the measurement of the normal activation of voltage-gated processes, such as the removal of the magnesium block of the NMDA channels, that are suppressed in voltage-clamp mode. In many studies of ongoing activity, in slices or in vivo, current-clamp mode is preferable due to a desire not to disrupt the firing pattern of the neuron being measured. For such cases, the voltage-deconvolution method described here provides a useful tool for the acquisition of data with high temporal detail.

APPENDIX
Cable theory analysis of voltage deconvolution

The effect of dendritic filtering on voltage-clamp current and voltage deconvolution measurements will be illustrated through the analysis of a simple cable model neuron with two dendrites. This two-dendrite extension of the Rall model (Rall 1969, 1977) is necessary since a soma with a single dendrite misses the current lost from the soma to other dendrites. To ease the notation and to keep the analysis general, time will be measured in units of the passive membrane time constant $\tau$, voltage will be measured from its resting value $v = V - E_{\text{rev}}$, and distance along the dendrite $x$ (with $x = 0$ at the soma) will be measured in units of the space constant $\lambda$. The voltage in a dendrite obeys

$$ \frac{\partial v}{\partial t} = -v + \frac{\partial^2 v}{\partial x^2} + \delta(x - y)\alpha(t) \quad (A1) $$

where it is assumed that there is a current injection with waveform $\alpha(t)$ at a position $x = y$ (in dendrite $a$ only). A synapse is of course better modeled as a conductance change. However, it is considered that the conductance is sufficiently small such that the voltage dependence of the synaptic drive can be safely neglected (Richardson and Gerstner 2005b). This was shown to be a good approximation in the experiment for EPSPs in Fig. 7.

The soma is modeled as an isopotential compartment of area $A_s$, with the same passive membrane properties as the dendrites

$$ \frac{d v_s}{d t} = -v_s + \frac{\partial v_s}{\partial x_s} \bigg|_{x_s=0} + \rho \frac{\partial v_h}{\partial x_s} \bigg|_{x_s=0} \quad (A2) $$

where the ratio $\rho = A_s/A_d$ (where $A_d$ is the surface area of a space constant’s worth of dendrite) measures the relative effective sizes of the dendritic and somatic compartments.

The dynamics of the system are therefore governed by three equations: two of the Eq. A1 type, for each dendrite $v_a$, $v_b$ (with no...
synapse on dendrite \( a \) and one synapse on dendrite \( b \); and one of the Eq. A2 type for the somatic voltage (see Fig. 6A). These are supplemented by the matching condition that at \( x_{a,b} = 0 \), then \( v_{a} = v_{b} = v_{s} \).

**DECONVOLUTION.** Equation A2 shows that the somatic voltage deconvolution is proportional to the net current flowing into the soma

\[
D(t) = \rho \frac{\partial v_{s}}{\partial x_{s}} \bigg|_{x_{s}=0} + \rho \frac{\partial v_{a}}{\partial x_{a}} \bigg|_{x_{a}=0} + \rho \frac{\partial v_{b}}{\partial x_{b}} \bigg|_{x_{b}=0}
\]

which for the two-dendrite case here is equivalent to the difference between the magnitudes of current flowing into the soma from the activated dendrite \( b \) and out of the soma to the inactivated dendrite \( a \).

**VOLTAGE-CLAMP CURRENT.** In voltage-clamp mode the somatic voltage is kept fixed at rest, \( v_{s} = 0 \), by the injection of a current

\[
I_{c} = -\frac{1}{R_{d}} \frac{\partial v_{a}}{\partial x_{a}} \bigg|_{x_{a}=0}
\]

where \( R_{d} \) is the membrane resistance of one space-constant’s worth of dendrite and where it should be noted that the voltage-clamp current is independent of the somatic area.

Two limits will now be considered: that of large somata for which \( \rho \to 0 \) and small somata for which \( \rho \to \infty \).

**A LARGE SOMA.** In this case the voltage solutions for the deconvolved voltage can be expanded perturbatively as a series in the small quantity \( \rho = A_{f}/A_{s} \). For example, the somatic voltage is written

\[
v_{s} = v_{s0} + \rho v_{a1} + \rho^{2} v_{a2} + \ldots
\]

At zero order in \( \rho \), Eq. A2 gives immediately that \( v_{s0} = 0 \). Electrophysiologically, this means that the soma is sufficiently large that it is not significantly depolarized by any current flowing from the dendrites. Because of this, no current flows from the soma to dendrite \( a \) and so \( v_{s0} = 0 \). To the next order in the approximation, it is seen that Eq. A2 can be written

\[
\frac{\partial v_{a1}}{\partial t} + \frac{\partial v_{a1}}{\partial x_{a}} = \frac{\partial v_{s0}}{\partial x_{a}} \bigg|_{x_{a}=0}
\]

The left-hand side of this equation is the deconvolved somatic voltage and the central term is the current flowing into the soma. However, because to zero order the somatic voltage remains constant, this current is equivalent to that flowing into the soma if it were clamped at \( v = 0 \) (the term on the right-hand side of Eq. A6). This current is the inverse of the balance current that would be injected in a voltage-clamp experiment, so it is seen that in the limit of a large soma the deconvolved voltage is directly proportional to the current recorded in current clamp.

**A SMALL SOMA.** In this limit, \( \rho = A_{f}/A_{s} \to \infty \), the somatic Eq. A2 reduces to a gradient matching between the two dendrites. Physiologically, the somatic conductance and capacitance are so small that the current passes from dendrite \( b \) directly into dendrite \( a \) without attenuation. Thus the problem reduces to a single dendrite, for which the voltage is measured at \( v = 0 \) (the putative soma), with a current injection at \( y \) described by Eq. A1. Clearly, this model is also applicable to voltage deconvolution on a long dendrite.

**General mathematical solution.** It will be useful in the following to calculate the response to an instantaneous charge injection, equivalent to replacing \( \alpha(t) \) with the Dirac delta function \( \delta(t) \) in Eq. A1. The solution of this equation on an infinite cable

\[
\phi_{0}(x, t) = \Theta(t) \frac{e^{-t}}{\sqrt{4 \pi t}} e^{-\frac{x^2}{4t}}
\]

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\]
It should be noted that these arguments may not hold unqualified for more detailed membrane models that include voltage-gated currents, or that treat the effect of nonhomogeneous channel densities (London et al. 1999; Stuart and Spruston 1998). For such detailed models the precise relation between voltage clamp and deconvolution remains a topic for further analysis.

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