Integration of Excitatory Postsynaptic Potentials in Dendrites of Motoneurons of Rat Spinal Cord Slice Cultures

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Larkum, Matthew E., Thomas Launey, Alexander Dityatev, and Hans-R. Lüscher. Integration of excitatory postsynaptic potentials in dendrites of motoneurons of rat spinal cord slice cultures. J. Neurophysiol. 80: 924–935, 1998. We examined the attenuation and integration of spontaneous excitatory postsynaptic potentials (sEPSPs) in the dendrites of presumed motoneurons (MNs) of organotypic rat spinal cord cultures. Simultaneous whole cell recordings in current-clamp mode were made from either the soma and a dendrite or from two dendrites. Direct comparison of the two voltage recordings revealed that the membrane potentials at the two recording sites followed each other very closely except for the fast-rising phases of the EPSPs. The dendritic recording represented a low-pass filtered version of the somatic recording and vice versa. A computer-assisted method was developed to fit the sEPSPs with a generalized α-function for measuring their amplitudes and rise times (10–90%). The mean EPSP peak attenuation between the two recording electrodes was determined by a maximum likelihood analysis that extracted populations of similar amplitude ratios from the fitted events at each electrode. For each pair of recordings, the amplitude attenuation ratio for EPSP traveling from dendrite to soma was larger than that traveling from soma to dendrite. The linear relation between mean ln attenuation and distance between recording electrodes was used to map 1/e attenuations into units of distance (μm). For EPSPs with typical time course traveling from the somatic to the dendritic recording electrode, the mean 1/e attenuation corresponded to 714 μm; for EPSPs traveling in the opposite direction, the mean 1/e attenuation corresponded to 263 μm. As predicted from cable analysis, fast EPSPs attenuated more in both the somatofugal and somatopetal direction than did slow EPSPs. For EPSPs with rise times shorter than ~2.0 ms, the attenuation factor increased steeply. Compartmental computer modeling of the experiments with biocytin-filled and reconstructed MNs that used passive membrane properties revealed amplitude attenuation ratios of the EPSP traveling in both the somatofugal and somatopetal direction that were comparable to those observed in real experiments. The modeling of a barrage of sEPSPs further confirmed that the somato-dendritic compartments of a MN are virtually isopotential except for the fast-rising phase of EPSPs. Large, transient differences in membrane potential are locally confined to the site of EPSP generation. Comparing the modeling results with the experiments suggests that the observed attenuation ratios are adequately explained by passive membrane properties alone.

INTRODUCTION

In the intact and normally functioning animal, single neurons are bombarded by trains of impulses that vary in frequency for any given afferent fiber and that are, in general, asynchronous across the population of input fibers. To understand how this barrage of activity is transformed by the neuron into the postsynaptic pattern of action-potential discharge, it is important to learn how the postsynaptic potentials are propagated through the branching dendritic tree and finally summed at the site of spike initiation, which most likely is the initial segment of the axon (Araki and Terzuolo 1962; Colbert and Johnston 1996; Mainen et al. 1995). These naturally occurring synaptic events are only partly mimicked in experiments in which the afferent fibers, or at least some of them, are electrically stimulated because the impulses in each volley are conducted synchronously in each activated fiber (Lipowsky et al. 1996; Magee and Johnston 1995; Stuart and Sakmann 1995). Simulation of the synaptic input by extracellular local application of the transmitter (e.g., glutamate) may suffer from similar shortcomings (Schwindt and Crill 1995, 1997). The use of direct dendritic current injection in the form of an α-function neglects the fact that postsynaptic currents are the result of conductance changes and thus are dependent on the instantaneous driving force of the ions involved (Bernander et al. 1994; Stuart and Sakmann 1995). It has also been pointed out that synaptic background activity influences synaptic integration in the dendritic tree (Bernander et al. 1991). All the aforementioned studies and many other similar ones have laid and refined the foundations for the concept of synaptic integration. This concept of integration at the cellular level has always been related to the collective mechanisms leading to summation of spontaneous synaptic potentials in space and time (Gogan and Tyc-Dumont 1990; Midtgaard 1996; Redman 1976). A more refined understanding of integrative properties of branching dendrites is derived largely from theoretical predictions by using cable analysis and compartmental computer modeling (Agmon-Snir 1995; Bras et al. 1987; Holmes and Rall 1992; Rall 1962, 1964; Rall and Rinzel 1973; Rinzel and Rall 1974; Segev 1995). Many of the basic principles and concepts come from experiments on motoneurons (MNs), often supported by modeling studies (Bras et al. 1993; Rall et al. 1992; Redman 1973; Ulrich et al. 1994). These concepts have been refined and expanded in their application to neurons thought to be involved in higher brain functions, notably the neocortical and hippocampal pyramidal cells (Bernander et al. 1991; Major et al. 1994; Midtgaard 1994). The discovery of a multitude of voltage- and ligand-gated ion channels in the dendrites of many neurons has widened the original concept of integration (passive) to complex computational (active) properties of the dendrites (Huguenard et al. 1989; Johnston et al. 1996; Kim and Connors 1993; Larkum et al. 1996; Markram et al. 1995; Regehr et al. 1993; Stuart and Sakmann 1994). It is, however, not known whether spontaneous excitatory postsynaptic potentials (sEPSPs) of small amplitude activate the different dendritic conductances.
In this study we analyze spontaneously occurring synaptic activity recorded simultaneously from two spatially separated locations on the somato-dendritic tree of cultured MNs by using patch electrodes in whole cell configuration. From these recordings we could estimate the attenuation of the EPSPs along the dendritic segment intercalated between the two recording electrodes in both the somatotopical and somatopetal directions. The experimental results confirm a number of theoretical predictions derived from cable analysis and compartmental modeling (Carnevale and Johnston 1982; Nitzan et al. 1990; Rall 1962, 1967; Rall and Rinzel 1973; Redman 1973; Rinzel and Rall 1974; Zador et al. 1995). In addition, the relative distribution of excitatory synaptic input to distinct dendritic territories could be inferred. Computer modeling of the experiments suggests that the dendritic processing of synaptic input observed in the cultured MNs could be sufficiently explained by passive membrane properties alone. Furthermore, these simulations give insight into the spatial and temporal evolution of the membrane voltage during ongoing spontaneous synaptic activity. On one hand, they demonstrate how each individual synapse, independent of its location, contributes similarly to the overall excitatory state of the MN. On the other hand, they also show how each individual active synapse leads to a prominent, locally confined and short-lived depolarization that would allow focusing temporal and spatial coincidence between individual EPSPs and the back-propagated dendritic action potential. Synaptic integration is not confined to the spike initiation zone but rather appears as a distributed phenomenon in the entire somato-dendritic apparatus with different functional properties in the somatic region or distal dendrites. The importance of these distinct computational properties in different dendritic compartments most likely differ in other neurons and might be reflected in the vast diversity of dendritic morphology.

**METHODS**

**Organotypic spinal cord cultures**

Organotypic spinal cord slice cultures were prepared by a method similar to that developed by Gähwiler (1981) as previously described (Braschler et al. 1989; Larkum et al. 1996). Briefly, 225-μm transverse slices were made from spinal cords of 2-week-old rat fetuses and fixed on coverslips with the use of reconstituted chicken plasma (Sigma P3266) coagulated with thrombin. The cultures were incubated at 36°C and rotated in roller drums at 120 rpm. The medium contained 50% Dulbecco’s modified Eagle’s medium, 35% Hanks’ balanced salt solution, 0.18% glucose, 10% fetal calf serum, and 5 ng/ml nerve growth factor; osmolarity 300 mosM, pH = 7.3. This medium was changed twice each week. On day 5, fluordeoxyuridine, uridine, and cytosine-β-d-arabinofuranoside (concentration 10 μM) were added to the cultures for 24 h to prevent mitosis without which the cultures became too overgrown with glial cells to allow easy access for microelectrodes. The slices were kept in culture for 14–21 d before use.

**Electrophysiology**

Patch pipettes were pulled from borosilicate glass capillaries (1.5 mm OD; 0.86 mm ID) with inner filament (Clark Electromedical) and filled with a solution containing (in mM) 120 K-gluconate, 20 KCl, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 10 ethylene glycol-bis(β-aminomethylene ether)-N,N,N’,N’-tetraacetic acid (EGTA), 2 MgCl₂, and 2 Na₂-ATP, pH 7.3. The extracellular solution contained (in mM) 150 NaCl, 4 KCl, 1 MgCl₂, 2 CaCl₂, 5 HEPES, 2 pyruvic acid, and 5 glucose, pH 7.4. Neurons were chosen from the ventral area of the slice culture and were selected on the basis of their morphology and accessibility to electrodes. Cells that had many dendritic branches and were larger than average were preferred as it was noted that similar neurons stained positive for choline acetyl transferase (CAT) in other experiments (not shown) and were therefore most probably MNs. Simultaneous electrical recordings from the soma and a dendrite were made with use of an Axoprobe microelectrode.
amplifier 1A (Axon Instruments) and high-resistance patch electrodes (somatic electrode: 11.4 ± 1.2 MΩ; means ± SE; dendritic electrode: 15 ± 0.7 MΩ) in the whole cell configuration. The electrical signals were recorded on a DAT recorder (JVC XD-ZS07) modified for recording DC signals (Lüscher et al. 1992). Signals were filtered with a low-pass filter at 10 kHz and digitized off-line at 20 kHz with 12-bit resolution with a CED 1400 plus A/D converter. They were then analyzed by using PV-Wave (Visual Numerics) and routines written at the Physiology Department at the University of Bern (Bern, Switzerland). All experiments were performed at room temperature (22–25°C).

**Estimating amplitude and rise time of EPSPs with the a-b function**

sEPSPs were extracted from continuous voltage recordings and fitted with a computer-assisted method with a template function of the form

\[ f(t) = ct^a e^{-bt} \]  

(1)

that is a generalization of an α-function and is referred to here as an α-b function. This was the function with the smallest number of parameters that could give a approximate description of the shape of typical EPSPs in the dendrites. The usefulness of this function as an approximation to actual dendritic events is demonstrated in Fig. 1. Figure 1A shows an isolated EPSP recorded simultaneously from an MN with two electrodes, one at the soma and the other one at a dendrite, separated by ~100 μm. The α-b function could accurately describe the form of both events. To show that this function can closely approximate the predicted cable filtering of EPSPs by dendrites we used it to fit a simulated EPSP.

Figure 1B shows the filtering of current in the form of an α-function injected at the soma after traveling to a dendritic location. Here again, the shape was well fitted by the α-b function. The time to peak (tp) of the α-b function is given by

\[ tp = \frac{a}{b} \]  

(2)

The fitting procedure involved an interactive computer program (Interactive Data Language, Cr ceso, Gilching, Germany) in which the user determined the onset, τ, amplitude and the parameter a. For a = 1, this equation has exactly the same shape as an α-function. By adjusting a, a close approximation to the cable filtering of an α-function can be achieved in most cases (see Fig. 1A and B).

Having determined a and τ, p can then be calculated by using Eq. 2. Because the amplitude and τp can be fitted independently of the shape, the cable filtering effect can be adjusted with one parameter that makes this a quick and robust method for estimating the rise time and half-width of sEPSPs in dendrites. Each fit was subtracted (‘‘peeled’’) from the data to allow accurate fitting of subsequent events (Fig. 1A). Figure 1C illustrates a short segment of a typical recording. The two electrodes were located on two different dendrites of an MN. Computer-assisted fits of all the distinguishable EPSPs are shown as the sum (Fig. 1C) of all the component EPSPs shown in Fig. 1C3. The subtraction of the summed fits from the original data leaves a straight line (not shown). For the results presented in this paper, care was taken not to fit EPSPs too close to each other to avoid possible nonlinear summation (Rall 1964), and only EPSPs that showed minimal overlap were used.

**Estimating average attenuation**

Given two recording electrodes, the neuron is divided into three regions: one region distal to each electrode and the region in between. To estimate average attenuation, the neuron is divided into three regions: one region distal to each electrode and the region in between.
tween (including interposing subbranches of the dendritic tree). The ratio of the EPSP amplitude at the first electrode to the EPSP amplitude at the second electrode is one measure of the attenuation of the signal between the two electrodes. We used the maximum likelihood estimator (Dityatev and Clamann 1993; Lüscher et al. 1994; Stricker and Redman 1994) to find the best linear regression fits to up to three populations of these amplitude ratios. We assumed that the probability of the recorded events was normally distributed and dependent on the following three factors: 1) the probability of being in the i-th population \( p_i \), 2) the mean attenuation factor for that population \( a_i \), and 3) the standard deviation for that population \( \sigma_i \). The probability of each recorded event being in each of the three populations given \( p_i, a_i \), and \( \sigma_i \) could thus be determined. The sum of the natural logarithms of these probabilities was then used as the likelihood function to be maximized. This analysis was used to provide estimates for the proportion of events in each population \( \left( p_1, p_2, p_3 \right) \) and their attenuation factors \( \left( a_1, a_2, a_3 \right) \). Monte Carlo simulations were used to test the accuracy of the estimates. A detailed account of the technique will be published elsewhere.

**Histological staining**

The cells that were used for reconstruction were filled with biocytin (3% in the patch solution). The cultures were fixed thereafter in 4% paraformaldehyde. The cells were then visualized with the Vectastain ABC Kit (Vector Laboratories, Burlingame, CA). Briefly, the cultures were washed with phosphate-buffered saline (PBS), incubated with Avidin horseradish peroxidase in 0.1% Triton X-100 for 2 h, and washed again in PBS. The final staining was achieved by incubating the tissue with diaminobenzidine, \( \text{H}_2\text{O}_2 \), and \( \text{NiCl} \) for 2 h, followed by another washing step with PBS. The cultures were mounted in 95% glycerol and reconstructed within the next 48 h.

**Reconstruction**

Biocytin stained MNs were reconstructed by means of a computer-assisted reconstruction system (Eutectic 3D NTS, Eutectic Electronics, Raleigh, NC) as previously described (Ulrich and Lüscher 1993).

Comparison of the soma outline and dimensions of the proximal dendrites in the video image that was taken during the experiments (before the staining procedure) with the corresponding outlines following the staining procedure showed no evidence for tissue shrinkage. This is consistent with observations on tissue shrinkage by other researchers using the same histological procedures (Thurbon et al. 1994; Ulrich et al. 1994).

**Modeling methods**

The simulator NEURON (Hines 1993) was used running under Windows95 on a 90-MHz Pentium-based computer. An integration time step of 25 \( \mu \text{s} \) was used. The cell’s morphology coded in a Eutectic file format was translated by CABLE into a file that could be used directly as an input file to NEURON specifying the 3-D morphology of the reconstructed cells. Each dendritic section was

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**FIG. 3.** Simultaneous recordings of sEPSPs recorded from 2 dendrites. A: phase-contrast video montage of a large neuron. Axon arises from the dendritic segment between the soma and electrode 2 (white arrow). No major dendritic branches arise from the soma or the dendrites between the 2 electrodes. Schematic representation of the recording arrangement is shown in the inset of C. Electrodes 1 (filled circle) and 2 (red open circle) are located 85 and 184 \( \mu \text{m} \) from the center of the soma, respectively. B: superimposed spontaneous synaptic activity recorded simultaneously at both electrodes and the difference of the 2 traces shown below. C: maximum likelihood analysis for estimating the attenuation ratios (see Fig. 2 for definition of plot symbols). Three distinct populations are found for EPSPs traveling from electrodes 1 to 2 and in the opposite direction and also for EPSPs probably arising from the somatic region. D and E: relation between attenuation ratios and rise time of the EPSPs propagating in each directions.
subdivided into segments of \( \pm 10-\mu m \) length. The NEURON files used are available from the authors on request.

**RESULTS**

**Simultaneous recording of spontaneous EPSPs from dendrite and soma**

A phase-contrast image of an MN with one electrode at the soma and one on a dendrite is shown in Fig. 2A. The length of the dendritic segment between the two electrodes was 218 \( \mu m \). The arrow points to a possible dendritic branch point. Spontaneous synaptic activity recorded simultaneously at the soma and in the dendrite is shown superimposed in Fig. 2B. The third line is a measure of the voltage difference between the two electrodes and the dashed line indicates no difference. Addition of 10 \( \mu M \) 6-cyano-7-nitroquininal-2,3-dione (CNQX) and 10 \( \mu M \) bicuculline in similar experiments abolished virtually all synaptic activity in these neurons. The membrane potentials at the two recording sites follow each other very closely except for fast-rising EPSPs.

To measure the attenuation of individual EPSPs it was necessary to extract them from continuously recorded data by using a computer-aided procedure fitting each EPSP with a modified \( \alpha \)-function as described in the METHODS section. Figure 2C plots the amplitudes of 210 spontaneous EPSPs recorded at the soma against the amplitude of the same EPSPs recorded at the dendritic location. Three distinct clusters of points can be seen that can be determined by only three classes of synaptic input as follows: 1) synapses located at the dendrites distal to the dendritic electrode, 2) synapses located at the soma and on other dendrites inserting into the soma, and 3) synapses located between the two electrodes or on a dendritic branch inserting into this section. EPSPs arising from synapses located in the first two populations would therefore travel the entire distance, albeit in the opposite direction, between the two electrodes. In a passive dendritic tree, signals of the same shape but varying amplitude will attenuate by the same proportion when traveling in the same direction over the same section of dendritic tree. In this case it would be possible to fit such signals with a regression line where the gradient represents the attenuation ratio. On the other hand, signals arising from synapses between the two electrodes would vary according to the respective distances between the synapse and each electrode. Because the dendritic segment between the two electrodes constitutes only a small proportion of the total dendritic length, we expect that only a few synapses were located between the electrodes. However, if a dendrite is inserted into the dendritic segment between the recording electrodes, a third distinct population should be present.

We used a maximum likelihood analysis and Wilks' statistic (Dityatev and Clamann 1993; Lüscher et al. 1994; Stricker and Redman 1994) with hypotheses designed to test the possibility of there being one, two, or three populations of attenuated signals. The least complex model with the greatest likelihood for the cell shown in Fig. 2 was that there were three populations of signals each with approximately the same standard deviation (Wilks’ statistic, \( P > 0.05 \)). Note that the classification of events based on the maximum likelihood (indicated with the regression lines) was completely independent from the method that used delay times for distinguishing EPSPs arising first at the somatic or dendritic recording site, respectively (indicated with different plot symbols and colors). The attenuation ratios for signals traveling in the somatofugal and somatopetal direction were 0.82 and 0.43, respectively. This result indicates that EPSPs attenuate approximately twice as much spreading from dendrite to soma as from soma to dendrite. We found a third population of EPSPs with an attenuation ratio of 0.70. This population most likely represents EPSPs generated in the dendrite inserting between the two electrodes at a fixed distance (dashed dendrite in the inset of Fig. 2C and arrow in Fig. 2A). From the population weights found by the maximum likelihood estimator we estimated the percentage of the EPSPs belonging to each of the three populations. The analyzed EPSPs were distributed among the three populations with attenuation factors of 0.43, 0.70, and 0.82 in the proportion of 25, 29, and 46\%, respectively.

Figure 2, D and E, shows the relationship between the rise time of the signals (10–90% amplitude of rising phase) and the attenuation ratio for signals traveling from the soma to the dendrite and vice versa. Signals were generally faster...
at the dendritic electrode than at the somatic electrode. For EPSPs with rise times < 2.0 ms the attenuation ratio increased steeply, especially for EPSPs traveling from dendrite to soma (Fig. 2E). The attenuation ratio is less dependent on rise time for EPSPs spreading from soma to dendrite (Fig. 2D).

It is interesting to note that the peak amplitude of fast EPSPs strongly attenuates in both directions but reaches the same amplitude in both the dendritic and somatic recording during the repolarization phase (Figs. 2B and 3B). This result and the efficient back-spreading of slow EPSPs from soma to dendrites make the somato-dendritic tree almost isopotential on a slow timescale.

This suggestion can be tested directly by recording simultaneously from two dendrites (Fig. 3). Electrodes 1 and 2 are located 184 and 85 μm from the center of the soma, respectively. Figure 3B shows the superimposed membrane potential fluctuations recorded from the two electrodes. The recording from electrode 1 reflects precisely the filtered recording from electrode 2 and vice versa. The maximum likelihood analysis finds three populations with attenuation ratios of 0.41, 0.77, and 0.52 with population weights of 40, 48, and 12%, respectively. The axon of this neuron appears to arise from the dendritic segment between the soma and electrode 2 (Fig. 3A, arrow). The middle population probably represents EPSPs arriving at the somatic region and traveling to the two electrodes; the other two populations probably represent EPSPs arising from the dendritic location distal to electrode 2 (red circles) and distal to electrode 1 (black circles). Again, EPSPs with rise times < 2.0 ms tend to attenuate more than EPSPs having rise times > 2.0 ms (Fig. 3, D and E). In this nearly symmetric recording, the dependence of the attenuation ratio on rise time is similar for EPSPs spreading in either direction. Note that the attenuation factor for both populations results from a somatofugal as well as from a somatopetal contribution and therefore cannot be compared with the attenuation factors obtained in the previously illustrated experiment. From the population weights obtained from the maximum likelihood estimation, it can be concluded that 12% of the synapses are located in the dendritic domain distal to electrode 1, 40% in the dendritic domain distal to electrode 2, and 48% in the region between the two electrodes or on dendritic branches in this region.

Figure 4 summarizes the results for 12 neurons of soma-dendrite recording configuration. We used the definitions
Computer simulation of the experiments

Simulations were carried out with the use of reconstructions of cells from organotypic slice cultures stained with biocytin. The simulations were intended to match the experimental observations qualitatively because the precise details of passive properties of these cells are unknown.

Figure 5 duplicates the experiment depicted in Fig. 2. Here we modeled all the possible outcomes for that experiment by testing what should be recorded at two electrodes, one at the soma and one 126 μm from the soma, for excitatory inputs located in every segment on the reconstructed and compartmentalized neuron. Synaptic input was modeled as a conductance change in the form of an α-function with peak conductances from 1–5 nS (steps of 1 nS) and applied sequentially to every segment of the dendritic tree. The EPSP amplitudes recorded at the soma are plotted against the EPSP amplitudes recorded at the dendritic location as plotted in Fig. 2. Three different clusters of points can easily be distinguished. The red symbols are from synapses located distal to the dendritic recording electrode. The green symbols are from synapses located on the soma and the other dendrites. The yellow symbols are from synapses located on the dendritic branch that projects into the dendritic section located between the two recording electrodes. The blue symbols are from synapses located on the dendritic section between the recording electrodes. Depending on the precise location of the synapse and their peak conductance, these points could theoretically be anywhere in the gray-shaded area. The colors of the symbols match the colors of the dendritic domains of the MN in the inset of Fig. 5A. Straight lines were fitted through the red, green, and yellow populations and the attenuation ratios were calculated for each population. Similarly to the experimental results, EPSPs spreading from dendrite to soma attenuate more than in the other direction (attenuation ratios 0.315 vs. 0.848). The regression line through the yellow population has a slope 1 because the insertion point of the dendritic branch is closer to the dendritic than to the somatic electrode.

In the red population five distinct subpopulations can be seen that result from the five different synaptic peak conductance changes. It becomes evident by inspecting one of these subpopulations that the amplitude ratios are not constant. The determining factor is the filtering and therefore the rise time of the EPSPs as they pass one of the two electrodes. Accordingly, EPSPs coming from more distal locations are filtered more when arriving at the first (dendritic) electrode and thus attenuated less on their way to the second electrode. They are also smaller at the first electrode than EPSPs arriving nearer the first electrode. This result is supported by Fig. 5C in plotting the amplitude ratio of the dendritic over the somatic EPSP amplitude versus the ttp of the dendritic EPSP. Attenuation increases with decreasing ttp; for longer ttp, attenuation is almost constant. The same analysis for EPSPs spreading from soma to dendrite is illustrated in Fig. 5B. As in the experimental results, in this situation there is much less dependence of the attenuation on the ttp of the EPSP. The EPSPs recorded first in the soma are, in general, slower and their ttp is always >1 ms.

This simulation example makes it clear that the attenuation calculated from the slope of the regression line is only approximately correct, because the closer to the recording electrode the EPSPs are generated (and thus the faster they are as they enter the dendritic segment intercalated between the

FIG. 6. Simulation of spontaneous synaptic activity recorded simultaneously from different somato-dendritic locations. A: reconstructed MN used for the simulation together with the color-coded recording sites. Same passive membrane parameters as in Fig. 5. At each dendrite and soma 5 synapses were placed at a random location. Peak conductance change varied at random with integral steps between 0 and 5 nS. Each synapse was activated at a random time in the simulation interval of 500 ms. B: example of the time course of an EPSP elicited and recorded at the soma (g_{syn} = 5 nS). C: superimposed recordings from the 4 somato-dendritic locations. Black and red traces most closely simulate the experiment shown in Fig. 2. Large, fast signals labeled with asterisks correspond to EPSPs generated close to their respective recording sites.
recording sites), the larger the attenuation. Most EPSPs recorded in the soma are already slow because they are filtered while spreading along the attached dendrites. This explains why there is less dependence of the attenuation ratio for the EPSP spreading from the soma out into the dendrite than in the opposite direction.

Figure 6 simulates the sEPSPs recorded simultaneously from the soma and three different dendritic locations. Synaptic conductance changes were placed at random locations, five on the soma and five on each dendrite. The peak conductance change varied at random between 0 and 5 nS in steps of 1 nS. The tip of the conductance change (τ) was 0.3 ms. A synapse at the soma (g_syn = 5 nS) produced the EPSP at the soma shown in Fig. 6B. The barrage of sEPSPs recorded simultaneously from the four locations are shown superimposed in Fig. 6C. The color code matches the colors of the recording electrodes in Fig. 6A. The red and black traces correspond most closely to the experimental situation (distance between the 2 electrodes = 126 μm). The blue and green electrodes (distances from the soma = 296 and 305 μm, respectively) record from distal dendritic segments and could not be realized in the experiments, with one exception. On a slow timescale the four recordings superimpose, suggesting that the entire dendritic tree is close to isopotential. Deviations from isopotentiality occur only transiently near the site of the synaptic conductance, notably in the four instances indicated by asterisks. Synaptic conductance changes produce very short-lived, locally confined depolarizations; those amplitudes increase the more distally the synapses are located on the dendritic tree. These observations are further analyzed in the simulations illustrated in Fig. 7.

The morphology of the MN used for this simulation is shown in Fig. 7A, together with the location of the synaptic inputs (arrows 1–9 and S). The potential profiles along the two dendrites (from 1 to 9) produced by each synapse after 0.3 ms are plotted in Fig. 7B. As predicted, the peak amplitude at time 0.3 ms increases with more distal location of the synaptic input. The spatial potential drop is very fast in either direction. The potential profiles are not symmetrical in the two dendrites because of geometric differences in the two dendritic branches. Simulations with lower intracellular resistivity (R_i = 75 Ωcm) lead to somewhat smaller and broader peaks (not shown). We have used different sets of passive cable parameter values. The basic findings, however, do not depend critically on these values. The same potential profiles are plotted as they develop in time after 5.0 ms in Fig. 7C. The profiles are flattened, especially the ones produced by synapses close to the soma (4, 5, 6, 7, and S; note the different voltage scale). This simulation and the one shown in Fig. 6 illustrate how the barrage of synaptic input leads to a short depolarization with large and steep (but locally restricted) voltage gradients along the dendrite at the site of the actual synaptic input. As time progresses, the potential redistributes and thus flattens out quickly, rendering the entire somato-dendritic apparatus almost isopotential.

**DISCUSSION**

The results taken as a whole confirm a number of theoretical predictions about passive dendritic integration that are based on concepts whose foundations were laid in the late fifties and early sixties (Rall 1967; Rall and Rinzel 1973; Rinzel and Rall 1974). The observations on the temporal and spatial progression of spontaneous synaptic potentials over the entire somato-dendritic tree may foster our understanding of the complex input-output relations in MNs and may even be generalized to other CNS neurons. In particular, the experiments together with the supporting simulations demonstrate that the attenuation of sEPSPs traveling in both the somatofugal and somatopetal directions can be deter-
mined directly with simultaneous dendritic and somatic whole cell recordings. Emphasis is placed on the fact that this is not simply a statement about the electrotonic properties of the neuron but a way of assessing how naturally occurring synaptic input is integrated at different points in the cell. The technique depends on the ability to extract the component single EPSPs of complex synaptic activity. We will first briefly discuss the limitations and possible pitfalls of the technique.

**Methodological considerations**

In principle, attenuation factors of EPSPs could also be obtained by injecting an \( \alpha \)-current at either electrode while simultaneously recording the resulting voltage transients at both electrodes. This approach gives the additional information on the exact location of current application (Stuart and Sakmann 1995). With this method it is also possible to vary signal amplitude and time course in a controlled manner. However, it is technically very difficult to reliably compensate the large access resistance of the current injecting electrode that is a necessary part of dendritic whole cell recording. This makes it very difficult to compare the voltage transients from the two electrodes, because the voltage signal recorded with the current passing electrode may be contaminated by an unknown voltage drop across the under- or overcompensated electrode resistance. Furthermore, mimicking synaptic input with an \( \alpha \)-current may not be appropriate, if the expected large synaptic depolarizations in the distal dendrites approach the reversal potential of the synaptic conductance, thus introducing significant nonlinearities. Perhaps most importantly, this method can make no statement about the shape and location of typical synaptic input that, as we have shown, considerably affects the average attenuation across any given segment. We therefore preferred to make a careful analysis of the sEPSPs recorded at two different sites.

The \( \alpha-b \) function introduced in this paper was used solely as a heuristic to extract useful parameters of each EPSP. We do not claim that the function is a mathematical equivalent of a cable-filtered \( \alpha \)-function. The accuracy of the computer algorithm for fitting the \( \alpha-b \) function as a template to the EPSPs was studied extensively with simulated EPSP data. The results of this evaluation will be published elsewhere.

The fitting procedure and the maximum likelihood analysis for identifying the different EPSP populations and their respective attenuation factors make assumptions about the linearity of signal attenuation. These assumptions are known to be false in some details. First, the conductance change caused by the synaptic input reduces the membrane resistance briefly. For events spatially and temporally close to one another, this change affects the apparent electrotonic length transiently. Also, a depolarization in the membrane potential produced by the conductance change causes a reduction in the driving force for subsequent EPSPs elicited in close spatial and temporal relations. Therefore it is suggested that only well-isolated events be chosen for the computer-assisted fitting algorithm. However, this could produce biased estimates of \( p_e \).

Another important source of nonlinearity is the relationship between rise time and attenuation. The faster the rise time the more attenuation. However, events of different amplitudes but the same rise times will all attenuate equally (given a passive dendritic tree). This result was demonstrated by simulations (Fig. 5A). Therefore the effect of varying rise times will simply be to increase the variance around a regression line. The last possible nonlinear contribution could come from voltage-dependent conductances.

MNs in these cultures have dendritic voltage-dependent sodium and calcium conductances that are activated during the back-propagation of the action potential that invades a large part of the dendritic tree (Larkum et al. 1996). In neocortical pyramidal cells, which appear to have the same sodium and calcium conductances in the apical dendrite (Stuart and Sakmann 1994), there is a small boosting effect during the falling phase of EPSPs that appears to be due to Na\(^+\) channels located near the soma or possibly near the axon (Stuart and Sakmann 1995). This effect would be invisible to the maximum likelihood analysis, which deals only with the amplitude, if only well-isolated events are used. It should also be noted that EPSPs in adult cat MNs summate linearly or to less than the sum of the component EPSPs, implying that there are no boosting effects for subthreshold signals (Burke 1967).

The population weights obtained from the maximum likelihood analysis give additional information on the percentage distribution of the origins of the analyzed EPSPs with respect to the electrode location. They allow a coarse spatial mapping of the synapse location onto the dendritic domains as defined by the particular locations of the two electrodes (see Fig. 5A). For certain cell types this mapping may be a more significant result of this analysis. For example, by using the same method with a pyramidal neuron (with electrodes on both the soma and apical dendrites) it would be possible to determine the proportions of inputs arriving on the basal dendrites and on the oblique dendrites and tufts.

Because of the small diameter of the dendrites, seal resistance of the recording pipette might have been less than perfect. Because we did not use voltage-clamp amplifiers we were not in a position to measure the seal resistance accurately, and the seal might even have changed significantly after we established the whole cell configuration. A leak introduced by the electrode, be it at the soma or at a dendrite, might significantly influence EPSP attenuation. In a computer simulation we analyzed the effects of additional leaks at the recording locations on the amplitude and time course of the EPSP (Fig. 8). From this simulation we concluded that although a leak of 1.0 nS at one electrode reduces the amplitude of the EPSP somewhat, the attenuation factor is not necessarily affected much and the conclusions remain the same.

This simulation study does not exclude a possible capacitive load of the electrode on the EPSPs.

**Passive spreading of EPSPs**

The attenuation for EPSPs spreading in the somatopetal direction was much more pronounced than for EPSPs traveling in the somatofugal direction. This asymmetry in voltage spread has been predicted from cable analysis (Carnevale and Johnston 1982; Rall and Rinzel 1973). EPSPs were also found to have a shorter rise time at the dendritic electrode.
attenuation is valid for EPSPs with the time course observed in our experiments and will be different for slower or faster synaptic potentials. This result can be interpreted as for typical EPSPs traveling toward the soma, the dendrites appear to be electrotonically three times as long. By using steady-state equivalent cylinder model analysis the mean electrotonic length ($L$) was found to be 0.7 in a population of similar cells (Ulrich et al. 1994). This classical analysis, however, could only give estimates for a "somatofugal $L"."

So far the discussion has neglected the fact that the dendrites of the MNs studied are equipped with voltage-sensitive Na$^+$ channels responsible for the active back-propagation of the action potential, as well as Ca$^{2+}$-channels leading to calcium inflow during the back-propagated action potential (Larkum et al. 1996). These voltage-sensitive ion channels could in principle provide inward currents to actively boost the EPSPs traveling along the dendrites. Experiments done with pipettes containing the intracellular Na$^+$-channel blocker lidocaine N-ethyl bromide (QX-314; 1–10 $\mu$M) did not provide attenuation factors significantly different from the ones obtained without the blocker. We conclude that the amplitude of the sEPSP was probably not sufficient for Na$^+$-channel activation. It is, however, conceivable that the locally restricted large depolarizations at the site of the EPSP generation might activate voltage-gated currents. On the other hand, simulations done with and without "weakly" excitabile dendrites ($g_{Na} = 3$ mS/cm$^2$) revealed that the excitabile dendrite showed slightly less attenuation than passive ones for EPSPs traveling from dendrite to soma, but attenuation was unaffected for EPSPs spreading from the soma back into the dendrite. The very small difference in the simulation would have been masked in the actual experiment by the large standard deviation within the population and could not have been detected.

Signal attenuation along the dendrite is, of course, dependent on the actual values of $R_m$, $R_i$, and $C_m$. We did not attempt to estimate these values to get a quantitative agreement between the experimentally determined attenuation factors and the values obtained from the simulations. Instead, we used ranges of values for the passive cable parameters obtained previously from similar cells (Ulrich et al. 1994). In principle, it should be possible to estimate these parameters by systematically changing them and comparing the resulting simulations with experimental data. There is, however, a strong interdependence between the parameters and substantial nonuniqueness in the possible solutions must be expected. We chose not to undertake this potentially futile and certainly very laborious exercise.

Possible implications on input-output relations

In addition to the more static picture of EPSP attenuation discussed in the previous section, the results provide a more dynamic representation of the spatial and temporal progression of the EPSP signal over the entire somato-dendritic tree. Most importantly, the entire somato-dendritic tree converges to isopotentiality on a long timescale. Only on a short timescale can we observe transient, locally restricted, large depolarizations deviating from the slow isopotential fluctuations. The amplitude of these short-lived transients increases in more distal dendritic compartments. Therefore the mem-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure8.png}
\caption{Influence of leak conductance introduced by the electrode on the amplitude and time course of the EPSPs recorded simultaneously from a dendritic and somatic location. \textit{A}: reconstructed MN used for simulation, together with the recording arrangement. One $\alpha$-synapse is simulated at the dendritic location indicated. The following passive membrane properties apply: $R_m = 17$ k$\Omega$cm$^2$, $R_i = 150$ $\Omega$cm, $C_m = 1.0$ $\mu$F/cm$^2$. The parameters for the simulation of the $\alpha$-synapse were as follow: $\tau = 0.3$ ms; $g_{Na} = 5$ nS, $E_{Na} = 0.0$ mV. A point conductance ($g_{Na} = 70$ mV), simulating an imperfect electrode seal, was located either at the soma (\textit{B}) or at the dendritic recording site (\textit{C}). Thick solid lines, control EPSPs (without additional shunt); stippled lines, $g_{Na} = 0.1$ nS; thin line, $g_{Na} = 1$ nS.}
\end{figure}
brane fluctuation dynamics show a different picture at the soma than in the dendrites. At the soma and axon hillock—initial segment region, where spike initiation most likely occurs (Larkum et al. 1996), the low-amplitude fluctuations are filtered, whereas out in the distal dendrites (cf. Fig. 6C) large amplitude, very rapid, locally restricted fluctuations can be seen. The results suggest that the dendritic tree of MNs is optimized for linear temporal and spatial integration of low-frequency synaptic input. This input leads to slow fluctuations of the somatic membrane potential that, if driven above threshold, provokes the MN to fire trains of action potentials as determined by its intrinsic membrane properties (Viana et al. 1993a,b). The small high-frequency fluctuation riding on the membrane potential at the soma may govern the reliability and precision of spike generation, as was suggested for neocortical pyramidal cells (Mainen and Sejnowsky 1995) and MNs (Calvin and Stevens 1968).

The fast, locally restricted EPSPs of large amplitude in the distal dendrites are well suited for coincidence detection of the back-propagated action potential with active synaptic input. They not only provide the necessary temporal information but precise spatial information as well. In cortical neurons, coincidence of the back-propagated action potential with active synapses was implicated in the regulation of synaptic plasticity (Markram et al. 1997). Similar mechanisms might be important during the formation of the spinal network during development. Our results suggest that the dendritic tree of MNs is endowed with distinct computational properties that are spatially segregated in different compartments according to the functional needs of the somato-dendritic tree. The spatial distribution and functional diversification of the computational properties of the dendrites most likely differs in other neurons and might be reflected in the vast diversity of the architecture of dendritic trees.

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