Effect of Nonlinear Summation of Synaptic Currents on the Input–Output Properties of Spinal Motoneurons

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Cushing, S., T. Bui, and P. K. Rose. Effect of nonlinear summation of synaptic currents on the input–output properties of spinal motoneurons. J Neurophysiol 94: 3465–3478, 2005. First published August 3, 2005; doi:10.1152/jn.00439.2005. A single spinal motoneuron receives tens of thousands of synapses. The neurotransmitters released by many of these synapses act on ionotropic receptors and alter the driving potential of neighboring synapses. This interaction introduces an intrinsic nonlinearity in motoneuron input–output properties where the response to two simultaneous inputs is less than the linear sum of the responses to each input alone. Our goal was to determine the impact of this nonlinearity on the current delivered to the soma during activation of predetermined numbers and distributions of excitatory and inhibitory synapses. To accomplish this goal we constructed compartmental models constrained by detailed measurements of the geometry of the dendritic trees of three feline motoneurons. The current “lost” as a result of local changes in driving potential was substantial and resulted in a highly nonlinear relationship between the number of active synapses and the current reaching the soma. Background synaptic activity consisting of a balanced activation of excitatory and inhibitory synapses further decreased the current delivered to the soma, but reduced the nonlinearity with respect to the total number of active excitatory synapses. Unexpectedly, simulations that mimicked experimental measures of nonlinear summation, activation of two sets of excitatory synapses, resulted in nearly linear summation. This result suggests that nonlinear summation can be difficult to detect, despite the substantial “loss” of current arising from nonlinear summation. The magnitude of this “loss” appears to limit motoneuron activity, based solely on activation of ionotropic receptors, to levels that are inadequate to generate functionally meaningful muscle forces.

INTRODUCTION

The current delivered by an ionotropic synapse is determined by the product of two factors: the conductance change caused by opening of ligand-dependent channels and the driving potential (the difference between the equilibrium potential and the instantaneous membrane potential). The dependency on driving potential imposes an intrinsic nonlinearity on the summation of synaptic currents and the resulting postsynaptic potentials generated by two or more simultaneously active synapses. The goal of the present study is to determine the impact of this nonlinearity on the input–output properties of spinal motoneurons where the input is defined by the number and proportion of active excitatory and inhibitory synapses and the output is defined by the current reaching the soma.

Previous studies of nonlinear summation in spinal motoneurons have used two different strategies: predictions based on cable theory or experimental observations. All of the experimental data lead to the conclusion that nonlinear summation of synaptic inputs to motoneurons is negligible or, at the most, modest (Burke 1967; Burke et al. 1971; Clements et al. 1986; Kuno and Miyahara 1969; Powers and Binder 2000; Rall et al. 1967; Skydsgaard and Houmongaard 1994; however, see Curtis and Eccles 1959). For example, near the threshold for repetitive firing, the current reaching the soma generated by combined activation of pairs of inputs (e.g., Ia afferents and axons from Dieters nucleus) was, on average, within 7% of the linear sum of the currents generated by each input (Powers and Binder 2000). In contrast, predictions based on cable theory (i.e., analytically tractable models of neurons with arbitrary geometry, innervation patterns, and synaptic conductances) conclude that interactions between neighboring synapses resulting from changes in synaptic driving potential could profoundly reduce the summation of postsynaptic potentials (Holmes and Woody 1989; Jack et al. 1975; Koch 1999; MacGregor 1968; Rall 1964, 1967; Segev and Parnas 1983; Spruston et al. 1999) and the amplitude of the synaptic current reaching the soma (Abbott 1991; Koch 1999). These conclusions have been reinforced by simulations that incorporate more realistic descriptions of motoneuron geometry, synaptic density, magnitude, and time course of synaptic conductances (Barrett 1975; Barrett and Crill 1974b; Binder et al. 1996; Korogod et al. 2000; Powers and Binder 2001; Ulrich et al. 1994; however, see Segev and Burke 1990). For example, the sum of the currents delivered by simultaneous activation of four synapses on a distal dendrite was only 50% of the linear sum of the currents delivered by each synapse (Barrett and Crill 1974b).

The large discrepancy between experimental data and predictions based on cable theory leaves the significance of nonlinear summation open to debate. It could be argued that the experimental data deserve a greater weight in this debate, but the interpretation of the experimental data is confounded by several poorly constrained variables, that is, the number and distribution of activated synapses. Moreover, it is not clear what role voltage-dependent channels play in fixing the nonlinear summation “bug” (cf. Cash and Yuste 1998, 1999; Clements et al. 1986; Urban and Barrionuevo 1998). On the other hand, the predictions based on cable theory are also subject to several caveats. With few notable exceptions (Koro-
stained process, leading to curling of dendrites that travel in the soma; differential shrinkage where the tissue shrinks more than the dendritic beading was absent; there was no evidence of damage to the sites, as opposed to a gradual, proximal to distal, loss of staining; following criteria: distal dendrites could be traced to abrupt termina-

Major 2001) and tissue shrinkage. Each motoneuron fulfilled the described in detail elsewhere (Rose and Cushing 2004). These methods for intracellular staining and tissue processing have been elaborated by Bui et al. (2003). The number of compartments ranged from 4,000 to 5,000. Specific internal resistivity was set at 70 Ωcm, the value of specific membrane capacitance was 1 μF cm⁻², and specific membrane resistivity was fixed at 6,000, 15,000, or 60,000 Ωcm².

The conductance change caused by each synapse was modeled by means of the following equation (cf. Bernander et al. 1991)

\[
g(t) = g_{peak}(t_{peak}) \exp[1 - (t/t_{peak})]
\]

For excitatory synapses, \( t_{peak} \) was set at 0.2 ms and \( g_{peak} \) was assigned a value of 5.0 nS based on the experimental data reported by Finkel and Redman (1983). For inhibitory synapses, \( t_{peak} \) and \( g_{peak} \) were increased to 0.65 ms and 9.0 nS, respectively. These values correspond to the time course and magnitude of the conductance caused by activation of single axons of Ia inhibitory interneurons (Stuart and Redman 1990). To mimic tonic, asynchronous activation of many synapses, these time-dependent synaptic conductances were replaced by a constant, equivalent time-averaged conductance change, \( \bar{g} \), as described by Bernander et al. (1991). \( \bar{g} \) is the integral of \( g(t) \) where

\[
\bar{g} = g_{peak}t_{peak} \exp(1)\int P_{release}
\]

and \( n \) is the number of synapses, \( f \) is the activation frequency, and \( P_{release} \) is the probability of neurotransmitter release (cf. Walmsley et al. 1998).

For most simulations, synapses were distributed uniformly (i.e., same number of synapses per unit area) over the entire dendritic tree and soma. Synaptic densities of excitatory and inhibitory synapses were equal and were limited to a maximum of one synapse/28.6 μm² based on the electron microscopic observations of Rose and Neuber-Hess (1991). The positions of the synapses were assigned using the following protocol. Beginning at the cell body, the cumulative area of successive compartments was determined. If addition of the next distal compartment resulted in a cumulative area exceeding a pre-defined value (e.g., for a density of 2% of the maximum number of excitatory synapses, there is one synapse/1,430 μm²), a synapse was randomly placed in that compartment or in one of the proximal compartments contributing to the predefined area value. If the cumulative area exceeded the prespecified area value, \( \bar{g} \) was adjusted to take into account the “excess area” contributed by the distal compartment. At low synaptic densities, the area of the last sequence of compartments that included the termination of a dendrite was invariably less than the predefined area interval. A “partial” synapse was randomly assigned to one of these compartments, where \( \bar{g} \) was determined by the ratio of the area of the compartments to the predefined area interval. The random assignment of synapses to the compartments contributing to each prespecified area was designed to avoid a systematic bias in synapse location that would have occurred if the synapses were placed on either the first or the last compartment within each prespecified area zone. An alternative scheme whereby each compartment was assigned a fraction of a synapse, based on the area of the compartment, would have achieved the same goal, but was rejected because it conflicted with our goal of building a model that was as anatomically realistic as possible. The current generated by each synapse (\( I_{syn} \)) was defined by

\[
I_{syn} = \bar{g}(E_{rev} - V_m)
\]

where \( V_m \) is the membrane potential and \( E_{rev} \) is the equilibrium potential. \( E_{rev} \) was set at 0 and −81 mV, respectively, for excitatory synapses and inhibitory synapses. The difference between \( E_{rev} \) and \( V_m \) is the driving potential.

Simulations were performed using Saber (Synopsys, Mountain View, CA) running in the Windows NT environment on a Pentium-

METHODS

The motoneurons used in the present study were selected from a collection of motoneurons stained in previous experiments (Rose and Neuber-Hess 1991; Rose et al. 1995). All experiments were conducted on adult cats. The experimental protocols were approved by the Queen’s University Animal Care Committee and were consistent with guidelines established by the Canadian Council of Animal Care. Motoneurons were identified antidromically by stimulating nerves that supply the dorsal neck muscles, biventer cervicis, and complexus. The methods for intracellular staining and tissue processing have been described in detail elsewhere (Rose and Cushing 2004). These methods were designed to minimize artifacts caused by osmotic stress (cf. Major 2001) and tissue shrinkage. Each motoneuron fulfilled the following criteria: distal dendrites could be traced to abrupt terminations, as opposed to a gradual, proximal to distal, loss of staining; dendritic beading was absent; there was no evidence of damage to the soma; differential shrinkage where the tissue shrinks more than the stained process, leading to curling of dendrites that travel in the z-axis (cf. Jaeger 2001), was absent.

Construction of compartmental models

The methods used to reconstruct and measure the lengths and diameters of intracellularly stained dendrites have been described in detail by Rose and Cushing (2004). These data were used to build compartmental models as delineated by Bui et al. (2003). The number of compartments ranged from 4,000 to 5,000. Specific internal resistivity was set at 70 Ωcm, the value of specific membrane capacitance was 1 μF cm⁻², and specific membrane resistivity was fixed at 6,000, 15,000, or 60,000 Ωcm².

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Simulations were performed using Saber (Synopsys, Mountain View, CA) running in the Windows NT environment on a Pentium-

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based computer (Carnevale et al. 1990). To calculate the total current reaching the cell body as a result of synaptic activity, the membrane potential of the cell body was voltage clamped to the resting membrane potential (assumed to be −64 mV). This procedure is equivalent to the experimental technique developed by Heckman and Binder (1988) and widely used by Binder and colleagues (for a review, see Binder et al. 1996) to determine the effective synaptic current produced by tonic activation of a wide variety of segmental and descending connections to lumbar sacral motoneurons. Thus the values of the simulated currents can be compared directly to those obtained experimentally. The use of $g$ as a substitute for $g(t)$, as defined in Eq. 1, greatly reduced the computational time required to calculate the current reaching the soma. To verify the equivalence of these versions of synaptic conductance, we compared the behavior of several models in which $g(t)$ was substituted for $g$.

In the $g(t)$ models, the synapses were activated asynchronously at the same frequency as in the $g$ models. The average current reaching the soma in the $g(t)$ models was identical to the steady-state current calculated using $g$.

Data analysis

The current reaching the soma was determined after activating 4, 8, 16, 32, 48, 64, or 100% of the total number of excitatory or inhibitory synapses. $f$ and $P_{\text{release}}$ were fixed at 100 Hz and 0.5, respectively, to mimic a high level of presynaptic activity and experimental data showing that $P_{\text{release}}$ of synapses on motoneurons can range from 0 to 1 (Clamann et al. 1989; Edwards et al. 1976; Redman and Walmsley 1983). Because we used a time-averaged conductance change (see Eq. 2), for the same $g$, $f$, $P_{\text{release}}$, and percentage of activated synapses are reciprocally related (e.g., activation of 16% of the excitatory synapses at 100 Hz with a $P_{\text{release}}$ of 0.5 is equivalent to activation of 8% of the excitatory synapses at 200 Hz with a $P_{\text{release}}$ of 0.5).

The derivative of the relationship between the current reaching the soma and the number of active synapses provides a measure of the current delivered by each successive synapse. Calculation of the derivative involved two steps. 1) If the synapses were excitatory or a mixture of excitatory and inhibitory synapses, the data were fitted using

$$I_{\text{soma}} = y_0 + a \ln(n - x_0)$$

(4)

where $I_{\text{soma}}$ is the current reaching the soma. The regressions were performed by SigmaPlot (SPSS, Point Richmond, CA) and the $r^2$ values were $>0.999$. If the synapses were all inhibitory or the value assigned to $g_{\text{peak}}$ of excitatory synapse depended on the distance from the soma, this equation provided a poor fit of the data. For these simulations, the data were fitted using

$$I_{\text{soma}} = y_0 + a \ln(n - x_0) + b[\ln(n - x_0)]^2 + c[\ln(n - x_0)]^3$$

(5)

The $r^2$ values based on this equation were always $>0.999$. Equations 4 and 5 were used to generate values of the current reaching the soma at intervals of 0.4% of the total number of excitatory or inhibitory synapses. 2) A “diff” function supplied by SigmaPlot was used to calculate the derivative of these data and thus the current delivered by each successive synapse as a function of the number of active synapses (see Fig. 3B). The derivative of the relationship between current added with each successive synapse and the number of active synapses is equal to the current lost as a result of nonlinear summation with each successive synapse. To calculate this derivative, the derivative of Eq. 4 or Eq. 5 at 4, 8, 16, 32, 48, 64, or 100% of the total number of excitatory or inhibitory synapses was fitted using

$$\text{Current added with each successive synapse} = a \exp[b(n + c)]$$

(6)

The “diff” function was used to calculate the derivative of Eq. 6. The current lost as a result of nonlinear summation was expressed as a percentage of the current delivered to the soma in the absence of nonlinear summation (see Fig. 3C).

RESULTS

Morphological database

Compartmental models were constructed for three motoneurons. These motoneurons were selected for two reasons. 1) Their qualitative (i.e., dendritic distribution) and quantitative (i.e., dendritic tree surface area) features were typical of a much larger population of motoneurons innervating the same muscle group (Rose 1981; Rose and Odlozinski 1998). 2) Although similar in many respects, each cell had one or more distinctive features. Thus the characteristics of these cells reflected the variability in dendritic tree structure that is commonly seen within motoneurons innervating the same muscle (Cameron et al. 1983; Cullheim et al. 1987; Kernell and Zwaagstra 1989; Moritani et al. 2003; Rose 1982). These three motoneurons have also been the subjects of previous compartmental modeling studies designed to determine the attenuation of current and voltage signals within the dendritic trees of motoneurons (Bui et al. 2003; Rose and Cushing 2004).

Figure 1 shows the dendritic tree of one of the motoneurons. This cell (LVN 2-1) had a somatic surface area of 5,461 μm$^2$ and dendritic tree surface area of 451,580 μm$^2$. The other two cells had, respectively, somatic and dendritic tree surface areas of 6,019 and 387,020 μm$^2$ (LVN 4-1) and 7,718 and 430,040 μm$^2$ (LAD 5-4). The dots in Fig. 1A represent the locations of 640 synapses based on a uniform distribution pattern (i.e., same number of synapses per unit surface area throughout the neuron). Assuming a total innervation density of seven synapses/100 μm$^2$ (see METHODS), these synapses represent only 4% of the total number of excitatory synapses on this cell. If the locations of 48% of all excitatory synapses ($n = 7679$) are added to the dendritic tree, the dots depicting each synapse provide a detailed outline of the dendritic tree (Fig. 1B). At the maximum density of synapses, seven synapses/100 μm$^2$, the three motoneurons selected for this study had a total of 27,512 (LVN 4-1), 30,103 (LAD 5-4), and 31,611 (LVN 2-1) synapses, of which <2% were located on the soma.

The linear versus nonlinear summation of synaptic currents will be strongly influenced by the distance between adjacent synapses (e.g., Rall 1964; Rall et al. 1967). Based on our assumption of a uniform distribution, the distance between synapses is determined by the density of synapses and the diameter of the dendrites. As shown in Fig. 2A, at a density of 4%, the distance between synapses on the same branch varied from <50 to >400 μm. These distances decreased inversely with synaptic density. The distribution of intersynapse distances was similar for all three cells. However, because of slightly smaller dendritic diameters, intersynapse distances for LVN 4-1 were typically greater than those of the other two cells [e.g., at a density of 4%, the median values were 130 μm (LVN 4-1) vs. 101 and 111 μm for LAD 5-4 and LVN 2-1, respectively; Fig. 2A]. The extent of the voltage clamp into the dendritic tree will also influence the nonlinearity of synaptic current summation because summation of synaptic currents...
will be linear if the membrane potential is constant (i.e., a perfect voltage clamp). In a passive dendritic tree, this parameter is determined by the electrotonic length of the dendrite (Rall 1977). In our sample population of three motoneurons, significant variability in electrotonic length was noted, reflecting the importance of even subtle differences in motoneuron morphology, such as dendritic surface area and branching pattern. This is shown in Fig. 2B. The electrotonic distance from the soma to the location of an “average” synapse (defined by the distance along a dendritic trajectory from the soma to a dendritic terminal at which 50% of the surface area is proximal and 50% is distal; cf. Rose and Cushing 2004) was 30% longer for synapses on LVN 4-1 than on LAD 5-4. The electrotonic distance to an “average” synapse on the dendritic tree of LVN 2-1 was typically between those of LAD 5-4 and LVN 4-1.

The relationship between excitatory synaptic activity and the magnitude of synaptic current reaching the soma

Figure 3A shows the magnitude of current reaching the soma of LVN 2-1 under three different conditions. In the first condition, all synapses were arbitrarily placed on the soma and assigned a constant driving potential (i.e., equivalent to constant current injection devices). As expected from the parameters assigned to each synapse (see METHODS), each synapse delivered 8.7 pA to the soma. Thus the total current from all
synapses and the current reaching the soma was highly nonlinear and the maximum current delivered by all synapses fell to 39.2 nA. This decrease was a direct result of current “lost” (indicated by the dark gray zone in Fig. 3A) as a result of decreases in the driving potential.

The slopes of the relationships shown in Fig. 3A represent the current added with each successive synapse (see METHODS). As shown in Fig. 3B, under the more physiological condition of a variable driving potential, the current added with each successive synapse diminished as additional synapses were activated. As a means of quantifying the impact of nonlinear summation on the current reaching the soma, we calculated the number of synapses that were active when the current/synapse reaching the soma fell to 50% of the current delivered with a constant driving potential. For LVN 2-1, this number (4,312) corresponded to 26.6% of the total number of synapses. At this level of activity, the current lost as a result of nonlinear summation, 2.9 pA/synapse, was identical to the current lost because of cable properties (indicated by the light gray zone on Fig. 3B). At maximal synaptic activity, the current lost as a result of nonlinear summation (indicated by the dark light gray zone on Fig. 3B) was almost 80% of the current delivered by the first active synapse. We also calculated the percentage of current lost as a result of nonlinear summation after successive activation of groups of 100 synapses (see METHODS). As shown in Fig. 3C, the most pronounced impact of nonlinear summation occurred at the lowest levels of synaptic activity. However, in absolute terms, this impact was relatively modest (e.g., the current delivered by the first set of 100 synapses was only 2.3% less than that delivered by the same synapses in the absence of nonlinear summation) and it decreased to <0.5% when more than 5,000 synapses were activated. Thus at intermediate to higher levels of synaptic activity, the relationship between the current reaching the soma and the number of active synapses became progressively more linear.

The transition from nonlinear to linear summation was a direct result of progressively smaller changes in the driving potential as more synapses were recruited. Figure 4 shows the relationship between the membrane potential and distance along a representative dendrite of LVN 2-1. At low levels of synaptic activity, doubling the number of active synapses, say from 4 to 8%, increased the mean membrane potential (weighted by the area of each dendritic compartment) by 4.0 mV. This increase was almost equal to the increase in mean membrane potential caused by increasing the number of active synapses from 0 to 4%, 5.0 mV. In contrast, raising the number of active synapses from 20 to 24% (i.e., the same increase in absolute number of synapses) changed the mean membrane potential by only 1.8 mV. At higher levels of synaptic activity, say from 60 to 64%, the membrane potential throughout the dendrite approached a voltage-clamp state where the mean change in membrane potential was only 0.9 mV. Under these conditions, the driving potential is, in effect, constant and each synapse delivers the same current regardless of the number of active synapses.

Table 1 compares the delivery of excitatory synaptic current to the soma for three motoneurons. The current lost as a result of nonlinear summation played a major role in reducing the synaptic current reaching the soma in all three cells. However, the magnitude of this role varied from cell to cell. In terms of current lost as a result of nonlinear summation and cable

![Figure 2](https://example.com/figure2.png)

**Fig. 2.** A: cumulative histograms of the distance between adjacent synapses for each of the motoneurons used to construct compartmental models. Innervation density was 4% of all excitatory synapses. Small differences between each cell stemmed from small differences in dendritic diameters. B: cumulative histograms of the electrotonic distance between an “average” synapse and the soma. To generate these data, the dendritic tree of each motoneuron was subdivided into many dendritic paths where each path began at the soma and ended at a different dendritic terminal. Average electrotonic distance of synapses on each dendritic path was defined as the distance from the soma where 50% of the surface area was proximal and 50% was distal. “Average” synapses on LVN 2-1 were closer to the soma than those on LVN 4-1, but further from the soma than those on LAD 5-4.
properties, LVN 2-1 (data illustrated in Figs. 3 and 4) was midway between the other two cells. Table 1 also summarizes the current reaching the soma when the membrane potential at the soma was voltage clamped to \(-55\) mV. These simulations were designed to mimic the quasi–voltage clamp that exists at the soma during rhythmic activity of motoneurons (Koch et al. 1995). Under these conditions, the current delivered to the soma by maximal excitatory synaptic activity (i.e., activation of 100% of all excitatory synapses activated at 100 Hz, \(P_{\text{release}} = 0.5\)) was as little as 23.9 nA (LVN 4-1, Table 1).

The relationship between inhibitory synaptic activity and the magnitude of synaptic current reaching the soma

The time-averaged conductance (\(g\)) of our model of inhibitory synapses was almost sixfold larger than \(g\) of the excitatory synapses. This difference, when combined with a driving potential that was four times smaller (at a resting membrane potential of \(-64\) mV), and assuming a constant driving potential, resulted in a 50% increase in \(I_{\text{syn}}\). However, as shown in Fig. 5A and summarized in Table 1, if the constraint of a constant driving potential was removed, the total inhibitory current reaching the soma was less than the total excitatory synaptic current. This was the consequence of a precipitous decline in the current added with each successive synapse as more inhibitory synapses were activated (Fig. 5B), such that after activating only 4.7% of all available excitatory synapses, the next

FIG. 3. Relationships between the number of active excitatory synapses and the current reaching the soma of LVN 2-1. All synapses were activated at 100 Hz with \(P_{\text{release}} = 0.5\). As shown in the lowest x-axis, these results were equivalent to activating 100% of the synapses over the frequency range of 0 to 100. A: total current reaching the soma. Light gray zone indicates the loss of current attributed to cable properties. Dark gray zone illustrates the additional loss arising from decreases in driving potential. B: current delivered to the soma with each additional active synapse. Gray zones are as described in A. Open circle indicates that point at which the current added with each successive synapse fell to 50% of the current delivered by synapses with a constant driving potential. This point corresponded to activation of 26.6% of all available excitatory synapses. C: loss of current resulting from nonlinear summation after activation of successive groups of 100 synapses. This loss was expressed as the percentage of the current reaching the soma in the absence of nonlinear summation. Note that the largest loss occurred at the lowest levels of excitatory synaptic activity.
Synaptic Input | Parameter | LAD 5-4 | LVN 2-1 | LVN 4-1
--- | --- | --- | --- | ---
Excitatory (inhibitory) | Current at 100% active synapses (nA) | 47.9 (−34.2) | 39.2 (−25.7) | 28.9 (−19.0)
 | % current loss due to cable properties | 25.6 | 32.6 | 39.5
Soma voltage clamp −64 mV | % of synapses active at 50% current loss due to nonlinear summation | 35.9 (6.8) | 26.6 (4.7) | 22.5 (4.6)
 | % current loss due to cable properties | 25.6 | 32.6 | 39.5
 | % of synapses active at 50% current loss due to nonlinear summation | 35.9 (6.8) | 26.6 (4.7) | 22.5 (4.6)
 | % current loss due to nonlinear summation at 100% active synapses | 71.1 (88.4) | 77.9 (91.2) | 79.3 (91.8)
 | Maximum % of current lost due to nonlinear summation/100 synapses | 1.8 (8.0) | 2.3 (10.3) | 2.9 (12.3)
Excitatory Soma voltage clamp −55 mV | Current at 100% active synapses (nA) | 39.6 | 32.1 | 23.9

Nonlinear summation was greater for currents generated by pairs of inhibitory inputs than by pairs of excitatory inputs. For example, the ratio of actual to predicted current fell to 0.9 if two equal sets of inhibitory inputs, each consisting of 240 synapses, were activated on LVN 2-1. However, despite the more obvious nonlinear summation of inhibitory synaptic currents, the maximum degree of nonlinearity as expressed by the ratio of actual to predicted current was 0.70 to 0.73. This ratio is much less than expected because, at this level of synaptic activity (100% activation), the inhibitory synaptic current lost as a result of nonlinear summation reached 90% (Table 1).

There was one combination of inputs where nonlinear summation was easily detected using the ratio of actual to predicted current. As shown in Fig. 6C, combinations of excitatory and inhibitory synapses invariably generated less current than predicted by the linear sum of the currents generated by each input alone. The disparity between actual and predicted current was obvious at low levels of synaptic activity. For example, the predicted sum of a combination of only 792 inhibitory synapses (5% of all inhibitory synapses) and 930 excitatory synapses (5.9% of all excitatory synapses) was −4.8 + 4.8 = 0 nA. The actual current delivered to the soma was −1.2 nA.

**Sensitivity analysis**

**EFFECT OF CHANGES IN SPECIFIC MEMBRANE AND SPECIFIC INTERNAL RESISTIVITY ON EXCITATORY SYNAPTIC CURRENT REACHING THE SOMA.** All of the previous simulations were conducted in models with a specific internal resistivity of 70 Ωcm and a specific membrane resistivity of 15,000 Ωcm². These values may underestimate the true value of these parameters (Barrett and Crill 1974a; Svirskis et al. 2001; Thurbon et al. 1998; Ulrich et al. 1994). To investigate the consequences of higher values of specific membrane and specific internal resistivity, we constructed two additional models for each motoneuron. One model had a specific internal resistivity of 300 Ωcm and the other model had a specific membrane resistivity of 60,000 Ωcm².

Figure 7A summarizes the effects of these changes on the current reaching the soma of LVN 2-1. Cable theory predicts that increasing specific membrane resistivity should decrease the current lost en route to the soma (Rall 1977). In keeping with this prediction, the current delivered to the soma by the first active synapse was 30.7% greater in the model with a
specific membrane resistivity of 60,000 Ωcm². However, this gain rapidly dissipated as a result of nonlinear summation. By the 5,000th synapse, the current added per synapse was the same as that in the 15,000 Ωcm² model. As a consequence, the total current reaching the soma at maximal excitatory synaptic activity was 42.1 nA, a net gain of only 1.9 nA. Qualitatively similar results were found for LVN 4-1 and LAD 5-4.

Increasing specific internal resistivity to 300 Ωcm increased the current lost as a result of cable properties and nonlinear summation (Fig. 7A). The current delivered by the first active synapse was 43.0% smaller than that in the model with a specific internal resistivity of 70 Ωcm. The current added with each successive synapse fell further because of nonlinear summation. As a result, the maximum current reaching the soma was only 19.2 nA, a reduction of 20.0 nA. Changes in the current delivered to the somata of LVN 4-1 and LAD 5-4 followed a similar pattern.

NONLINEAR SUMMATION OF SYNAPTIC CURRENTS IN THE PRESENCE OF "BACKGROUND" EXCITATORY AND INHIBITORY SYNAPTIC ACTIVITY. The starting point of all of the previous simulations was zero synaptic activity. Because it is very unlikely that synaptic activity is zero at any time, we developed three models that were designed to mimic different levels of background synaptic activity. Each model consisted of a combination of excitatory and inhibitory synaptic activity where the net synaptic current was zero at a membrane potential of −64 mV. A “low” level of background synaptic activity was generated by coactivating 5% of the inhibitory synapses at 50 Hz (P_release = 0.5) and 4% of the excitatory synapses at 100 Hz (P_release = 0.5). “Intermediate” and “high ” levels were mimicked by doubling or quadrupling the number of active synapses used in the “low”-level model. The inclusion of background synaptic activity had two consequences, which are illustrated for LVN 2-1 (Fig. 7B). First, the current added with each successive synapse decreased as the level of background synaptic activity increased. Second, at the high level of background synaptic activity, the current added with each successive synapse was relatively immune to the number of active synapses. Similar results were observed for the other two cells. Thus in the presence of background synaptic activity, the membrane potential of the dendritic tree approaches a quasi–voltage clamp and, because of the absence of large changes in driving potential, nonlinear summation is greatly reduced. This effect reduced the ability to detect nonlinear summation in typical experimental settings where the current delivered by coactivating two inputs is compared with the linear sum of the currents delivered by each input alone (i.e., Fig. 6A). For example, in the presence of the high level of background synaptic activity on LVN 2-1, the ratio of actual to predicted currents was 0.9 if two inputs, each consisting of 4,042 synapses, were activated (data not shown). In contrast, it required only 1,236 + 1,236
active synapses to achieve the same level of nonlinear summation in the absence of background synaptic activity.

**EFFECT OF INCREASING **$g_{\text{peak}}$** OF EXCITATORY SYNAPSES LOCATED ON THE DISTAL ONE THIRD OF THE DENDRITIC TREE.** In all of the previous models, we assumed that the peak conductance per synapse is independent of synapse location. However, based on measurements of excitatory postsynaptic potentials (EPSPs) generated by single Ia afferents, Iansek and Redman (1973) and Jack et al. (1981) suggested that synapses located on distal dendrites of motoneurons have larger peak conductances (see also Pierce and Mendell 1993). In other types of neurons there is very compelling evidence for synaptic scaling whereby the peak conductance per synapse increases with distance from the soma (Adrasfalvy et al. 2001; Magee and Cook 2000). Because our estimate of $g_{\text{peak}}$ was based on measurements of somatic excitatory postsynaptic currents (EPSCs) (cf. Finkel and Redman 1983), the relatively small values of total synaptic current may be a consequence of underestimating the peak conductance of synapses located distally.

To address this possibility, the peak conductance for all synapses on the distal one third of the dendritic tree was increased two- or fourfold (i.e., $g_{\text{peak}}$ 10 or 20 nS). The additional current arriving at the soma as a consequence of the increase in $g_{\text{peak}}$ was very modest. At maximal synaptic activity, the increase ranged from 0.3 to 1.0 nA for the 10-nS models and 0.5 to 1.8 nA for the 20-nS models. This seemingly counterintuitive result was a consequence of the interaction between the increase in current resulting from the larger values of $g_{\text{peak}}$ and the greater loss of current resulting from a much larger nonlinear summation of synaptic currents. At low levels of synaptic activity on LVN 2-1, the current per synapse delivered to the soma increases in proportion to the increase in $g_{\text{peak}}$ (Fig. 7C). However, as more synapses were activated the additional current per synapse fell sharply until all three models delivered nearly the same current per synapse to the soma. Indeed, after nearly 15% of all of the available synapses were activated, the additional current from each synapse was less in the models with higher peak conductances for synapses on the distal one third of the dendritic tree.

**COMPARISON OF CURRENT LOSS AS A RESULT OF CABLE PROPERTIES AND NONLINEAR SUMMATION FOR SYNAPSES DISTRIBUTED ON PROXIMAL ONE THIRD, MIDDLE ONE THIRD, AND DISTAL ONE THIRD OF THE DENDRITIC TREE.** Because of the proximity of synapses on proximal dendrites to the voltage clamp applied at the soma, it would be expected that changes in driving potential arising

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**FIG. 6.** Current reaching the soma during concurrent activation of 2 sets of synapses (i.e., A and B, actual sum) compared with the linear sum of the currents generated by each set alone (i.e., A + B, predicted sum). A: summation of combinations of A and B where A consisted of $n$ excitatory synapses and B consisted of $n$, $2n$, or $4n$ excitatory synapses. Each data point corresponds to a simulation where $n$ was assigned a multiple (1, 2, 4, 8, etc.) of 62 (0.4% of all excitatory synapses). Maximum number of activated synapses corresponded to 100% of all excitatory synapses. LVN 2-1 was used in these simulations. B: summation of excitatory (to the right of the origin) or inhibitory (to the left of the origin) inputs for all 3 motoneurons. A and B contained the same number of synapses ($n$). Each data point corresponds to a simulation where $n$ was assigned a multiple of 0.4% of all excitatory or inhibitory synapses. C: summation of 2 inputs, one excitatory, the other inhibitory, on LVN 2-1. All synapses were activated at 100 Hz. $P_{\text{release}} = 0.5$. For each level of inhibitory synaptic activity, 396 vs. 792 vs. 1,584 active synapses, the number of active excitatory synapses was varied from 62 to 2,418, 62 to 2,976, and 62 to 3,944, respectively, in multiples of 62.
from synaptic activity will be less on proximal compared with distal dendrites. Thus nonlinear summation should be larger for more distal synapses. To test this prediction, we divided the dendritic tree into three equal zones. Each zone contained one third of the total dendritic area and the boundaries between the zones corresponded to the distance from the soma at which the cumulative surface area reached 33.3 or 66.7% of the total dendritic surface area. Within each zone, the synapses were distributed uniformly (i.e., same number per unit area). No synapses were placed on the soma in these models.

Figure 8A illustrates the key findings for LVN 2-1. When expressed as a ratio of the current delivered by the first active proximal synapse, the relative efficacy of current delivery for proximal, intermediate, and distal synapses was 1:0.80:0.62 for LVN 2-1. This result is a simple consequence of the increase in current loss en route to the soma, as predicted by cable theory (Rall 1977). The effect of nonlinear summation also depended on the distribution of the synapses. The current added with each successive synapse on the proximal one third of the dendritic tree was relatively independent of the number of active synapses. In contrast, the current added with each successive synapse fell sharply as more synapses were activated in the intermediate and distal dendrites. As a consequence, nonlinear summation caused a large decrease in the relative efficacy of current delivery from synapses on intermediate and distal dendrites, such that at maximal synaptic activity, the relative efficacies of the current delivered per synapse were 1:0.37:0.17. Qualitatively similar results were found for LVN 4-1 and LAD 5-4.

We also extended the simulations of experimental measures of nonlinear summation (i.e., activation of two sets of synapses each consisting of the same number of synapses) to synapses distributed on the proximal one third, middle one third, and distal one third of the dendritic tree. Nonlinear summation was more easily detected if the active synapses were located on the distal one third of the dendritic tree (Fig. 8B). However, this result is deceptive. To generate a predicted sum of currents equal to 5 nA (corresponding to a ratio of actual to predicted current of 0.82), 709 synapses had to be activated. These synapses represented 25% of all excitatory synapses found on the distal one third of the dendritic tree.

DISCUSSION

The study was designed to resolve a paradox: experimentally, nonlinear summation of synaptic currents in motoneurons is negligible or, at the most, modest (e.g., Binder and Powers 2000), yet predictions based on cable theory (e.g., Koch 1999) make a compelling case that changes in driving potential should lead to large nonlinearities in the summation of synaptic currents. Is this discrepancy a result of fundamental flaws in the models based on cable theory (i.e., simplified or artificial versions of the actual geometry of motoneuron dendritic trees) or the presence of other factors (i.e., voltage-dependent channels) found in real neurons, but absent in models based solely on cable theory? To address these questions, we constructed compartmental models of motoneurons whose dendritic anatomy, physiological characteristics of synaptic conductances, and number of synapses were highly constrained by experimental data. Voltage-dependent membrane properties were deliberately excluded from these models. Thus we could di-
directly determine whether the presence of these channels was a necessary prerequisite for masking of nonlinear summation. We also took advantage of a unique opportunity afforded by compartmental models to build a control representation of synapses that generated current whose magnitude was independent of changes on driving potential or, as in the case of physiological synapses, directly related to the driving potential. These control models provided a direct means of measuring the impact of changes in driving potential on the delivery of synaptic current to the soma.

The results of this study demonstrate that the synaptic current reaching the somata of spinal motoneurons is not a simple linear sum of the current delivered by each synapse activated in isolation. Instead, changes in driving potential arising from activity in nearby synapses cause a highly sublinear summation of synaptic currents. As a consequence, the total current reaching the soma is a small fraction of the expected current based on each synapse acting as an independent unit. Unexpectedly, when the same model was used to measure currents generated by two populations of excitatory synapses, each designed to mimic typical inputs to motoneurons, the response of the two inputs activated together approached the linear sum of the responses seen when the inputs were activated in isolation. Thus despite the presence of a highly sublinear summation of synaptic currents, this nonlinearity is largely invisible in typical experimental settings.

**Many synapses, little current**

Estimates based on electron microscopic observations and quantitative analyses of the size of motoneuron dendritic trees suggest that a single feline neck motoneuron receives 30,000 synapses, of which approximately one half are excitatory (Rose and Neuber-Hess 1991). Our simulations show that the total current reaching the soma from all excitatory synapses is <50 nA. In the presence of an intermediate level of background synaptic activity with a somatic potential of −55 mV, the maximum current falls to 30 nA. To put these numbers in perspective, the average rheobase current in triceps motoneurons in cats deeply anesthetized with pentobarbital is 21, 12, and 5 nA for fast fatigable, fast fatigue-resistant, and slow motoneurons, respectively (Zengel et al. 1985). The threshold for sustained discharge is 50\% higher (Kernell 1965). Thus our results suggest that it would require activation of all 15,000 excitatory synapses just to reach the threshold for sustained discharge, assuming that some of the motoneurons examined in this study were type FF and that the rheobases of neck and hindlimb motoneurons are similar. If the motoneurons examined in this study belong to slow or fast fatigue-resistant motor units, the minimum number of active synapses required for sustained discharge is smaller, but would still represent 30\% (n = 4,000) to 60\% (n = 9,000), respectively, of all excitatory synapses.

These results appear to defy common sense and raise questions regarding the validity of the compartmental models used in this study. Our models were based on three biventer cervicis/complexus motoneurons. The morphological characteristics of these cells are representative of a much larger sample of motoneurons innervating the same muscle group (discussed in detail by Rose and Cushing 2004). Moreover, differences between the electrotonic distances to an average synapse largely accounted for the cell-to-cell variability in the current reaching the soma. Thus it is unlikely that the three cells chosen for this study underestimate current delivery to a typical
biventer cervicis/complexus motoneuron. In addition, the currents predicted by our models are in good agreement with experimental measurements of synaptic currents recorded in hindlimb motoneurons (Powers and Binder 2001) and are similar to estimates based on simpler models of hindlimb motoneurons (Binder et al. 1996; Powers and Binder 2001). Adjusting key parameters of the compartmental models, such as the specific membrane resistivity, the distribution of synapses, and $\delta$peak of the distal synapses (cf. Iansek and Redman 1973; Magee and Cook 2000), did not significantly increase the synaptic current delivered to the soma. Indeed, the value assigned to internal resistivity, 70 $\Omega$cm, is low compared with many estimates (Rose and Cushing 2004) and increasing this parameter caused a large decrease in the current reaching the soma. All of these observations attest to the validity of our models and therefore justify the conclusion that despite thousands of excitatory synapses, the current generated by these synapses is insufficient to generate a meaningful level of force production.

The logistics of tonically activating 5,000 to 10,000 synapses would seem to preclude the possibility that motoneurons rely on this massive synaptic bombardment as a routine mechanism for achieving sustained activity. Perhaps the most obvious solution to this problem is the activation of persistent inward currents. It is now well established that in the presence of monoamines, L-type calcium channels and persistent sodium channels on the dendrites of motoneurons generate sodium and calcium currents that can amplify synaptic currents by four- to sixfold (for recent reviews see, Heckman et al. 2003, 2004). Our results suggest that these intrinsic currents are not just an alternative for the current generated by ionotropic synapses; they constitute an essential prerequisite for the execution of purposeful movement. This conclusion is also consistent with the relatively modest magnitude of currents generated by activation of many descending and segmental afferents in the absence of monoaminergic drive (Binder et al. 1996; Powers and Binder 2001).

There are other solutions. The models used in this study assumed that the inputs were asynchronous. Even modest levels of synchrony in the discharge of afferents could be expected to increase motoneuron activity (Bernander et al. 1991). Segev et al. (1990) showed that simultaneous or nearly simultaneous activation of 300 synapses with the same properties as those used in this study could generate peak synaptic currents of more than 50 nA in triceps surae motoneurons. In contrast, asynchronous activation of 300 synapses in our models produced a mean current of <2 nA. However, it is unclear to what degree and under what circumstances synchronization of afferent activity is used to boost motoneuron activity. It is known that synchronization of Ia afferents is relatively weak (Durbaba et al. 2003; Hamm et al. 1985). Thus this mechanism of boosting the influence of synaptic currents may be specific to certain types of afferents. Because of their voltage-dependent properties, N-methyl-D-aspartate (NMDA) channels may represent another means of amplifying synaptic currents (Ascher and Nowak 1988). These channels are activated by stimulation of dorsal roots (Pinco and Lev-Tov 1993) and some descending systems (Pinco and Lev-Tov 1994). Furthermore, by modulating the activity of other voltage-dependent channels, such as L-type calcium channels, activation of NMDA channels may further increase the total current reaching the soma (Guertin and Hounsgaard 1998; MacLean et al. 1997). Nevertheless, like synchronization, the exact contribution of NMDA channels is unclear because the number of synapses using NMDA receptors and the time course and peak conductance associated with individual synapses are unknown.

**Input–output properties of motoneurons**

The nonlinear relationship between the input, defined here as the number of active excitatory synapses, and the output, defined here as the synaptic current reaching the soma, means that the same input generates a progressively weaker output as the total synaptic activity increases. Because the steady-state frequency of motoneuron discharge is related in a quasi-linear fashion to the synaptic current reaching the soma (Granit et al. 1963, 1966; Schwindt and Calvin 1973; for a review see Binder et al. 1996), the response of the motoneuron, in terms of its steady-state discharge frequency, will also saturate. This would appear to impose a serious problem for the control of motoneuron activity and demand the addition of compensating voltage-dependent membrane properties, such as those that have been reported for pyramidal neurons (Bernander et al. 1994; Cash and Yuste 1998, 1999; Urban and Barionuevo 1998). However, our results unexpectedly provide a partial solution to this problem based solely on passive membrane properties. Although the current added with each successive synapse decreases as the total number of active synapses increases, this decrease becomes progressively smaller, such that over the last part of the input–output relationship, the current delivered to the soma on a per synapse basis becomes relatively constant and the percentage of current lost as a result of nonlinear summation with each successive set of 100 synapses diminishes to <0.5%. This transition is particularly striking for inhibitory synapses. In the presence of a modest level of balanced excitatory and inhibitory activity, as might be expected to occur under most physiological conditions (Barrett 1975; Bernander et al. 1991; Berg-Graham et al. 1998; Des-texhe and Paré 1999; Paré et al. 1998), the relationship between the number of active synapses and the total synaptic current reaching the soma is also more linear, albeit with a lower value of total current. It is also possible to linearize the input–output properties of motoneurons by distributing the synapses on proximal synapses.

However, perhaps the most striking example of a linear input–output relationship is the summation of currents generated by two sets of excitatory inputs. In this typical experimental setting, it is common practice to compare the response of two inputs activated alone to the response of the two inputs activated together. Our simulations of these types of experiments indicate that summation of excitatory or inhibitory synaptic currents is remarkably close to linear, especially over the range of currents that are commonly recorded in motoneurons (Binder et al. 1996). These results parallel direct measurements of the summation of synaptic currents (Powers and Binder 2000) and indicate that the effective relationship between the number of active synapses and the total synaptic current reaching the soma is largely immune to nonlinearities introduced by the loss of synaptic current arising from changes in driving potential.
Experimental measures of the current lost as a result of nonlinear summation of synaptic currents

The results of this study demonstrate that nonlinear summation of synaptic currents leads to a significant decrease in the delivery of synaptic current to the soma. Yet, it is also apparent that this decrease is largely invisible in experiments that compare the summation of current evoked by stimulating two sets of synaptic inputs alone versus concurrently. The loss of synaptic current is most obvious if small groups of synapses (ideally one at a time) are activated sequentially as simulated in this study. However, it is difficult to imagine how this protocol could be repeated in an experimental setting. There is one experiment that is more tractable; the nonlinear addition of excitatory and inhibitory inputs. The sum of these inputs is highly nonlinear. Assuming that excitatory and inhibitory inputs can be activated separately and the distributions of the excitatory and inhibitory synapses overlap, this special case provides an experimental means of demonstrating the loss in current as a result of nonlinear summation. However, it should be recognized that this is only a partial solution in that this measure of nonlinearity underestimates the true current lost as a result of nonlinear summation. Thus a complete solution to this dilemma awaits the development of experimental techniques that provide an estimate of the current delivered to the soma in the absence of changes in driving potential, i.e., equivalent to changing physiological synapses into constant current injection devices as implemented in our compartmental models.

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