Effect of non-linear summation of synaptic currents on the input/output properties of spinal motoneurons

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Running head: Non-linear summation of synaptic currents

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ABSTRACT

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 neighbouring synapses. This interaction introduces an intrinsic non-linearity 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 non-linearity 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’ due to local changes in driving potential was substantial and resulted in a highly non-linear 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 decreased the current delivered to the soma further, but reduced the non-linearity with respect to the total number of active excitatory synapses. Unexpectedly, simulations that mimicked experimental measures of non-linear summation, activation of two sets of excitatory synapses, resulted in nearly linear summation. This result suggests that non-linear summation can be difficult to detect, despite the substantial ‘loss’ of current due to non-linear 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 dependence on driving potential imposes an intrinsic non-linearity 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 non-linearity 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 non-linear summation in spinal motoneurons have employed two different strategies: predictions based on cable theory or experimental observations. All of the experimental data leads to the conclusion that non-linear summation of synaptic inputs to motoneurons is negligible or, at the most, modest (Burke 1967; Burke et al. 1971; Clements at al. 1986; Powers and Binder 2000; Kuno and Miyahara 1969; Rall et al. 1967; Skydsgaard and Hounsgaard 1994; see however, 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 (Binder and Powers, 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 neighbouring synapses due to 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 and 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; see however, Segev and Burke 1990). For example, the sum of the currents delivered by simultaneous activation of 4 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 non-linear summation open to debate. It could be argued that the experimental data deserves a greater weight in this debate, but the interpretation of the experimental data is confounded by several poorly constrained variables, namely the number and distribution of activated synapses. Moreover, it is not clear what role voltage-dependent channels play in fixing the non-linear summation ‘bug’ (c.f. 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 (Korogod et al., 2000; Segev and Burke 1990), theoretical predictions have been based on simplified or artificial morphological versions of the dendritic tree of motoneurons. Since interactions between neighbouring synapses depend on dendritic structure (Abbott 1991; Koch, 1999), the applicability of these generic predictions to motoneurons is questionable. Furthermore, in most of the theoretical studies, the number of active synapses was not explicitly defined or only certain groups of synapses (e.g. distal) were considered.
In the present study, we have taken advantage of detailed measurements of the dendritic geometry of dorsal neck motoneurons in the cat (Rose and Odlozinski 1998) to construct morphologically realistic compartmental models. These models were further constrained by electron microscopic studies of the density of synapses on these cells (Rose and Neuber-Hess 1992) and physiological studies of the conductance and time course of excitatory and inhibitory synapses on hindlimb motoneurons (Finkel and Redman 1983; Stuart and Redman 1990). In a further effort to mimic experimental conditions, we adopted the same protocol developed by Heckman and Binder (1988) and widely used by Binder and colleagues (for a review see Binder et al. 1996) to measure the current reaching the soma in response to tonic activation of excitatory and inhibitory synapses. This protocol has two distinct advantages. First, the current reaching the soma is the primary factor governing the steady-state frequency of action potentials generated by a tonic barrage of presynaptic activity (Granit et al. 1963, 1966; Schwindt and Calvin 1973; for a review see Binder et al. 1996). Secondly, in a simulation, synapses can be represented by constant current injection devices (i.e. constant driving potential) or ‘physiological’ synapses where the driving potential depends on the local membrane potential. Thus, we could directly compare the current lost due to non-linear summation versus that lost due to leakage en route to the soma. Our results indicate that the non-linearity introduced by variable driving potentials at synapses on spinal motoneurons is substantial and plays a major in determining their input-output properties. Preliminary accounts of this study have appeared in abstract form and in a brief review (Rose and Cushing 1998, 1999).
METHODS

Experimental procedures

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 µFcm⁻¹ and specific membrane resistivity was fixed at 6000 or 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):

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

(1)

For excitatory synapses, \( t_{\text{peak}} \) was set at 0.2 msec and \( g_{\text{peak}} \) was assigned a value of 5.0 nS based on the experimental data reported by Finkel and Redman (1983). For inhibitory synapses, \( t_{\text{peak}} \) and \( g_{\text{peak}} \) were increased to 0.65 msec 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-dependant 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:

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

(2)

\( n \) is the number of synapses, \( f \) is the activation frequency and \( P_{\text{release}} \) 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 \( \mu \text{m}^2 \) 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/1430 \( \mu \text{m}^2 \)), a synapse was randomly placed in that compartment or in one of the proximal compartments that contributed to
the pre-defined area value. If the cumulative area exceeded the pre-specified area value, \( g \) 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 pre-defined area interval. A 'partial' synapse was randomly assigned to one of these compartments, where \( g \) was determined by the ratio of the area of the compartments to the pre-defined area interval. The random assignment of synapses to the compartments contributing to each pre-specified 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 last compartment within each pre-specified 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, \( i_{sy} \), was defined by:

\[
i_{sy} = \bar{g} (E_{rev} - V_m)
\]

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 Inc., Mountain View, CA) running in the Windows NT environment on a Pentium based computer (Carnevale et al. 1990). In order to calculate the total current reaching the cell body due to 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 his 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 lumbosacral motoneurons. Thus, the values of the simulated currents can be compared directly to those obtained experimentally. The use of $\bar{g}$ as a substitute for $g(t)$, as defined in equation 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 behaviour of several models in which $g(t)$ was substituted for $\bar{g}$. In the $g(t)$ models, the synapses were activated asynchronously at the same frequency as in the $\bar{g}$ models. The average current reaching the soma in the $g(t)$ models was identical to the steady-state current calculated using $\bar{g}$.

**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). Since we used a time-averaged conductance change (see equation 2), for the same $\bar{g}$, $f$ and $P_{\text{release}}$ and percent 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)

\( i_{\text{soma}} \) is the current reaching the soma. The regressions were performed by SigmaPlot (SPSS, Point Richmond, CA) and the \( r^2 \) values were above 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 above 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 due to non-linear summation with each successive synapse. To calculate this derivative, the derivative of equations 4 or 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 equation 6. The current lost due to non-linear summation was expressed as a percentage of the current delivered to the soma in the absence of non-linear 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 5461 µm² and dendritic tree surface area of 451,580 µm². The other two cells had, respectively, somatic and dendritic tree surface areas of 6019 µm² and 387,020 µm² (LVN 4-1) and 7718 µm² and 430,040 µm² (LAD 5-4). The dots in Figure 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 7 synapses/100 µm² (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, 7 synapses/100 µm², 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 less than 2% were located on the soma.

The linear versus non-linear 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 Figure 2A, at a density of 4%, the distance between synapses on the same branch varied from less than 50 µm to over 400 µm. These distances decreased inversely with synaptic density. The distribution of inter-synapse distances was similar for all three cells. However, due to slightly smaller dendritic diameters, inter-synapse distances for LVN 4-1 were typically larger than the other two cells (e.g. at a density of 4%, the median values were 130 µm (LVN 4-1) versus 101 µm and 111 µm for LAD 5-4 and LVN 2-1, respectively; Figure 2A). The extent of the voltage clamp into the dendritic tree will also influence the non-linearity of synaptic current summation, since 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 Figure 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, c.f. 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 exceeded 120 nA. In the second condition, the synapses were distributed uniformly such that the number of synapses per 100 µm² was the same throughout the somato-dendritic surface of the cell. Under these conditions, the same synapses delivered less current to the soma (5.8 pA/synapse; maximum 93.9 nA). This difference (indicated by the light grey zone on Figure 3A), is due to the current lost en route to the soma due to the cable properties of the dendrites. In the final condition, the synapses were also distributed uniformly, but the constraint of a constant driving potential was removed. Instead, the driving potential was determined by the local membrane potential at the site of each synapse. Under these conditions, the relationship between the number of active synapses and the current reaching the soma was highly non-linear 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 Figure 3A) due to decreases in the driving potential.

The slopes of the relationships shown in Figure 3A represent the current added with each successive synapse (see Methods). As shown in Figure 3B, under the more physiological condition of a variable driving potential, the current added with each successive synapse fell as additional synapses were activated. As a means of quantifying the impact of non-linear
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 (4312) corresponded to 26.6% of the total number of synapses. At this level of activity, the current lost due to non-linear summation, 2.9 pA/synapse, was identical to the current lost due to cable properties (indicated by the light grey zone on Figure 3B). At maximal synaptic activity, the current lost due to non-linear summation (indicated by the dark light grey zone on Figure 3B) was almost 80% of the current delivered by the first active synapse. We also calculated the percentage of current lost due to non-linear summation following successive activation of groups of 100 synapses (see Methods). As shown in Figure 3C, the most pronounced impact of non-linear 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 non-linear summation) and it decreased to less than 0.5% when more than 5000 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 non-linear 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, for example 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, e.g. 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 due to non-linear summation played a major role in the 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 due to non-linear summation and cable properties, LVN 2-1 (data illustrated in Figures 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_{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 ($\bar{g}$) of our model of inhibitory synapses was almost 6 times larger than $\bar{g}$ of the excitatory synapses. This difference, when combined with a 4 fold smaller driving potential (at a resting membrane potential of -64 mV), and assuming a constant
driving potential, resulted in a 50% increase in $i_i$.

However, as shown in Figure 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 due to a precipitous fall 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 inhibitory synapses, the next synapse delivered only 50% of the current delivered by the first synapse (Table 1). At maximal synaptic activity, the current delivered by the last activated synapse was less than 10% of the current delivered by the first synapse (Table 1). Similar to the results seen with excitatory synaptic activity, the most pronounced impact of non-linear summation occurred at the lowest levels of synaptic activity (Fig. 5C). However, in absolute terms, this impact was much larger when expressed as the current lost due to non-linear summation due to the activation of each successive 100 synapses (typically 10% versus 1.8 to 2.9%, Table 1). However, this loss of current decreased to less than 0.5% when only 15% of all inhibitory synapses were activated (Fig. 5C). Thus, the relationship between the current reaching the soma and the number of active inhibitory synapses was close to linear over most of the range of inhibitory synaptic activity.

Simulations of experimental measures of non-linear summation

In a typical experiment, non-linear summation is measured by stimulating two pathways, A or B, and comparing the linear sum of the responses to the response evoked by stimulating both pathways together. To mimic these conditions, we activated two uniformly distributed sets of synapses, A and B, where each set contained the same number of synapses (n), and compared the current delivered to the soma to the predicted linear sum of the currents delivered by activating A or B alone. As shown in Figure 6A, the current delivered by A and B was very close
to the linear sum of the currents, unless the predicted sum exceeded 10 nA. The ratio of actual to predicted current did not fall to 0.9 until 1236 + 1236 synapses were activated. We also explored the summation of synaptic inputs that were not equal, i.e. A consisted of n synapses and B consisted of 2n synapses or 4n synapses. Once again, summation of these inputs was close to linear (i.e. a ratio of actual to predicted current ≤ 0.9) unless the total number of active synapses exceeded 3000 synapses (Figure 6A).

Figure 6B summarizes the predicted versus actual currents for equal pairs of excitatory or inhibitory inputs for LVN 2-1, LVN 4-1 and LAD 5-4. All synapses were activated at 100 Hz, with \( P_{\text{release}} = 0.5 \). The relationship between predicted and actual currents was similar for all three cells, with the data from LVN 2-1 lying midway between LVN 4-1 and LAD 5-4. Non-linear summation was greater for currents generated by pairs of inhibitory inputs than 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 non-linear summation of inhibitory synaptic currents, the maximum degree of non-linearity as expressed by the ratio of actual to predicted current was 0.70 to 0.73. This ratio is much less than expected given that at this level of synaptic activity (100% activation), the inhibitory synaptic current lost due to non-linear summation reached 90% (Table 1).

There was one combination of inputs where non-linear summation was easily detected using the ratio of actual to predicted current. As shown in Figure 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 $-4.8 + 4.8 = 0 \text{ nA}$. The actual current delivered to the soma was $-1.2 \text{ nA}$.

**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 $\Omega \text{ cm}$ and a specific membrane resistivity of 15,000 $\Omega \text{ cm}^2$. 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 $\Omega \text{ cm}$ and the other model had a specific membrane resistivity of 60,000 $\Omega \text{ cm}^2$.

Figure 7A summarizes the effect 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 $\Omega \text{ cm}^2$. However, this gain rapidly dissipated due to non-linear summation. By the 5000$^{th}$ synapse, the current added per synapse was the same as in the 15,000 $\Omega \text{ cm}^2$ 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 due to cable properties and non-linear summation (Fig. 7A). The current delivered by the first active synapse was 43.0% smaller than in the model with a specific internal resistivity of 70 Ωcm. The current added with each successive synapse fell further due to non-linear 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.

**Non-linear 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. Since 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 co-activating 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. These consequences 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. Secondly, 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, due to the absence of large changes in
driving potential, non-linear summation is greatly reduced. This effect reduced the ability to detect non-linear summation in typical experimental settings where the current delivered by co-activating two inputs is compared to 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 4042 synapses, were activated (data not shown). In contrast, it only required 1236 + 1236 active synapses to achieve the same level of non-linear summation in the absence of background synaptic activity.

**Effect of increasing $g_{peak}$ 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 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 (Adrasfaly et al. 2001; Magee and Cook 2000). Since our estimate of $g_{peak}$ was based on measurements of somatic 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 2 or 4 fold (i.e. $g_{peak}$ 10 or 20 nS). The additional current arriving at the soma as a consequence of the increase in $g_{peak}$ 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 counter-intuitive result was a consequence of the interaction between the increase in current due to the larger values of $g_{\text{peak}}$ and the greater loss of current due to a much larger non-linear 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 approximately 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 due to cable properties and non-linear summation for synapses distributed on proximal one-third, middle one-third and distal one third of the dendritic tree** Due to the proximity of synapses on proximal dendrites to the voltage-clamp applied at the soma, it would be expected that changes in driving potential due to synaptic activity will be less on proximal compared to distal dendrites. Thus, non-linear 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 non-linear 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, non-linear 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 non-linear 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. Non-linear summation was more easily detected if the active synapses were located on the distal one-third of the dendritic tree (Figure 8B). However, this result is deceptive. In order to generate a predicted sum of currents equal to 5 nA (corresponding to a ratio of actual to predicted current of 0.82), 709 + 709 synapses had to be activated. These synapses represented over 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, non-linear 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 non-linearities in the summation of synaptic currents. Is this discrepancy due to 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 directly determine if the presence of these channels was a necessary pre-requisite for masking of non-linear 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 due to 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 sub-linear summation of synaptic currents, this non-linearity 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 less than 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 nA, 12 nA, 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 rheobase 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=4000) to 60% (n=9000), 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 $g_{peak}$ 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 to 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 4 to 6 fold (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 are 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 would 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 less than 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. NMDA channels, due to their voltage-dependent properties, 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, since the number of synapses utilizing NMDA receptors and the time course and peak conductance associated with individual synapses are unknown.

Input-output properties of motoneurons

The non-linear 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. Since 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 Barrionuevo 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 due to non-linear summation with each successive set of 100 synapses diminishes to less than 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; Borg-Graham et al. 1998; Destexhe 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 non-linearities introduced by the loss of synaptic current due to changes in driving potential.

*Experimental measures of the current lost due to non-linear summation of synaptic currents*

The results of this study demonstrate that non-linear 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 non-linear addition of excitatory and inhibitory inputs. The sum of these inputs is highly non-linear. 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 due to non-linear summation. However, it should be recognized that this is only a partial solution in that this measure of non-linearity underestimates the true current lost due to non-linear 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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FIGURE LEGENDS

FIG. 1. Morphological features of the compartmental model based on LVN 2-1. The dendritic tree (in grey) is shown in the horizontal (top panels) and transverse (bottom panels) planes. Each black dot represents one synapse. The synapses are arranged such that the number of synapses per unit area is the same throughout the cell. A: Example of a low innervation density (i.e. 4% of all excitatory synapses). B: Example of a high innervation density (i.e. 48% of all excitatory synapses). Note that the synapses appear to be sparse in the low innervation density model, despite the fact that this model contained 640 synapses. R – rostral; C – caudal; M – medial; L – lateral; D – dorsal; V – ventral.

FIG. 2. A: Cumulative histograms of the distance between adjacent synapses for each of the motoneurons used to construct compartmental models. The innervation density was 4% of all excitatory synapses. The small differences between each cell were due to 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. The 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. The ‘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.

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_{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. The light grey zone indicates
the loss of current due to cable properties. The dark grey zone illustrates the additional loss due
to decreases in driving potential. B: Current delivered to the soma with each additional active
synapse. Grey zones are as described in A. The 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 due to non-linear summation following activation of
successive groups of 100 synapses. This loss was expressed as the percentage of the current
reaching the soma in the absence of non-linear summation. Note that the largest loss occurred at
the lowest levels of excitatory synaptic activity.

FIG. 4. Relationship between distance from the soma and the local membrane potential along
the path to the dendritic terminal indicated by the asterisk in the dendrogram. As the level of
excitatory synaptic activity increased the change in local membrane potential became
progressively smaller: compare 4% to 8%; 20% to 24%; 60% to 64%.

FIG. 5. Relationships between the number of active inhibitory 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. The light grey zone indicates
the loss of current due to cable properties. The dark grey zone illustrates the additional loss due
to decreases in driving potential. B: Current delivered to the soma with each additional active
synapse. Grey zones are as described in $A$. The 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 4.7% of all available inhibitory synapses. $C$: Loss of current due to non-linear summation following activation of successive groups of 100 synapses. This loss was expressed as the percentage of the current reaching the soma in the absence of non-linear summation.

FIG. 6. Current reaching the soma of during concurrent activation of two sets of synapses (i.e. A and B, actual sum) compared to 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 $n$ or $2n$ 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). The 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 three 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 two 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 versus 792 versus 1584 active synapses, the number of active excitatory synapses was varied from 62 to 2418, 62 to 2976, and 62 to 3944, respectively, in multiples of 62
FIG. 7. Sensitivity analysis of the relationship between the number of active excitatory synapses and the current delivered to the soma with each additional active synapse in models of LVN 2-1. 

A: Effect of different values of specific membrane resistivity ($R_m$) or specific internal resistivity ($R_i$). B: Effect of different levels of background synaptic activity. Note that in the presence of high background synaptic activity (activation of 16% of the excitatory synapses at 100 Hz, $P_{\text{release}}=0.5$, and 20% of the inhibitory synapses at 50 Hz, $P_{\text{release}}=0.5$), the current/synapse was relatively independent of the number of active excitatory synapses. C: Effect of increasing $g_{\text{peak}}$ of synapses on the distal one-third of the dendritic tree from 5 nS, to 10 nS or 20 nS. $g_{\text{peak}}$ of all other synapse was set to 5 nS.

FIG. 8. A: Relationships between the number of active excitatory synapses and the current delivered to the soma with each additional synapse in models of LVN 2-1 with synapses restricted to the proximal, middle, and distal one-third of the dendritic tree. The open circles indicate the points 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 16.8% and 34.7% of all available excitatory synapses in the models with synapses on the middle and distal one-third of the dendritic tree, respectively. In the model with synapses on the proximal one-third, the current/synapse was greater than 50% of the current delivered by synapses with a constant driving potential. B: Comparison of actual versus linear sum of two inputs, each consisting of $n$ synapses, distributed throughout the dendritic tree of LVN 2-1 versus the proximal or middle or distal one-third of the dendritic tree of LVN 2-1. Each data point corresponds to a simulation where $n$ was assigned a multiple of 0.4% of all excitatory or inhibitory synapses.
Table 1

Delivery of current to the soma with a uniform distribution of synapses

<table>
<thead>
<tr>
<th>Synaptic input</th>
<th>Parameter</th>
<th>Motoneuron</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LAD 5-4</td>
<td>LVN 2-1</td>
</tr>
<tr>
<td>excitatory</td>
<td>current at 100% active synapses (nA)</td>
<td>47.9 (-34.2)</td>
</tr>
<tr>
<td>(inhibitory)</td>
<td>% current loss due to cable properties</td>
<td>25.6</td>
</tr>
<tr>
<td>soma voltage</td>
<td>% of synapses active at 50% current loss due to non-linear summation</td>
<td>35.9 (6.8)</td>
</tr>
<tr>
<td>clamp -64 mV</td>
<td>% current loss due to non-linear summation at 100% active synapses</td>
<td>71.1 (88.4)</td>
</tr>
<tr>
<td>excitation</td>
<td>maximum % of current lost due to non-linear summation /100 synapses</td>
<td>1.8 (8.0)</td>
</tr>
<tr>
<td>soma voltage</td>
<td>current at 100% active synapses (nA)</td>
<td>39.6</td>
</tr>
<tr>
<td>clamp -55 mV</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1
Intersynapse distance on each branch (µm)

Cumulative percentage of intersynapse distances

LVN 2-1
LVN 4-1
LAD 5-4

Electrotonic distance between an 'average' synapse and the soma

Cumulative percentage of dendritic paths

LVN 2-1
LVN 4-1
LAD 5-4

Figure 2
Figure 3

A

Current reaching soma (nA)

0 3000 6000 9000 12000 15000

B

Current added with each succeeding synapse (pA)

0 3000 6000 9000 12000 15000

C

Percentage of active synapses or Frequency of active synapses (Prelease = 0.5), all synapses activated

Number of active synapses (frequency 100 Hz, P_release = 0.5)

0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500

Percentage of active synapses

0.0 0.5 1.0 1.5 2.0

Frequency of active synapses (P_release = 0.5), all synapses activated
Distance to soma (µm)

Membrane potential (mV)

Figure 4
A

Number of active synapses (frequency 100 Hz, Prelease= 0.5)

Percentage of current lost due to non-linear summation with each succeeding 100 synapses

B

Percentage of active synapses

Current added with each succeeding synapse (pA)

C

Percentage of current lost due to non-linear summation with each succeeding 100 synapses

Figure 5
Figure 6

A

B

C

Linear sum

-20 -10 0 10 20

-20 0 10 20

-8 -4 0 4 8

396 inhibitory synapses + variable excitation
792 inhibitory synapses + variable excitation
1584 inhibitory synapses + variable excitation
Figure 7

A

Number of active synapses (frequency=100 Hz, Prelease=0.5)

Current added with each succeeding synapse (pA)

Rm 15,000 Ω cm², Ri 300 Ω cm
Rm 15,000 Ω cm², Ri 70 Ω cm
Rm 60,000 Ω cm², Ri 70 Ω cm

B

Additional number of excitatory synapses (frequency 100 Hz, P_relsae=0.5)

Current added with each succeeding synapse (pA)

no background synaptic activity
5% inhibitory and 4% excitatory
10% inhibitory and 8% excitatory
20% inhibitory and 16% excitatory

C

Number of excitatory synapses (frequency 100 Hz, P_relsae=0.5)

Current added with each succeeding synapse (pA)

g_\text{peak} 5 nS
g_\text{peak} 10 nS
g_\text{peak} 20 nS
Predicted sum ($nA$)

Actual sum ($nA$)

Uniform distribution
Proximal 1/3 distribution
Middle 1/3 distribution
Distal 1/3 distribution
Linear sum

Figure 8