Summation of Effective Synaptic Currents and Firing Rate Modulation in Cat Spinal Motoneurons

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Powers, Randall K. and Marc D. Binder. Summation of effective synaptic currents and firing rate modulation in cat spinal motoneurons. J. Neurophysiol. 83: 483–500, 2000. The aim of this study was to examine how cat spinal motoneurons integrate the synaptic currents generated by the concurrent activation of large groups of presynaptic neurons. We obtained intracellular recordings from cat triceps surae motoneurons and measured the effects of repetitive activity in different sets of presynaptic neurons produced by electrical stimulation of descending fibers or peripheral nerves and by longitudinal vibration of the triceps surae muscles (to activate primary muscle spindle Ia afferent fibers). We combined synaptic activation with subthreshold injected currents to obtain estimates of effective synaptic currents at the resting potential ($I_{\text{rest}}$) and at the threshold for repetitive discharge ($I_{\text{Nthresh}}$). We then superimposed synaptic activation on suprathreshold injected current steps to measure the synthetically evoked change in firing rate. We studied eight different pairs of synaptic inputs. When any two synaptic inputs were activated concurrently, both the effective synaptic currents ($I_{\text{S}}$) and the synthetically evoked changes in firing rate generally were equal to or slightly less than the linear sum of the effects produced by activating each input alone. However, there were several instances in which the summation was substantially less than linear. In some motoneurons, we induced a partial blockade of potassium channels by adding tetraethylammonium (TEA) or cesium to the electrolyte solution in the intracellular pipette. In these cells, persistent inward currents were evoked by depolarization that led to instances of substantially greater-than-linear summation of injected and synaptic currents. Overall our results indicate that the spatial distribution of synaptic boutons on motoneurons acts to minimize electrical interactions between synaptic sites permitting near linear summation of synaptic currents. However, modulation of voltage-gated conductances on the soma and dendrites of the motoneuron can lead to marked nonlinearities in synaptic integration.

INTRODUCTION

The activity of a neuron is influenced by transmitter release from thousands of presynaptic inhibitory and excitatory contacts distributed throughout its dendritic tree. The net effect of synaptic activity on the postsynaptic cell can be quantified in terms of the total synaptic current reaching the soma (variously referred to as “effective somatic current” (Redman 1976), “effective synaptic current” (Heckman and Binder 1988), and $I_{\text{soma}}$ (Bernander et al. 1994]). The amount of synaptic current transferred to the soma from a given set of activated synapses will be affected by concurrent transmitter release from other synapses as a consequence of changes in the driving force for current flow at the original synaptic sites and increases in membrane conductance at the sites of the concurrently activated synapses. Both of these effects are likely to lead to less-than-linear summation of the currents produced by two separate sets of synapses (Jack et al. 1975; Rall 1964, 1967). However, the amount of synaptic current transferred to the soma also may be affected by the presence of voltage-gated dendritic conductances (Bernander et al. 1994; Clements et al. 1986) that can amplify synaptic currents (Schwindt and Crill 1995), possibly leading to greater-than-linear summation.

The widespread distribution of synaptic terminals on the dendritic tree may help isolate synapses from one another, minimizing the occurrence of nonlinear interactions. Indeed, measurements of summation of monosynaptic Ia excitatory postsynaptic potentials (PSPs) in mammalian motoneurons indicate that linear summation often is observed and departures from linearity are relatively small (i.e., generally <10%) (Burke 1967). Significant departures from linearity are more common when excitatory and inhibitory PSPs are evoked concurrently (Burke et al. 1971; Rall et al. 1967). In these instances, the timing of the activation of the two inputs is critical with the largest departure from linearity occurring when the voltage change produced by the excitatory input encounters the conductance change underlying the inhibitory input (Burke et al. 1971; Rall et al. 1967; Segev and Parnas 1983).

The magnitude of the deviation from linear summation is likely to depend both on the relative proximity of the two sets of synapses (e.g., Rall 1964, 1967; Rall et al. 1967) and on the extent to which the transfer of synaptic current to the soma is modified by voltage-sensitive dendritic conductances (Bernander et al. 1994; Clements et al. 1986). These two effects may compound the extent of nonlinear summation or counteract one another. For example, a recent study of summation of glutamate-evoked PSPs in cultured hippocampal neurons reported linear summation that was independent of the relative spatial location of the two inputs (Cash and Yuste 1998). In this case, linear summation resulted from the balanced activation of voltage-sensitive dendritic conductances carrying inward and outward currents in keeping with recent theoretical work (Bernander et al. 1994).

Most of the previous experimental work on synaptic input summation has been restricted to measurements of PSPs produced by the transient activation of two inputs. However, physiological activation of neurons generally is achieved by repetitive discharge in presynaptic fibers. Fortunately, repetitive activation of presynaptic afferents also affords a more straightforward method of quantifying synaptic inputs and their effects on the discharge of the postsynaptic cell (reviewed in Binder et al. 1996). The steady-state synaptic current reaching the soma ($I_{\text{s}}$) can be measured with a modified voltage-clamp...
technique (cf. Heckman and Binder 1988; Lindsay and Binder 1991; Powers et al. 1992), and synaptically evoked changes in firing rate can be predicted from the product of this effective synaptic current and the slope of the motoneuron’s steady-state, firing frequency-current relation (f-I) (Powers and Binder 1995; Powers et al. 1992).

The goal of the present study was to measure the effective synaptic currents and the changes in motoneuron firing rate evoked by the separate and concurrent activation of two distinct groups of presynaptic neurons. We obtained intracellular recordings from cat triceps surae motoneurons and measured the effects of repetitive activity in different sets of presynaptic neurons produced by electrical stimulation of descending fibers or peripheral nerves and by longitudinal vibration of the triceps surae muscles (to activate primary muscle spindle (Ia) afferent fibers) (Heckman and Binder 1988). We combined synaptic activation with subthreshold injected currents to obtain estimates of effective synaptic currents at the resting potential ($I_{\text{rest}}$) and at the threshold for repetitive discharge ($I_{\text{thresh}}$). We then superimposed synaptic activation on suprathreshold injected current steps to measure the synaptically evoked change in firing rate. In some motoneurons, we induced a partial blockade of potassium channels by adding tetraethylammonium (TEA) or cesium to the electrolyte solution in the intracellular pipette.

We studied eight different pairs of synaptic inputs. When any two inputs were activated concurrently, both the effective synaptic currents ($I_{\text{rest}}$) and the synaptically evoked changes in firing rate were generally equal to or slightly less than the linear sum of the effects produced by activating each input alone. However, with partial blockade of potassium channels, persistent inward currents were evoked by depolarization that led to instances of substantially greater-than-linear summation of injected and synaptic currents. Portions of this work have been presented previously in abstract form (Powers and Binder 1998; Powers et al. 1991) and in a symposium proceedings (Binder and Powers 1999).

**METHODS**

**Experimental preparation**

The data presented here were obtained from experiments on 21 adult cats of either sex, weighing between 2.5 and 3.5 kg. Twelve of these experiments were designed primarily to measure descending inputs from the pyramid tract fibers (identified by antidromic activation from their muscle nerves) (Heckman and Binder 1988; Powers and Binder 1985). In the experiments in which descending pathways were stimulated, we performed a craniotomy to allow placement of bipolar stimulating electrodes in the contralateral pyramidal tract (PT) (cf. Binder et al. 1998), the ipsilateral Deiters’ nucleus (DN) (cf. Westcott et al. 1995), or the contralateral red nucleus (RN) (cf. Powers et al. 1993). Before initiating the intracellular recordings, the animals were paralyzed with gallamine triethiodide and mechanically respired at a rate adjusted to maintain end tidal CO₂ between 3 and 5%. A bilateral pneumothorax was performed to increase recording stability. At the conclusion of the experiments, the animals were killed by administering a lethal dose of pentobarbital. Stimulation sites in the brain stem were confirmed by postmortem histological analysis as previously described (Binder et al. 1998; Powers et al. 1992, 1993; Westcott et al. 1995).

**Measurement and stimulation techniques**

In 16 of the 21 experiments, we used glass capillary microelectrodes filled with either 0.6 M potassium sulfate or 2 M potassium citrate to obtain intracellular recordings from MG and LGS motoneurons (identified by antidromic activation from their muscle nerves). The tips of the electrodes were broken to obtain in situ resistances of from 2 to 14 MΩ. Only motoneurons with resting potentials greater than −55 mV and antidromic action potentials with positive overshoots were accepted for study. We measured the effects of stimulating a number of different peripheral and descending inputs, individually and in combination, on the motoneurons. Selective activation of Ia afferent fibers was achieved by high-frequency, low-amplitude (200 Hz, 150 μm peak to peak) longitudinal vibration of the triceps surae muscles (Heckman and Binder 1988; Powers and Binder 1985). The remaining inputs were activated by 200-Hz bipolar electrical stimulation (0.1-ms pulse width). The strength of stimulation of descending inputs was adjusted to be supramaximal for the descending volley recorded at $L_s$. The SN was stimulated at five times the threshold for the appearance of a dorsal root volley, and the CP nerve was stimulated at 5–10 times threshold.

In the other five experiments, we filled our electrodes with 100 mM of the lidocaine derivative QX-314 to block voltage-gated sodium channels and either 0.5 − 1 M tetraethylammonium (TEA) or 3 M cesium chloride to block voltage-gated potassium channels. Our intent was to observe how the activation of persistent inward currents (Lee and Heckman 1996, 1998a,b; Schwindt and Crill 1977, 1980a–c) and plateau potentials (Hounsgaard and Kiehn 1985, 1993; Hounsgaard et al. 1984, 1988) in the motoneurons would affect synaptic integration. In these experiments, we combined steady-state Ia input with stimulated inhibitory or excitatory synaptic inputs produced by injecting 1-s current steps through the recording microelectrode (Poliakov et al. 1996, 1997; Powers and Binder 1996; Reyes and Fetz 1993). To simulate a synaptic input as a change in conductance rather than a fixed-amplitude current pulse, we operated the intracellular amplifier (Axoclamp 2B) in the discontinuous current-clamp configuration with a switching rate of 7 kHz. This provided a record of motoneuron membrane potential that was less subject to the effects of electrode capacitance and changing electrode resistance than a continuous current-clamp recording in bridge mode. The sampled membrane potential was sent to a separate amplifier that multiplied the amplitude of the injected current pulse by the difference between the membrane potential and a specified synaptic equilibrium potential [−80 mV for inhibitory PSPs (IPSPs) and 0 mV for excitatory PSPs (EPSPs)]. The output of this amplifier then was sent to the external current command.
input of the Axoclamp. Thus the amplitude of the simulated synaptic current varied with membrane potential just as a current produced by an actual change in synaptic conductance does. (This procedure is identical to the reactive current-clamp technique described in Hutchison et al. 1996.)

Membrane potential, injected current, the dorsal root and descending volleys, and a muscle length signal were all stored on VCR tape via a pulse code modulated digitizing unit. Membrane potential and injected current were digitized at 10 kHz using a MacAdios II data-acquisition board (GWI Instruments) for off-line analysis.

**Experimental protocol**

After a motoneuron was identified and judged acceptable by measurement of the antidromic action potential and resting potential, we determined its rheobase by adjusting the magnitude of depolarizing, 50-ms injected current steps to be just threshold for action potential initiation (cf. Zengel et al. 1985). We then used our modified voltage-clamp technique (Binder et al. 1998; Heckman and Binder 1988; Lindsay and Binder 1991; Powers et al. 1993; Westcott et al. 1995) to measure the effective steady-state synaptic current \(I_{ss}\) generated in the motoneuron by two sources of synaptic input, both activated individually and in combination. The basic protocol combined 1-s steps of injected current with 1-s periods of high-frequency activation of synaptic inputs and is illustrated in Fig. 1, A and D. The onset of synaptic current occurred 500 ms later than the onset of injected current, so that there were three consecutive 500-ms epochs consisting of injected current alone, injected plus synaptic current, and synaptic current alone. Each combination of injected and synaptic current was repeated four times to obtain an average response. We measured the mean membrane voltage over the last 300 ms of epochs 1 and 2 (indicated in Fig. 1A) to obtain estimates of the voltage response to injected current alone \(V_i\) and that to the combination of injected and synaptic current \(V_{i+ss}\), respectively. The steady-state synaptic potential \(\Delta V_s\) was taken as the difference between these two estimates \(V_{i+ss} - V_i\). We attempted to obtain average responses to two different synaptic inputs acting alone and in combination using several different subthreshold levels of injected current. After these subthreshold measurements, we increased the level of depolarizing current steps to produce steady repetitive discharge in the motoneuron.
(e.g., Fig. 4). (This portion of the protocol could not be done on those cells recorded with electrodes containing QX-314). Two trials with injected current alone were alternated with two trials of injected plus synaptic current. In each trial, we calculated the steady-state firing rate as the mean rate over the last 300 ms of the current step. The change in firing rate (ΔF) produced by the synaptic input was taken as the difference between the mean steady-state rate in response to synaptic plus injected current and the mean steady-state rate for the four bracketing control (injected current alone) trials. As in the case of subthreshold measurements, we attempted to obtain the firing rate responses to two different synaptic inputs individually and in combination using a number of different levels of injected current to produce different background ("control") firing rates.

Data analysis
We used the experimental measurements described above to derive three additional parameters describing the effects of a synaptic input on the motoneuron: the steady-state effective synaptic current measured at rest (I\textsubscript{Nrest}), the effective input resistance of the cell during the activation of a synaptic input (R\textsubscript{Nsyn}), and the effective synaptic current at the threshold for repetitive discharge (I\textsubscript{thresh} \text{Nthresh}). The latter quantity was estimated by "clamping" the membrane potential at its resting value during the combined application of injected and synaptic current (Heckman and Binder 1988; Lindsay and Binder 1991). This value was estimated by calculating a linear regression between current (Heckman and Binder 1988; Lindsay and Binder 1991). This latter quantity was estimated by adding an even larger degree of uncertainty (Powers and Binder 1995). Measurements of synthetically evoked changes in firing rate will be affected by slow changes in the cell’s repetitive firing capability. We attempted to minimize the impact of these changes by bracketing the responses to injected and synaptic current with control trials (i.e., responses to injected current alone). Nonetheless significant changes in the cells repetitive firing properties between the application of the different synaptic inputs will contribute to the degree to which the combined effects of two inputs differ from the linear sum of their individual effects. These various sources of error could easily cause departures from linearity on the order of ±15–30% in individual cases but should not have caused systematic deviations from linearity across the entire sample.

RESULTS
We measured the effects of activating two different synaptic inputs in 28 triceps surae motoneurons (21 MG and 7 LGS). From this sample, we obtained a total of 28 comparisons of the effective synaptic currents produced by concurrent and separate activation of different input systems and 18 comparisons of synthetically evoked changes in firing rate. We typically combined Ia afferent input produced by triceps surae vibration with the activation of one of the descending systems [contralateral PT (n = 6), ipsilateral DN (n = 3), or contralateral RN (n = 2)] or with one of the other peripheral inputs [afferent fibers in the CP (n = 12) or SN (n = 2)]. In three cases, we combined stimulation of a descending input with one of the other peripheral inputs (DN + CP, DN + SN, and RN + CP). The results in these cases were similar to those obtained with combinations of Ia afferents and other inputs.

In another 12 triceps motoneurons, we measured the effects of combining Ia afferent input produced by triceps surae vibration with depolarizing or hyperpolarizing "conductance steps" injected into the motoneuron to mimic steady-state EPSPs and IPSPs, respectively. In these cells, the microelectrode electrodes were filled with a solution containing 100 mM QX-314 to block voltage-gated sodium channels and with varying amounts of tetraethylammonium (TEA) or cesium (Cs) salts substituted for potassium to block potassium channels: (1 M K acetate and 1 M TEA acetate: 6 cells; 0.5 M TEA Cl and 2.5 M KCl: 2 cells, 3 M CsCl: 4 cells). The results obtained without potassium channel blockers will be presented first followed by a description of the effects of the blockers.

Summation of effective synaptic currents
Figure 1 illustrates how we measured and evaluated the effective synaptic currents (I\textsubscript{N}) generated by two different
input systems in the same triceps motoneuron. Figure 1, left, shows the effects of activating Ia afferents alone (green trace), pyramidal tract fibers alone (blue trace), and the two inputs concurrently (red trace). Figure 1A shows the averaged voltage responses to the different inputs, together with a 6.2-nA hyperpolarizing current step. The mean voltages calculated over the last 300 ms of the period of combined injected and synaptic current (Vt+I+, marked by line 2 in the figure) are plotted as a function of injected current magnitude in Fig. 1B. The responses to injected current alone (Vt) are represented by the black circles. As described in METHODS, the slopes of the best-fit linear regression lines to the Vt+I+ versus I relations represent the effective input resistance in the presence of synaptic input (Rsyn), whereas the fit to the Vt versus I relation represents the steady-state input resistance in the absence of synaptic input (RNe). The values of Rsyn were 96, 90, and 84% of RNe for the Ia, PT, and Ia + PT inputs, respectively, indicating that even when the two inputs were activated concurrently a relatively modest change in input resistance occurred. The magnitudes of the effective synaptic currents (ISyn) calculated as −1 times the zero-voltage intercept of the fit to the Vt+I+ versus I relations (see METHODS) were 4.4 nA for the Ia input, 8.2 nA for the PT input, and 11.0 nA for the combined inputs. In this case, ISyn was measured for the combined inputs was 88% of the sum of their individual effects, indicating near-linear summation. Figure 1C shows the relation between the magnitude of the steady-state synaptic potentials (∆Vs, see METHODS) and the background membrane potential (Vb). Although the inputs were not constant, they did not show a clear dependence on somatic membrane potential. The synaptic currents flowing at threshold (ISyn,threshold) therefore were estimated by assuming a synaptic reversal potential of 0 mV and correcting for the change in driving force from the resting potential to the mean membrane potential at threshold (see METHODS). The estimates of ISyn,threshold were 3.9, 7.2, and 9.8 nA for the Ia, PT, and Ia + PT inputs, respectively. Because the same correction factor was applied in all three cases, the small departure from linearity was the same as for the ISyn values.

As mentioned in the INTRODUCTION, less-than-linear summation of effective synaptic currents could result from changes in the driving force for current flow at the synapses, increases in the conductance of the membrane between the synapses and the soma or a combination of the two factors. Thus departures from linearity might be expected to be more prominent in cases in which one or both of the synaptic inputs causes a large decrease in effective input resistance. Figure 1, right, shows the effects of activating Ia afferents alone (green), CP afferents alone (blue), and the two inputs together (red) in a different motoneuron. Figure 1D shows that the response to the combination of Ia and CP input is quite similar to the response to the CP input alone. The CP input acting alone and in combination with the Ia input causes a large reduction in input resistance as can be seen from the Vt+I+ versus I relations illustrated in Fig. 1E. The slope of this relation for the Ia input (green line) is nearly parallel to that for Vt versus I (black line), as was the case in Fig. 1B. However, the slopes are significantly lower for the CP (blue) and Ia + CP (red) inputs, indicating decreases in input resistance of 30 and 42%, respectively. The estimate of ISyn,threshold for the Ia + CP input is −5.6 nA, which is nearly identical to the value of ISyn for the CP input, and markedly different from the expected linear sum of the Ia and CP effective synaptic currents (−2.1 nA). The steady-state synaptic potentials produced by the CP and Ia + CP inputs showed a strong linear dependence on membrane potential (Fig. 1F), and this dependence was used to predict the values of ISyn,threshold for these inputs (see METHODS). The ISyn,threshold value for the combined Ia + CP inputs (−13.1 nA) was close to the predicted linear sum of the estimates for the individual inputs (2.8 − 13.1 = −12.0 nA), largely because the predicted Ia current was small.

Figure 2 illustrates the relations between the estimates of effective synaptic current during combined activation of two inputs versus the predicted linear sum of the currents produced by each input alone. Each of the eight different combinations of inputs we studied is denoted by a different symbol. The filled symbols indicate cases in which the combined inputs caused a decrease in steady-state input resistance <20%, whereas the open symbols are cases in which a decrease ≥20% occurred. Figure 2A shows that the observed values of ISyn,threshold produced by combined activation of any two of these inputs are generally slightly below the predicted linear sum of their individual effects (The bold diagonal line is the line of identity.) For the entire sample, the observed value was 76% of the predicted linear sum (mean difference = −1.1 nA, paired t = 3.16, P < 0.01). The mean difference between observed and predicted effective synaptic currents was similar for cases in which the combined inputs produced a >20% decrease in input resistance and those in which the inputs produced a smaller change in input resistance (−1.1 nA in both groups). However, there was a weak but significant correlation between the percentage resistance change (∆RNe) produced by synaptic activation and the magnitude of the difference between the measured and predicted effective synaptic currents (ISyn,threshold − ISyn,thresholdpr). The values of ISyn,threshold were, on average, 93% of those predicted from the linear sum of the values estimated when each input was activated separately. The observed values of ISyn,threshold were, on average, 93% of those predicted from the linear sum of the values estimated when each input was activated separately. There was no significant difference even when the sample was restricted to cases in which a large synthetically evoked change in input resistance occurred. It is possible that the increased measurement error associated with our estimates of ISyn,threshold (see METHODS) may have obscured a trend toward less-than-linear summation.

**Contribution of presynaptic factors to less-than-linear summation**

There are two types of presynaptic factors that can contribute to less-than-linear summation of concurrent synaptic inputs. If two polysynaptic pathways use some common interneurons, their concurrent activation may result in less-than-linear summation through occlusion. In the simplest example, if each pathway activated >50% of the common interneurons, then concurrent activation would produce a smaller effect on the target neuron than the linear sum of the individual inputs. Intraneuronal occlusion was unlikely to have made a signifi-
cant contribution to the pattern of results obtained here because monosynaptic Ia excitation was one of the inputs in all of the cases in which synaptically evoked changes in firing rate were measured and in all but three of the cases in which effective synaptic currents were measured.

The most likely presynaptic factor contributing to less-than-linear summation is presynaptic inhibition of transmitter release. The most common combination of synaptic input systems we used was activation of Ia afferents from the triceps surae and afferents from the CP nerve (12 cases). The CP nerve includes afferent fibers that innervate the flexor muscles tibialis anterior and extensor digitorum longus, and group I afferents from flexor muscles often have been used to induce presynaptic inhibition of Ia afferents from the triceps surae muscles (Rudomin 1990).

To determine whether or not presynaptic inhibition might have contributed to the less-than-linear summation we observed during concurrent Ia and CP activation, we measured the composite Ia EPSP produced in a MG motoneuron by stimulating the synergist LGS nerve with and without a preceding conditioning train of three shocks (at 200 Hz) to the CP nerve at 10 times threshold. Figure 3A illustrates the synaptic potentials measured in the MG cell in response to stimulation of the CP nerve alone (gray trace), the LGS nerve alone (thin black trace), and the LGS nerve when preceded by stimulation of the CP nerve 45 ms earlier (thick black trace). The CP stimulation produces a large IPSP, and when the LGS stimulation is applied 45 ms later, the “area” (i.e., charge) of the “conditioned” monosynaptic EPSP was 30% less than that of its “control” value. The difference can be seen more clearly in Fig. 3C, which shows the control EPSP (thin trace) and the conditioned EPSP (thick trace) after subtracting the CP response. However, virtually all of this 30% decrease in the EPSP area can be attributed to the conductance change associated with CP stimulation. Figure 3, B and D, shows the effect of CP stimulation on the amplitude and time course of the voltage response to a 1-ms injected current pulse. The CP stimulus produced a 30% reduction in the area of the response to the current pulse,
suggesting that the reduction in the LGS EPSP produced by the preceding CP stimulus should be attributed to changes in the conductance of the postsynaptic cell. In the same motoneuron, the effective synaptic current added by the Ia input was reduced by 28% of its control value when the CP input was applied. These results suggest that presynaptic inhibition did not make a significant contribution to the nonlinearity in the summation of effective synaptic currents from Ia and CP inputs. Further, in the five trials in which stimulation of the contralateral PT produced mixed excitation and inhibition, its summation with Ia afferent input was also significantly less than linear. All of these results suggest that postsynaptic factors were primarily responsible for the observed less-than-linear summation of effective synaptic currents.

Differences in the summation of effective synaptic currents and steady-state PSPs

The deviations from linear summation we found for effective synaptic currents from CP and Ia afferents were smaller than those for the accompanying EPSPs and IPSPs. Activation of low- and high-threshold afferents in the CP nerve generally produced a PSP containing both excitatory and inhibitory components with inhibition predominating. The steady-state PSPs resulting from CP stimulation all exhibited a strong dependence on somatic membrane potential with reversal potentials ranging from +1.8 to −23.6 mV relative to the resting potential (−5.9 ± 6.8 mV; mean ± SD). The somatic depolarization produced by the Ia excitatory input (mean 4.1 ± 2.4 mV; range: 0.8–7.7 mV) thus would be expected to significantly increase the driving force for inhibitory synaptic current flow. As a result, the net depolarization added by a Ia EPSP should be reduced significantly when it is superimposed on a CP IPSP. In contrast, when the somatic membrane potential is clamped at the resting potential, current flow through inhibitory synapses located on or electrotonically close to the soma should be unaffected by concurrent excitatory input.

The difference between summation of synaptic potentials and effective synaptic currents can be seen in Fig. 4, A and B. The amplitudes of the steady-state PSPs recorded during membrane hyperpolarization (Fig. 4A) for the Ia, CP, and Ia + CP inputs were 4.6, −0.5, and 1.5 mV, respectively. The additional depolarization produced by the Ia input when superimposed on the CP input was 2.0 mV; equal to 43% of the depolarization it produced when applied alone. The

FIG. 3. Effects of common peroneal nerve (CP) input on monosynaptic Ia excitatory postsynaptic potentials (EPSPs). A: membrane responses to 4 shocks to the CP nerve at 10T (gray trace), a single shock to the LGS nerve at 2T (thin black trace), and CP stimulation followed 45 ms later by LGS stimulation (thick black trace). B: same paradigm as A except that a 1-ms injected current pulse was substituted for the LGS input. C: expanded view of response to LGS alone (thin black trace), and the LGS input conditioned by the CP stimulus (thick black trace; estimated by subtracting the response to CP from that to CP + LGS. D: effects of CP stimulation on the response to injected current pulse.
effective synaptic currents produced by the three inputs were 1.4.3 nA for the Ia input, 2.2.5 nA for the CP input, and 2.0.2 for the combined input. The additional depolarizing current supplied by the Ia input when it was concurrently activated with the CP input was 2.3 nA, which represents 54% of the current it supplies when applied alone. A similar pattern was observed in the other 11 cases of combined Ia and CP input. When activated at the resting potential (i.e., in the absence of injected current), Ia activation applied in combination with CP input produced an average additional depolarization that was 32% of the depolarization it produced when activated alone. In contrast, the effective synaptic current added by the Ia input during CP activation was on average 56% of the amount produced during Ia activation alone, representing significantly less deviation from linear summation than that observed for the respective synaptic potentials (paired t = 2.25, P < 0.05). Overall, during activation of other inputs, the Ia input added 76% of the synaptic current it produced when activated alone (vs. 58% of the depolarization it produced when activated alone).

**Summation of synaptically evoked changes in firing rate**

Measurement of the changes in discharge rate produced by synaptic activity should reflect the effective synaptic current flowing at threshold (\(I_{\text{Nthresh}}\)) more accurately than estimates based on \(I_{\text{Nrest}}\) (Powers and Binder 1995; Powers et al. 1992) and also provides a more functionally relevant test of the linearity of synaptic input summation. We measured both the effective synaptic currents and synaptically evoked changes in firing rate produced by separate and combined activation of two inputs in 16 motoneurons. Figure 4 illustrates the effects of repetitive activation of Ia afferents (green traces), high-threshold afferents in the CP (blue traces), and combined activation of the two inputs (red traces) during the injection of subthreshold (\(A\) and \(B\)) and suprathreshold (\(C\) and \(D\)) current steps. As illustrated previously (Fig. 1, \(D\) and \(E\)), the CP input, whether activated alone or in combination with Ia afferent input, significantly decreased the measured input resistance. In addition, the effective synaptic current (\(I_{\text{Nrest}}\)) produced by combined Ia and CP input was less than their linear sum (Ia: 4.3 nA; CP 2.5 nA; Ia + CP 0.2 nA).
After collecting the subthreshold data (Fig. 4, A and B), we repeated the synaptic activation during injection of current steps of different magnitude. The black trace in Fig. 4C illustrates a 1-s epoch of repetitive discharge produced by a 19.3-nA injected current step. On subsequent trials, we superimposed Ia afferent input (green trace), CP input (blue trace), or Ia + CP activation (red trace), starting 0.5 s after the onset of current injection. Each synaptic input was applied twice at each level of injected current and bracketed by control trials (injected current alone). The synthetically evoked change in firing rate was calculated as the difference between the mean firing rate over the last 300 ms of the injected current step during the synaptic activation trials and the mean rate measured over the same portion of control trials. Figure 4D illustrates the time course of instantaneous discharge rate during the synaptic activation trials after subtracting the mean steady-state rate measured during bracketing control trials. The Ia input produced an increase in discharge rate of 11.4 imp/s, the CP input decreased the rate by 17.2 imp/s, and the combined input produced a decrease in rate of 15 imp/s. Thus as was the case for summation of effective synaptic currents (Fig. 4B), the synthetically evoked change in firing rate produced by combined activation of these two inputs was significantly less than the predicted linear sum of their individual effects (−15 vs. −5.8 imp/s).

As mentioned in the preceding text, the less-than-linear summation observed during concurrent activation of the Ia and CP inputs may have resulted in part from the fact the depolarization produced by the monosynaptic Ia excitatory input increased the driving force for synaptic current flow through the inhibitory synapses activated by CP stimulation. Our voltage-clamp protocol should minimize these changes in driving force for somatic synapses but would have less effect on electrotonically remote synapses. Increased membrane depolarization during repetitive discharge also should produce an increase in the driving force for CP synaptic currents. Because the threshold for spike initiation and the mean level of membrane depolarization increase with increasing firing rate (Schwindt and Crill 1982), the effects of CP stimulation might be expected to increase with the background discharge rate of the motoneuron. Figure 5 shows one example in which the decrease in firing rate produced by CP stimulation increased with the background discharge rate of the motoneuron. When the background discharge rate of the motoneuron was 20.7 imp/s (Fig. 5A), the CP input produced a decrease in firing rate of −7.1 imp/s (gray lines), whereas the same input decreased firing rate by −9.0 imp/s when the background firing rate was 27.2 imp/s (Fig. 5B). The effects of Ia input on firing rate (dashed lines) were the same at the two different background discharge rates (6.9 and 6.7 imp/s). At both background discharge rates, the effects of combined Ia and CP input (thick solid lines) was less than the expected linear sum of the effects of each individual inputs (at 20.7 imp/s, observed change of −2.7 imp/s vs. predicted change of −0.2 imp/s; at 27.2 imp/s observed change of −4.1 imp/s vs. predicted change of −2.3 imp/s).

The example presented in Fig. 5 suggests that even though the decrease in firing rate produced by an inhibitory synaptic input may vary with the background discharge rate of the motoneuron, less-than-linear summation occurs at different background discharge rates. Although we generally only tested input summation at one or two different background discharge rates, in six motoneurons we were able to examine summation at three or more different firing rates. Figure 6 illustrates one such case, in which the effects of Ia and CP synaptic inputs were examined over a wide range of background discharge rates (−10–80 imp/s). The decrease in firing rate produced by the CP input (filled triangles vs. control, open circles) tended to be largest at the highest background discharge rates. As a result, the slope of the frequency-current relation in the presence of the CP input was lower than that of the control frequency-current relation (1.41 vs. 1.75 imp/s), although this difference did not reach statistical significance (t = 1.83, df = 81, P > 0.05). Nonetheless, regardless of the background firing rate, combined activation of the Ia and CP inputs (asterisks) produced a change in firing rate close to that produced by the CP input alone and less than that expected from the linear sum of the Ia and CP effects.

Figure 7 illustrates the relation between the observed changes in firing rate during concurrent activation of two inputs and the predicted linear sum of the effects of each input alone (n = 18). Less-than-linear summation of synthetically evoked changes in firing rate was observed commonly (14 of 18 points fell below the line of identity). The observed change in firing rate during concurrent activation of two inputs was on average 77% of the predicted linear sum (observed − pre-
Voltage-dependent amplification of effective synaptic currents after partial blockade of potassium channels

Although the less-than-linear summation described in the preceding text is consistent with the nonlinear interactions expected in passive membranes, these results do not rule out the presence of active conductances that, under certain conditions, may lead to greater-than-linear summation. Voltage-dependent amplification of synaptic current is seen in motoneurons recorded in decerebrate cats due to the activation of a persistent inward current (Bennett et al. 1998; Lee and Heckman 1996, 1998b). This effect may depend on the suppression of certain potassium conductances because pharmacological blockade of potassium channels with internal TEA can lead to amplification of synaptic inputs (Clements and Redman 1986). In 12 cells, we measured the interaction of injected current and effective synaptic currents produced by repetitive activation of Ia afferents in the presence of internal TEA or cesium. Voltage-dependent amplification of Ia input occurred in 10 of these cells, resulting in a clear increase in the amplitude of the steady-state Ia synaptic potential once a critical level of depolarization was reached.

Figure 8 illustrates an example of this voltage-dependent amplification in an LGS motoneuron impaled with an electrode containing QX-314 and TEA. Figure 8A illustrates the averaged voltage responses (top) to muscle vibration in combination with −6, +6, and +9.5 nA of injected current (bottom). The amplitude of the steady-state Ia synaptic potential (ΔV̄) clearly increases when muscle vibration is superimposed on the depolarization produced by the +6 nA injected current step (middle). The initial response to muscle vibration (right horizontal arrow) is similar in amplitude to that seen at the hyperpolarized membrane potential. This is followed by an increase in depolarization over the next 30 ms (see inset, bottom) leading to a steady-state synaptic potential amplitude that is nearly twice that obtained at the hyperpolarized membrane potential. A further increase in the magnitude of depolarizing injected current leads to amplification before the onset of muscle vibration (left horizontal arrow). The additional depolarization produced by muscle vibration is quite small in this case, but it is sufficient to enhance the synaptic response.
case. The membrane potential thus appears to be “clamped” at a more depolarized level as previously reported for plateau potentials obtained in the decerebrate cat preparation (Hounsgaard et al. 1984, 1988).

Figure 8B illustrates the relation between the amplitude of the injected current and the change in voltage produced by injected current alone ($V_i$; circles) and injected plus synaptic current ($V_{i+s}$; squares) for a set of responses obtained from 11 to 16 min after impalement. For hyperpolarizing currents, the slopes of the linear fits between injected current and $V_i$ and $V_{i+s}$ are nearly identical (2.27 and 2.30 MΩ, respectively), indicating that over this range of membrane potentials the activation of Ia afferents does not produce a detectable change in steady-state input resistance. Similar findings were obtained in the other 11 cells recorded with TEA- or Cs-containing electrodes: the average value of $R_{Nsyn}$ was 2.37 ± 1.68 MΩ, which was not significantly different from that of $R_{Nss}$ (2.20 ± 1.39 MΩ; paired t value = 1.35, P = 0.203). In contrast, under control conditions, Ia input caused a slight but statistically significant increase in input resistance ($R_{Nsyn} = 1.15 ± 0.62$ MΩ, $R_{Nss} = 1.00 ± 0.53$ MΩ, n = 25, paired t =4.35, P < 0.01). In the presence of potassium channel blockers, both the average $R_{Nsyn}$ and $R_{Nss}$ values were significantly higher than comparable values for the control sample (t values of 3.25, P < 0.01 and 3.82, P <0.01). These findings suggest that internal TEA or Cs leads to a decrease in a resting potassium conductance (see also Campbell and Rose 1997). The presence of QX-314 also may have contributed to the increase in steady-state input resistance of blocking $I_h$ (Perkins and Wong 1995). Simulations based on a cable model of the motoneuron with the conductance mediating $I_h$ present on both the soma and dendrites (Powers and Binder 1998) suggest that the presence of $I_h$ leads to a decrease in $R_{Nss}$ and to a value of $R_{Nsyn}$ that is greater than $R_{Nss}$.

The steady-state amplitude of the Ia synaptic potential exhibited a steep dependence on membrane potential, first in-
creasing as the membrane was depolarized from the resting potential and then decreasing sharply once a critical level of depolarization was reached. Figure 8C illustrates this relation for the same LGS cell. The filled circles represent synaptic potentials recorded from 11 to 16 min after impalement when the resting membrane potential remained fairly stable (−61.2 to −60.7 mV). The peak amplitude of the steady-state synaptic depolarization was 10.6 mV, which was 2.8 times greater than the mean amplitude recorded at hyperpolarized membrane potentials. For the 10 cells in which voltage-dependent amplification occurred, the maximum amplitude of the steady-state synaptic potential ranged from 1.6 to 7.3 (mean = 2.8 ± 1.8) times greater than the amplitude recorded at membrane potentials equal to or less than the resting potential. The synaptic current flowing during maximum amplification could be calculated by dividing the steady-state synaptic potential amplitude by $R_{\text{Nss}}$ (see METHODS). This estimated value ranged from 0.6 to 5.1 (mean = 2.3 ± 1.5) times greater than the synaptic current estimated at the resting potential.

The development of distinct plateau potentials generally occurred within 5 min after impalement and did not require long depolarizing current pulses. The same relation between membrane potential and the amplitude of the synaptic potential generally was seen during the entire time course of the recording, as illustrated by the different symbols in Fig. 8C, which show responses obtained before (upward triangles) and after (inverted triangles) the data presented in Fig. 8, A and B. However, the membrane potential generally depolarized during the course of the recording period, and in the cell of Fig. 8, the resting membrane potential varied from −66.5 mV at the beginning of the recording period to −56.4 mV at the end. If the resting potential is assumed to depend on the relative magnitudes of currents flowing through a resting potassium conductance with a reversal potential of −80 mV and through a nonspecific, impalement-induced shunt conductance with a reversal potential of 0 mV, then this progressive depolarization may reflect an increasing block of the resting conductance, an increasing impalement leak, or a combination of the two effects (cf. Campbell and Rose 1997). However, if a progressive block of the resting conductance was the predominant factor, then an increase in $R_{\text{Nss}}$ would be expected, whereas an increased shunt conductance would lead to a decrease in the value of $R_{\text{Nss}}$. In five cells in which the membrane potential exhibited ≥5 mV of depolarization over the recording period, the input resistance tended to increase on average (15 ± 23%; range −8 to +51%), suggesting that an increasing block of the resting leak conductance predominated over changes in shunt conductance. A progressive block of the conductance mediating $I_{\text{K}}$ also would be expected to increase the input resistance.

The time-dependent changes in resting potential and input resistance probably reflect an increasing block of dendritic channels as TEA (or Cs) and QX-314 diffuse from the presumed somatic site of impalement. This increasing block was associated with a lower voltage-threshold for the onset of the plateau as well as a slower decay of the amplified response to the synaptic and/or injected current input. Figure 9 illustrates examples of this time-dependent change in responsiveness in two different motoneurons. The thin black trace in Fig. 9A is the response to the combination of a 7.7-nA step of injected current combined with muscle vibration, obtained ~6.5 min after the cell was impaled with an electrode containing 0.1 M QX-314 and 1 M TEA. The initial depolarization represents the response to the injected current alone and exhibits no sign of voltage-dependent amplification. However, within 10 ms of the onset of muscle vibration, the membrane potential quickly depolarizes to a level that is 11.4 mV above the steady-state depolarization produced by injected current alone, so that the steady-state synaptic potential amplitude is over twice that obtained at more hyperpolarized membrane potentials. At the end of the injected current step, the response to muscle vibration alone is initially greater than that obtained at hyperpolarized membrane potentials but decays over the next 500 ms so that the membrane potential reaches the “control” level just before the offset of vibration. The green trace is the next response to the identical set of stimuli, obtained ~5 s later. The time course of depolarization produced by current alone and vibration plus current is nearly identical to that in the previous response, but there is less decay of the membrane potential after the offset of the current step. In the following response (blue trace), the injected current alone produces a plateau potential, so that the addition of muscle vibration produces very little additional depolarization. The sustained response to muscle vibration alone (last 300 ms) is larger than the previous two responses and also decays more slowly back to the resting potential after the offset of muscle vibration. The thick black and red traces are two consecutive responses to the combination of muscle vibration and a slightly smaller (7.4 nA) injected current step, obtained ~1.5 min later. The resting membrane potential depolarized by ~3 mV and the decay of the depolarization after the offset of muscle vibration is slower.

Plateau potentials generally decayed within a few hundred milliseconds after the end of stimulation except in a few cases in cells impaled with electrodes containing 3 M Cs. Figure 9B shows examples of plateaus evoked by a 2.5-s, 8.9-nA current step 33–42 min after impalement with a Cs-containing electrode. The initial voltage response to the current step (green trace) declined back to the resting potential within ~0.5 s after the offset of the current. Six minutes later the voltage response to the same injected current step (blue trace) remained at a depolarized level for 15 s after the offset of the current step, and the subsequent response (red trace) exhibited a voltage plateau lasting 30 s after current offset. The black trace on the right is a response to the same stimulus obtained 2.5 min later, and in this case, the voltage plateau could be terminated only by applying a hyperpolarizing current step. This stable plateau state indicates that potassium currents have been blocked sufficiently to lead to a region of net inward current in the cell’s steady-state current-voltage ($I\text{-}V$) relation (cf. Schwindt and Crill 1980c).

Time-dependent variations in the degree of channel block made it difficult to determine the voltage threshold for the plateau onset. The insets in Figs. 8A and 9A show the plateau onset in response to either the injected current alone or the combination of injected and synaptic current. In the example presented in Fig. 8A, the plateau threshold for the response to injected current alone (solid line) is ~2 mV higher than for the response to injected plus synaptic current (dashed line). For the set of responses illustrated in Fig. 9A, the plateau thresholds (marked by arrows in the inset) of the responses to injected plus synaptic currents exhibited about a 2.5-mV range that included the voltage threshold for the plateau onset in response to injected current alone. It was not always possible to obtain reliable estimates of the voltage thresholds for plateaus evoked in response to both injected current alone and injected plus...
synaptic current. For five cells in which both measurements could be obtained, their values did not differ significantly (paired $t = 0.240$, $P = 0.82$). For the entire sample of cells, the mean threshold values for activating the plateau potentials were similar for injected current alone and injected plus synaptic current \([-49.0 \pm 4.2 \text{ mV} (n = 6)\) and \(-48.2 \pm 5.4 \text{ mV} (n = 9)\), respectively]. The development of block of sodium channels by QX-314 also exhibited considerable variation between cells. In eight cells, we were able to estimate the voltage threshold for spike initiation during the initial measurement of rheobase before the action potential was significantly attenuated. The onset of the spike was determined as the voltage at which its first derivative first exceeded 10 mV/ms (cf. Brownstone et al. 1992). The mean voltage threshold for spike initiation was slightly more depolarized \((-45.9 \pm 2.7 \text{ mV})\) than the plateau thresholds, but paired comparisons of threshold values revealed no significant differences between the spike threshold and the plateau threshold in response to either injected current alone or injected plus synaptic current.

The similarity of the voltage thresholds for plateau initiation in response to injected current alone and injected plus synaptic current suggests that a significant proportion of the channels contributing to voltage-dependent amplification are located close to the somatic recording site. However, inferences about the precise spatial distribution of the channels are limited by the potential errors in the estimates of plateau thresholds. An alternative approach is to compare amplification of steady-state Ia synaptic potentials with that of potentials produced by a simulated, somatic excitatory conductance. Because Ia afferent terminals are distributed widely over the dendritic tree (Burke and Glenn 1996), dendritic channels contributing to amplification would be expected to have a more marked effect on Ia synaptic potentials than on potentials produced by a somatic conductance change. We compared amplification of Ia input with that of a simulated somatic excitatory conductance (see METHODS) in three cells impaled with TEA-containing electrodes. Figure 10A illustrates results from one of these cells. In this case, the steady-state amplitudes of the synaptic potentials produced by a simulated excitatory conductance (blue traces) and by muscle vibration (green traces) were similar both at the original resting potential (bottom traces) and after subsequent depolarization (top traces). At the depolarized potential, both responses are amplified and are followed by a slow decline of membrane potential after the offset of the synaptic input. In one of the other cells, the response to the Ia input appeared to be amplified to a greater degree than the response to the somatic conductance and in the remaining cell, neither response was amplified. A more systematic comparison of the

![FIG. 9. Progressive changes in plateau onset and offset. A: series of responses to muscle vibration and injected current obtained from \(-6–9 \text{ min after impalement with an electrode containing } 1 \text{ M TEA. Inset: onset of plateau potentials (marked by arrows) evoked by injected plus synaptic current (top traces) or injected current alone (bottom traces). B: progressive changes in plateau offset from 33–42 min after impalement with an electrode containing 3 M CsCl.}](http://jn.physiology.org/)
amplification the two types of responses is warranted because
computer simulations (Powers and Binder 1998) suggest that
the relative degree of amplification of dendritic and somatic
inputs can help distinguish between different spatial distribu-
tions of the responsible channels.

The pattern of voltage-dependent amplification observed
after partial block of potassium channels suggests that summa-
tion of synaptic potentials can range from sublinear to supra-
linear depending on the background membrane voltage. For
example, the dependence of Ia steady-state synaptic potential
amplitude on membrane voltage illustrated in Fig. 8 suggests
that the combined depolarization produced by two excitatory
synaptic potentials might be near the linear sum of their indi-
vidual effects at hyperpolarized membrane potentials, greater-
than-linear at slightly more depolarized potentials and less-
than-linear at potentials more depolarized than about −50 mV.
Voltage-dependent changes in the pattern of summation also
were observed when a simulated somatic inhibitory conduc-
tance was combined with Ia input. Figure 10B illustrates re-
sponses of a motoneuron to a 3.3-nA depolarizing current step
in combination with muscle vibration (green), a somatic inhib-
itory conductance (blue), or concurrent activation of both in-
puts (red). The additional depolarization produced when the Ia
input is superimposed on the inhibitory input is 2.8 mV, which
is only 26% of the depolarization it produces at this back-
ground membrane potential in the absence of the inhibitory
conductance. This marked degree of sublinear summation re-
sults from the fact that the hyperpolarization produced by the
inhibitory input brings the membrane potential out of the
region over which amplification of the excitatory input can
occur. In contrast, the inhibitory input can have the opposite
effect at more depolarized membrane potentials. Figure 10C
shows that under these conditions, the Ia input alone produces
very little additional depolarization (0.7 mV), whereas in the
presence of the inhibitory input the Ia input adds 5.2 mV of
depolarization, which is close to the depolarization that the Ia
input produces when it is applied alone at the resting potential
(5.8 mV). Figure 10D shows the dependence of synaptic po-
tential amplitude (ΔV) on membrane potential (relative to the
resting potential) for the Ia input alone (green), the inhibitory
conductance alone (blue), and their combination (red). When the Ia
input is applied alone, the synaptic potential amplitude exhibits
the typical dependence on membrane potential (cf. Fig. 8C). In
contrast, when it is superimposed on an inhibitory conduc-

FIG. 10. Interaction of Ia input with simulated conductances after partial blockade of potassium channels with internal TEA. A: comparison of synaptic potentials evoked by muscle vibration with those produce by a simulated somatic excitatory conductance.
B: responses of a different motoneuron to a depolarizing injected current step and muscle vibration alone (green), a simulated somatic inhibitory conductance (blue), and a combination of the 2 inputs (red).
C: same as B, but with a larger step of injected current. D: steady-state synaptic potentials (ΔV) vs. background membrane potential (V) for Ia input alone (green), the inhibitory conductance alone (blue), and the combination of the 2 inputs (red).
dance, the additional depolarization it produces is relatively independent of the background membrane potential.

**Discussion**

The present results indicate that when two sources of synaptic input are activated concurrently in the absence of potassium channel blockade, their combined postsynaptic effects on cat spinal motoneurons are generally slightly less than those predicted from the linear sum of their individual effects. This was true for measurements of both effective synaptic currents and for synaptically evoked changes in motoneuron firing rate. In contrast, when channel blockers were included in the intracellular electrodes, greater-than-linear summation occurred. Our discussion of these findings will be divided into four parts. First, we will compare our findings to those reported in previous studies of synaptic input summation. Next, we will compare the voltage-dependent amplification seen in the presence of internal channel blockers with that associated with high endogenous or exogenous levels of serotonin, noradrenaline and their agonists. We subsequently will consider the implications of these findings for the utility of measurements of effective synaptic currents and changes in motoneuron firing rate. Finally, we will review briefly the possible dendritic mechanisms contributing to synaptic integration.

**Previous studies of synaptic input summation**

Much of the previous work on summation of synaptic inputs in motoneurons has focused on the interaction of PSPs evoked by single volleys in different sets of presynaptic fibers. EPSPs produced by combined stimulation of different sets of Ia afferents are generally within 10% of the expected linear sum of the effects of each set (Burke 1967). This result is consistent with the fact that Ia boutons are distributed widely across a very large dendritic tree and account for a relatively small percentage of the total input to motoneurons (Burke and Glenn 1996; Lev-Tov et al. 1983; Segev and Parnas 1990). Although there has been less systematic study of the interaction between Ia EPSPs and IPSPs produced by different sets of afferents, summation is often close to linear and large departures from linearity are relatively rare (Burke et al. 1971; Rall et al. 1967).

In the present study, the steady-state depolarization produced in a motoneuron by repetitive activation of Ia afferents in the presence of concurrent activation of CP afferents was on average 32% of that produced during activation of Ia afferents alone. The greater departure from linearity observed under our experimental conditions can be attributed to the different effects of transient versus steady-state synaptic inputs. The low-pass filtering properties of dendrites (Jack et al. 1975) cause the transient depolarization produced by an excitatory dendritic input to decay rapidly with distance. Thus transient EPSPs will only affect the driving forces operating at concurrently activated synapses if they are in close proximity to the source of the EPSP. In addition, the increase in membrane conductance produced by transient activation of one set of synapses will only reduce the transfer of synaptic current from another set of synapses if the current passes by the “active patch” of dendritic membrane during the brief conductance change (Burke et al. 1971; Rall et al. 1967; Segev and Parnas 1983). Because both of these restrictive conditions are relaxed by repetitive synaptic activity, greater departures from linear summation are to be expected.

**Comparisons of the effects of neuromodulators and internal channel blockers on synaptic integration**

A number of recent studies suggest that active conductances in motoneuron dendrites can affect the transfer of synaptic current to the soma and that these conductances are under neuromodulatory control. The amplitude of somatically recorded current produced by glutamate iontophoresis onto the dendrites of turtle motoneurons is altered by the extracellular application of channel blockers and neuromodulators (Skydsgaard and Hounsgaard 1994, 1996). The evidence for the presence of active dendritic conductances in mammalian motoneurons is less direct but compelling nonetheless. After the intrathecal administration of a noradrenergic α-1 agonist, the synaptic current evoked by repetitive activation of Ia afferents is smaller at the resting potential than when the somatic voltage is clamped at a depolarized level (Lee and Heckman 1996). Under current-clamp conditions in decerebrate cat preparations with a high level of endogenous neuromodulators, the activation of a persistent inward current produces a depolarizing plateau (Hounsgaard et al. 1988), and the somatic voltage threshold for this plateau is reduced during tonic activation of Ia afferents (Bennett et al. 1998), again supporting a dendritic location for the responsible channels.

The dendritic conductances supplying inward current normally may be counteracted by voltage- and/or calcium-activated dendritic potassium conductances. Monoaminergic neuromodulators have been shown to reduce calcium-activated potassium conductances, a resting potassium leak conductance, and a hyperpolarization-activated mixed-cation conductance in a variety of types of motoneurons (reviewed in Binder et al. 1996). Serotonergic terminals also have been shown recently to be localized primarily on motoneuron dendrites (Alvarez et al. 1998). Thus the effects of neuromodulators may be mediated largely by their action on dendritic potassium and mixed-cation conductances. For this reason, it is not surprising that the effects of internally applied potassium channel blockers on motoneuron behavior seen here are qualitatively similar to those of neuromodulators. In both cases, there is voltage-dependent amplification of synaptic input starting at voltages slightly depolarized to the resting potential. Quantitative differences may arise due to the different spatial distributions of the modulation of channel behavior: potassium channel blockers diffusing from the somatic site of electrode impalement should have the most marked effect on proximally located channels, whereas the widespread distribution of serotonergic terminals (Alvarez et al. 1998) suggests that exogenous neuromodulators may exert more spatially uniform effects on dendritic channels. Further differences could arise if neuromodulators modify the behavior of the channels carrying the persistent inward current.

**Predictions based on measurements of effective synaptic currents and firing rates**

In the absence of channel blockers, we found that the summation of both effective synaptic currents and synaptically evoked changes in firing rate exhibit smaller departures from linearity than would be expected based on the summation of synaptic potentials. This result is a consequence of our restricting the membrane potential of the soma to a fairly narrow range of values both during measurements of effective synaptic
current and during repetitive discharge. This procedure ensured that changes in the driving force for synapses that are located on or near the soma were quite small. During concurrent activation of two sets of synaptic inputs, both the effective synaptic currents and evoked changes in firing rate are correlated with the expected linear sum of the effects of each input. Although there are systematic departures from linearity, the differences between the observed and expected results are relatively small; the effective synaptic current produced by two inputs is on average 1.1 nA or 26% less than predicted and the evoked change in firing rate is 2.6 imp/s or 23% less than predicted.

An alternative measure of the degree of nonlinearity can be obtained by comparing the amount of effective synaptic current or the increase in firing rate produced by activating Ia afferents alone and in combination with one of the other inputs. The effective synaptic current added by Ia afferent activation in the presence of another input was on average 76% of that produced when Ia afferents were activated alone, and the comparable figure for the synaptically-evoked change in firing rate was 78%. These findings suggest that predictions of motoneuron pool output based on the assumption of linear summation of inputs (e.g., Heckman and Binder 1991) will be reasonably accurate when synaptic inputs with magnitudes comparable with those previously characterized are considered (i.e., effective synaptic currents of ±10 nA) (rev. in Binder et al. 1996, 1998). However, synaptic inputs required to produce maximum output of the motoneuron pool are likely to be up to an order of magnitude larger than those studied to date so that nonlinear summation is likely to play a more significant role when large force outputs are considered.

Both the summation of synaptic currents and their effects on motoneuron firing rate are likely to depend on the state of active dendritic conductances. However, the presence of these conductances does not necessarily invalidate our simple model of the effects of synaptic inputs on firing rate. Our prediction of the effects of synaptic current on firing rate is based on an estimate of the effective synaptic current flowing during repetitive discharge \(I_{\text{thresh}}\). In dendrites with significant voltage-activated inward currents, \(I_{\text{thresh}}\) will be greater than the current measured at the resting potential \(I_{\text{rest}}\). Nonetheless the predicted change in firing rate \(\Delta f\) produced by this amplified current is still the product of \(I_{\text{thresh}}\) and the \(f-I\) slope, and this prediction has been shown recently to hold for neocortical pyramidal cells even though persistent inward currents on the dendrites amplify the effective synaptic current in a voltage-dependent manner (Schwindt and Crill 1996).

The effects of amplification of synaptic currents may depend both on the strength of the synaptic input and on the voltage range over which the persistent, inward dendritic currents are activated (Lipowsky et al. 1996). If the dendritic voltages reached during combined synaptic activation and repetitive discharge already lead to near maximal activation of these inward currents, then further changes in depolarization associated with increasing firing rates may not lead to any further amplification. In contrast, if individual excitatory synaptic inputs produce a level of dendritic depolarization that is subthreshold for activation of a persistent inward current but in combination produce a suprathreshold depolarization, then their combined effective synaptic current and effects on firing rate will be greater than the linear sum of their individual effects.

Dendritic mechanisms contributing to synaptic integration

We have initiated computer simulations of our experimental observations on the effects of internal channel blockers on synaptic integration (Powers and Binder 1998). Our modeling results are consistent with a broad distribution of synaptic boutons and voltage-activated conductances on motoneuron dendrites. However, the experimental results do not tightly constrain either the distribution of synaptic inputs or the magnitude and distribution of active dendritic conductances. Previous experimental work suggests that the density of the leak conductance may be lower on the dendrites than on the soma (Clements and Redman 1989; Fleshman et al. 1988). However, the data are consistent with either a step increase in specific membrane resistance from the soma to the dendrites (i.e., a step decrease in the leak conductance) or a monotonic increase in resistivity from the soma to the distal dendritic branches. Assuming that part of the effects of the internal potassium channel blockers are on a potassium leak conductance, the relatively rapid effects of the blockers that we observed here are consistent with the idea that a significant proportion of the blocked channels must be close to the presumed somatic recording site. However, both direct dendritic recordings and combined experimental and simulation studies in other types of neurons suggest that there is a distally increasing density of both leak (Redman et al. 1987; Stuart and Spruston 1998) and voltage-activated channels (Hoffmann et al. 1997; Stuart and Spruston 1998) in these cell types.

An accurate description of the spatial distribution of dendritic conductances in motoneurons will probably have to await immunocytochemical localization of identified channel subtypes (e.g., Westenbroek et al. 1998). However, our preliminary simulations (Powers and Binder 1998) suggest that a systematic comparison of the voltage-dependent amplification of Ia input with that of a simulated somatic conductance may help distinguish between different spatial distributions of the conductance mediating the persistent inward current.

The functional role of these dendritic conductances is unclear at present. Their effects on motoneuron behavior will no doubt depend on their spatial distribution, voltage sensitivity, and response to neuromodulators. In the absence of channel blockers, we found that the summation of effective synaptic currents is qualitatively similar to the summation of synaptically evoked changes in firing rate. This suggests that under these experimental conditions, the contribution of voltage-sensitive dendritic conductances to the transmission of synaptic currents to the soma is similar under subthreshold and suprathreshold conditions. It remains to be determined whether or not this similarity holds under experimental conditions in which the state of dendritic channels favors voltage-dependent amplification.

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SUMMATION OF SYNAPTIC INPUTS TO MOTONEURONS

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