The transformation of synaptic to system plasticity in motor output from the sacral cord of the adult mouse

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Jiang MC, Elbasiouny SM, Collins WF 3rd, Heckman CJ. The transformation of synaptic to system plasticity in motor output from the sacral cord of the adult mouse. J Neurophysiol 114: 1987–2004, 2015. First published July 22, 2015; doi:10.1152/jn.00337.2015—Summary:little is known about the transformation of synaptic plasticity at the input to a neuron into system plasticity at the output of a neural system. The goal of the present study was to evaluate each step in this transformation, using a combined experimental and computer simulation approach. For our model system, we studied the motor output of the mouse spinal cord evoked via independent, repeated activation of two synaptic systems converging on the spinal motor pool.

In the spinal motor system, plasticity is believed to play an important role in learning and maintaining motor skills, and its dysfunction in pathological conditions is the cause of motor impairment and disability (reviewed by Wolpaw and Tennissen 2001). To investigate mechanisms of plasticity in spinal circuits, previous studies utilized either in vivo preparations in anesthetized adult animals (Collins et al. 1986; Honig et al. 1983) or in vitro preparations in very young animals (Floeter and Lev-Tov 1993; Floeter et al. 1993; Lev-Tov and Pinco 1992; Pinco and Lev-Tov 1993). Here we utilized the in vitro preparation of the sacral segments of the adult mouse spinal cord. We developed this preparation (Jiang and Heckman 2006) based on the rat sacral cord preparations of Bennett and colleagues (Harvey et al. 2006b). A multiscale, high-fidelity computer model of the alpha motoneuron (MN) pool that examined the input/output transformation process from the synaptic to cellular to the system scale was developed and closely tuned to reproduce our experimental results and then used to identify the cellular components governing system plasticity.

We compared plasticity evoked from electrical stimulation of two input pathways converging on the sacral MN pool (Fig. 1). Stimulation of sensory afferents evoked STD in the corresponding MNs (Fig. 1, red traces). This sensory input includes a prominent low-threshold monosynaptic component, presumably from activation of muscle spindle Ia afferents (Honig et al. 1983). However, stimulation of ventromedial white matter activated descending axons (Fig. 1, blue traces) likely originated in brainstem and composed of both monosynaptic and polysynaptic connections to MNs (Floeter and Lev-Tov 1993; Fukushima et al. 1979; Grillner et al. 1970; Westcott et al. 1995). Previous plasticity studies in response to sensory stimulation in neonatal in vitro preparations demonstrated that depression dominated the responses (Lev-Tov and Pinco 1992; Pinco and Lev-Tov 1993). In contrast, limited studies showed facilitation dominated synaptic responses in descending pathway from brainstem (Floeter and Lev-Tov 1993; Floeter et al. 1993). Not only did our results demonstrate STD in the sensory

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input but we also uncovered frequency-dependent bimodal plasticity in the descending inputs: STF at high-stimulation frequencies but STD at low-stimulation frequencies. Using drugs, such as picrotoxin (PTX) and strychnine (STR) that block inhibitory synapses and mephenesin that blocks polysynaptic pathways (Lev-Tov and Pinco 1992), we pharmacologically blocked various synaptic components and examined the contribution of excitatory vs. inhibitory, and monosynaptic vs. polysynaptic inputs to system STP in both pathways. Further analysis with intracellular recording and computer simulation showed that the primary source of this striking difference in the two input pathways was due to activation of excitatory inputs from monosynaptic sources (in the sensory system) or local interneuronal networks (in the descending system).

MATERIALS AND METHODS

Adult B6SJL mice with age between 50 and 70 postnatal days were maintained at the Center for Comparative Medicine of Northwestern University Medical Center. All experimental procedures were reviewed and approved by the Northwestern University Animal Research Committee and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Sacral segments 1 to 3 (S1–S3) of the spinal cord were chosen for this study because of their reliable activity (Jiang et al. 2009; Jiang and Heckman 2006). Forty-eight male and female adult mice (female = 23, male = 25) were used in the present study.

Sacral Spinal Cord Preparation

The sacral spinal cord preparation was described in detail in earlier reports (Jiang et al. 2009; Jiang and Heckman 2006). The in vitro whole cord preparation was chosen because of its advantages in maintaining an intact and healthy network and in rapid drug delivery to study specific cellular functions. An animal was deeply anesthetized with intraperitoneal injections of urethane (0.18 g/100 g). Supplemental urethane was given depending on the animal’s response to a forcepts foot pinching. During the surgery, the animal was supplied with 95% O₂-5% CO₂ through a facemask. The vertebral column was cut open between the low thoracic and caudal segments. The exposed spinal cord was immediately perfused with modified artificial cerebrospinal fluid (mACSF), composed of the following (in mM): 20 NaCl, 140 sucrose, 3 KCl, 1 NaH₂PO₄, 1.5 MgSO₄, 1 CaCl₂, 10 glucose, 26 NaHCO₃, and 1 kynurenic acid. The mACSF osmolality was adjusted to 340 mosM by adding sucrose, and its pH was adjusted to 7.4 by adding NaHCO₃ to the mACSF when bubbled with 95% O₂-5% CO₂. The mACSF was supplied at rate of 5–7 ml/min. The dura below lumbar segments was removed with eye-surgical scissors. The animal was then decapitated, and the cord was transected at the L1–L2 lumbar regions. The transected caudal spinal cord with the attached roots that were cut at their spinal outlets was quickly transferred to a 100-mm petri dish filled with the mACSF and bubbled with 95% O₂-5% CO₂. After separating the ventral roots (S1–S3) and the dorsal root entering the S3 segment were separated, the cord was cut at the end of lumbar segment and transferred to a recording chamber. The surgery and the following recording were done at room temperature.

Experimental Setup and Protocols

The sacral cord was pinned to the center of a recording chamber at its rostral and caudal ends with the ventral side up. Each ventral root (3 on each side of the cord) was placed on two bipolar electrodes, the one proximal to the cord for recording and the distal one for stimulating, and both were separated by 5 mm. The dorsal root (one on each side of the cord) was placed on a bipolar stimulating electrode. Electrodes were made of stainless steel wire coated with Teflon and mounted on a plastic plate (−6 × 7 mm and 1 on each side). The roots were covered with a mineral oil/vaseline (2:1) mixture. A concentric bipolar electrode (NE-100; KOPF; with a 0.2-mm contact diameter and 0.5-mm shaft diameter) was placed at the medial side of ventral root entrance on the cord ventral surface and 2 mm rostral to the rostral end of S1. In mouse sacral segments, ventral anterior funiculus contains several descending tracts, which mostly originate in lateral vestibular nucleus, oral pontine reticular nucleus, and gigantocellular reticular formation (Liang et al. 2014, 2015a,b). Thus the evoked
descending inputs likely included the activations of these descending tracts. In addition, the descending stimulation might also evoke local descending pathways in the sacral cord. The chamber was perfused with ACSF composed of the following (unit in mM): 126 NaCl, 3 KCl, 1 NaH2PO4, 1.5 MgSO4, 2.5 CaCl2, 26 NaHCO3, and 10 glucose with osmolarity adjusted to 300 mosM and pH to 7.4 by carefully adding NaHCO3. The ACSF was bubbled with 95% O2-5% CO2 and circulated at 2.5–3 ml/min.

The six recording electrodes on the proximal ventral roots were individually connected to six amplifiers (DAM 50; WPI) in differential mode with 1,000 times gain, high-pass filtering at 300 Hz, and low-pass filtering at 20 kHz. For intracellular recording, the glass pipette (A-M Systems) was pulled (model P-97; Sutter Instrument) to render a sharp electrode with resistance of 16–20 MΩ when filled with 3 M solution of potassium acetate. The signal was amplified with Axoclamp-2A (Molecular Devices). The outputs of these amplifiers were transferred to an A/D interface (DT1322A; Molecular Devices) and the analog signals were digitized at 20–50 kHz and acquired into a Dell computer controlled by pCLAMP software (version 9.1; Molecular Devices). A MN was studied when its resting membrane potential was −60 mV or lower and its spike height was 70 mV or higher.

Synaptic inputs from either the sensory input root or the descending pathway and antidromic action potentials (APs) from the ventral root were activated by a single pulse or a train of five square pulses from an isolation unit (PSIU6E; Grass Instruments) that was connected to a stimulator (S88; Grass Instruments). The synaptic inputs were activated with 0.2-ms pulses, and the antidromic APs were activated with 0.05-ms pulses. Threshold intensity was determined as the minimum current that evoked minimum root or synaptic responses, with 0.05-ms pulses. Threshold intensity was determined as the minimum current that evoked minimum root or synaptic responses, with 0.05-ms pulses. Threshold intensity was determined as the minimum current that evoked minimum root or synaptic responses, with 0.05-ms pulses. Threshold intensity was determined as the minimum current that evoked minimum root or synaptic responses, with 0.05-ms pulses. Threshold intensity was determined as the minimum current that evoked minimum root or synaptic responses, with 0.05-ms pulses.

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**Pharmacological Studies**

To study the effect of excitatory synapses separately, we blocked both the GABAergic and glycinergic inhibitory synapses using concurrent administration of PTX (5 μM) and STR (0.25 μM). In a different set of experiments, the drug mephenesin (1 mM) was used to block polysynaptic inputs. A few MNs were loaded with 100 mM of mephenesin, and QX-314 were purchased from Sigma.

**Statistical Data Analysis**

To illustrate the plasticity taking place during repeated stimulation, the amplitudes of compound APs (coAPs; peak-to-peak measured at the ventral roots) and the excitatory postsynaptic potentials (EPSPs; measured intracellularly) were plotted as a percentage of the evoked synaptic potential at each pulse relative to the first pulse using the nonparametric rank sum test. The significance of whatever test was used.

**MN Pool Model**

Across the MN pool, excitatory and inhibitory synapses on individual cells interact with the MN intrinsic properties to produce the ventral root output. We, therefore, developed a multiscale (that bridged the synaptic, cellular, and system scales), high-fidelity (based on anatomically detailed MN data) computer model of the alpha-MN pool to reproduce our ventral root recordings in response to sensory and descending stimulation. We have used anatomically detailed MNs, as opposed to simplified computer models of MNs, because the simplification of dendritic morphology in the latter has been shown to generate large errors in simulating MN firing behaviors and active properties (Elbashouy 2014). The model was used to examine the changes in synaptic conductances (i.e., synaptic plasticity) and assess how they contributed to the changes in coAP firing recorded at the ventral root (i.e., system plasticity). The MN pool model consisted of 50 cells (see Fig. 6A) and was implemented using the NEURON simulation environment (Hines and Carnevale 1997). The model of individual MNs was based on that developed for alpha MNs by Elbashouy et al. (2005), which incorporated realistic alpha MN morphology (see Fig. 6B), realistic dendritic distribution of synaptic inputs, and somatic and dendritic active conductances. This model was used because it has been highly optimized to reproduce multiple electrophysiological datasets of spinal MNs obtained under different recording conditions (current and voltage clamp, MN activation via synaptic inputs, and current injection) (Elbashouy et al. 2005, 2006). The somatic active conductances included the fast Na+ and delayed rectified K+ channels (which mediate the AP spike) and the Ca2+-activated K+ channels and N-type Ca2+ channels [which mediate the afterhyperpolarization (AHP)]. The dendritic active conductances included the low voltage-activated L-type Ca2+ channels [which mediate Ca2+ persistent inward current (Ca2+ PIC)]. Detailed information on the equations governing these ion channels and how the model was adapted to replicate adult mouse MNs is available in the APPENDIX. The passive and active properties of the Elbashouy et al. (2005) model were varied to match the electrical properties of sacral MNs of different types (i.e., slow, fatigue-resistant, and fast-fatigable types) that matched our intracellular recordings from mouse MNs in the sacral cord. Each individual MN model was driven by both excitatory and inhibitory synapses whose distributions were uniform on the dendrites and whose conductances were the same across all neurons in the pool. This assumption is supported by the equal synaptic input limb MNs receive from inhibitory Remshaw and Ia-reciprocal inhibitions and the small variability in amplitude of inputs MNs receive from excitatory Ia-afferents and vestibulospinal input (Powers and Binder 2001) (see DISCUSSION).

To simulate the effects of electrical stimulation of sensory or descending pathways, the excitatory and inhibitory synapses were activated synchronously and repeatedly at frequencies comparable to the stimulation frequencies used in our experiments. In experiments where inhibition was blocked, only excitatory synapses were activated synchronously and repeatedly at the required stimulation frequency. Therefore, the synaptic conductance in the model accounted for the strength of the synapses on MNs and the probability of neurotransmitter release at that stimulation frequency. Because when activated spinal MNs fire APs, which are all-or-none events and the summation of which gives rise to compound APs measured at the ventral root, the amplitude of compound APs is proportional to the number of MNs activated by synaptic inputs. This assumption is supported by the linear summation of electromyography (EMG) signals and muscle force over a large range in response to an increased input to the MN pool (Fuglevand et al. 1993; Heckman and Binder 1991). Therefore, the number of MNs in the pool activated by synaptic inputs at each stimulation pulse was determined from Eq. 1 as followed:
number of spiking MNs,
\[
\text{coAP amplitude from orthodromic stimulation} = \frac{\text{amplitude of orthodromic coAP}_i}{\text{coAP amplitude from antidromic stimulation}} \times 50 
\]
where \(i\) is the pulse number; 50 is the total number of MNs in the pool model; the numerator term represents the output of MNs activated at pulse \(i\) in responses to sensory or descending stimulation; and the denominator represents the output of all MNs in the pool when the ventral root was stimulated.

Similarly, the output of activated MNs in the pool model as seen at the ventral root at each pulse was determined by converting the number of spiking MNs in the model to the amplitude of compound AP in mV using Eq. 2 as follows:

\[
\text{amplitude of orthodromic coAP}_i = \frac{\text{number of spiking motoneurons}}{50} \times \text{coAP amplitude from antidromic stimulation} 
\]

**Network Excitation**

During intracellular recordings, MNs were verified through antidromic electrical stimulation of the ventral root and the recording of AP spikes in the MN membrane potential. These antidromically evoked APs featured a spike followed by a period of AHP (see Fig. 4A, inset). However, when electrical stimulation of sensory or descending pathways was applied, the AHP disappeared and was replaced by afterdepolarization (see Fig. 4A, filled arrow), and the resting membrane potential between stimuli was depolarized by an amount that varied up to 3 mV (see Fig. 4, A and C, open arrows show when network excitation was evident). This behavior was called “network excitation” because the membrane depolarization was sustained between pulses and thus likely produced by activity in interneuronal networks (see Fig. 5 for more information on the origin of this activity). In the pool model, the effects of network excitation were simulated by 1) depolarizing the resting membrane potential of the MN between stimuli to account for the membrane depolarization resulting from poly-EPSP temporal summation (see next section on how this parameter is set), and 2) eliminating the AHP by depolarizing the reversal potential of the Ca\(^{2+}\)-activated K\(^+\) channels, which mediate the AHP, to simulate AHP disappearance and the appearance of afterdepolarization.

**Process for Adjustment of Model Parameters**

We used our pharmacological studies to separate the effects of excitatory and inhibitory synapses and the effects of monosynaptic and polysynaptic inputs to MNs, which helped in adjusting various model parameters. Under control conditions (when no drugs were applied), ventral root firing resulted from the effects of monosynaptic excitatory conductances \(G_{\text{excit}}\), disynaptic inhibitory conductances \(G_{\text{inhib}}\), and effects of network excitation from polysynaptic sources, which could be represented as:

\[
\text{control data} = G_{\text{excit}} + G_{\text{inhib}} + \text{Network Excitation} 
\]

Please also note that network excitation includes the combined effect of excitation and inhibition from polysynaptic pathways. However, our data (see Fig. 3, A–D) indicate that network excitation is resulting predominantly from excitatory synapses and that inhibition from polysynaptic sources is generally very weak because of the similar effects of PTX and STR on ventral root activity at 1.5 times T (1xT; when mono- and disynaptic inputs are fully activated without much activation of polysynaptic inputs) and 10 times T (10xT; when mono-, di-, and polysynaptic inputs are all fully activated).

When PTX and STR were administered, inhibitory conductances \(G_{\text{inhib}}\) were blocked and ventral root firing resulted from the effects of monosynaptic \(G_{\text{excit}}\) and polysynaptic network excitation, which could be represented as:

\[
\text{PTX/STR data} = G_{\text{excit}} + \text{network excitation} 
\]

When mephenesin was administered, effects resulting from polysynaptic pathways, such as network excitation, were blocked and ventral root firing resulted from the effects of monosynaptic \(G_{\text{excit}}\) and disynaptic \(G_{\text{inhib}}\), which could be represented as:

\[
\text{mephenesin data} = G_{\text{excit}} + G_{\text{inhib}} 
\]

By rearranging Eqs. 3–5, we can use the experimentally recorded ventral root data to assess the effects of monosynaptic \(G_{\text{excit}}\) alone on ventral root firing as follows:

\[
G_{\text{excit data}} = \text{PTX/STR data} + \text{mephenesin data} - \text{control data} 
\]

Accordingly, the following model tuning process was conducted at each stimulation frequency to adjust the three main model unknowns \(G_{\text{excit}}, G_{\text{inhib}}, \text{and network excitation}\) based on our ventral root recordings obtained with the pharmacological agents:

**Step I.** In a model with only excitatory synapses, \(G_{\text{excit}}\) on individual cells were varied such that the output of the pool model matched the firing data computed from Eq. 6. This step set the value of \(G_{\text{excit}}\).

**Step II.** In a model with both excitatory and inhibitory synapses, we used \(G_{\text{excit}}\) values obtained from step I and varied \(G_{\text{inhib}}\), which got activated 0.7 ms after \(G_{\text{excit}}\) to simulate the synaptic delay, on individual cells such that the output of the pool model matched the firing data obtained in presence of mephenesin (see Eq. 5). This step set the value of \(G_{\text{inhib}}\).

**Step III.** In a model with excitatory and inhibitory synapses, in addition to network excitation, we used \(G_{\text{excit}}\) and \(G_{\text{inhib}}\), values obtained from steps I and II, eliminated the AHP by depolarizing the reversal potential of the Ca\(^{2+}\)-activated K\(^+\) channels, and then varied the amount of membrane depolarization between pulses needed such that the pool model output matched control firing data obtained in absence of any blockers (see Eq. 3). This step set the value of membrane depolarization (in mV) mediated by network excitation.

**RESULTS**

Short-term plasticity was studied using three techniques. First, we studied plasticity at the system level by measuring the coAPs generated in ventral roots, which contain all the MN axons for a particular spinal segment (Fig. 1). Pharmacological agents, such as PTX and STR, were used to isolate the effects of excitatory vs. inhibitory components of the inputs, and mephenesin was used to isolate the effects of monosynaptic vs. polysynaptic components of the inputs. To understand cellular contributions to the system plasticity, we then performed intracellular recordings in MNs to identify the behavior of PSPs in single neurons. Finally, we developed a multiscale, high-fidelity computer model of the MN pool to estimate the synaptic conductances and to quantify how system behavior emerges from behavior at the synaptic level. In each case, we analyzed plasticity in response to electrical stimulation of both sensory and descending inputs (as illustrated in Fig. 2 for ventral root recordings).

**Differential Forms of System Plasticity in Response to Sensory and Descending Stimulation**

We first confirmed that the descending input depended mainly on AMPA receptors for fast excitatory synaptic transmission (demonstrated by effective block of the de-
Mild Effect on Synaptic Plasticity

Network Inhibition, Phase-Locked to Each Stimulus, Has Mild Effect on Synaptic Plasticity

To determine if inhibitory circuits affected the overall system plasticity, we first examined coAP behavior in the presence of inhibitory blockers. Bath application of 5 μM PTX (to block GABA receptors) and 0.25 μM STR (to block glycine receptors) changed the magnitude, but not the form of plasticity.
Fig. 3. The effect of stimulation intensity (A-D) and frequency (E-H) on the ventral root neural plasticity. A and B: coAP amplitudes, as percentage of responses at the 1st pulse, at different stimulation intensities (from 1xT to 10xT) in response to sensory and descending stimulation at 25 Hz, respectively (n = 17 for A; n = 16 for B). The same format is shown in C and D but when inhibitory synapses were blocked using picrotoxin and strychnine (n = 12 for C; n = 9 for D). E and F: coAP amplitudes, as percentage of responses at the 1st pulse, at different stimulation frequencies (from 0.05 to 100 Hz) in response to sensory and descending stimulation at 2xT, respectively (n = 9 for E; n = 10 for F). The same format is shown in G and H but when inhibitory synapses were blocked using picrotoxin and strychnine (n = 5 for G; n = 7 for H). In general, the number of asterisks indicates the level of statistical significance between the amplitude at each pulse relative to the 1st pulse for each stimulation intensity (A-D) or frequency (E-H) (using the nonparametric one-way Kruskal-Wallis test. *P < 0.05; **P < 0.01; ***P < 0.001).

Inhibitory blockers markedly reduced the degree of STD in response to sensory input, although depression remained statistically significant (Fig. 3. A vs. C, shows the effect of inhibition for various stimulus intensities; Fig. 3, E vs. G, shows the effect for various stimulation frequencies). An exception occurred at the very lowest stimulus intensity, where a slight STF emerged. For descending input, inhibition had a more modest effect on the magnitude, not the form, of plasticity (Fig. 3D) in which it dampened both the STF at high frequencies and the STD at medium frequencies (Fig. 3F). These results showed that network inhibition was activated by these stimuli and had a modest impact on system plasticity.

**Intracellular Recordings of EPSPs Also Showed Differential Plasticity in Sensory vs. Descending Inputs**

Sharp-electrode intracellular recordings were performed in MNs of the sacral cord preparation (Jiang and Heckman 2006) to compare the change in EPSP amplitudes in individual MNs to the change in coAP amplitudes at the ventral root output (Fig. 4). MNs were verified through the recording of antidromic APs in response to ventral root stimula-
tion. In these antidromic APs, the spike was followed by AHP (Fig. 4A, inset). A total of 30 individual MNs were recorded, each stimulated for many trials, at different sensory and descending stimulation intensities and frequencies. A MN was studied when its resting membrane potential was lower than $-60 \text{ mV}$ and AP amplitude was $70 \text{ mV}$ or higher.

Figure 4, A and C, shows examples of EPSP recordings at 25 Hz of sensory and descending stimulation, respectively (2xT intensity). In these recordings, EPSP amplitude was measured as the difference between the EPSP peak and the resting membrane potential before the delivery of the first pulse. The changes in baseline during the stimulus train are likely due to asynchronous excitatory network activity and are considered in the next section. The amplitudes of the EPSPs thus reflect the stimulus-locked effects of excitation and inhibition as well as the effect of the network excitation. In those cases where an EPSP evoked an AP, the cell was hyperpolarized to avoid evoking an AP that interferes with the measurement of EPSP amplitude. This occurred primarily with sensory stimulation, which produced larger EPSPs than descending stimulation, especially for the first pulse (and larger coAPs; compare coAPs amplitude in Fig. 2, B vs. E; note the differences in y-axis scale). Similar to ventral root recordings, sensory stimulation produced STD in the amplitude of evoked EPSPs over the five pulses at 25 Hz (Fig. 4B). This was observed consistently in 17/17 MNs. On the contrary, descending stimulation produced STF in the amplitude of evoked EPSPs (seen in 8/8 cells; Fig. 4D).

**Effect of Network Activity on Plasticity**

The changes in the amplitudes of the PSPs reflect effects of excitatory and inhibitory pathways that are time locked to each stimulus. In Fig. 4, A and C, it is also evident that considerable membrane depolarization developed between stimulus pulses (between the dotted line and membrane potential at the open arrows). The magnitude of this depolarization varied among pulses (see Fig. 4A, magnitude of membrane depolarization was very small at P1 and P5 but larger otherwise). Remarkably, when the EPSP triggered a spike, there was no sign of the usual AHP, and in fact, an afterdepolarization tended to appear (note filled arrow right after the spike in Fig. 4A). This suggests that neuromodulators released by local circuit activity might have suppressed the AHP, as occurred during activation of spinal central pattern generators for scratch and locomotion (Brown-
served that was proportional to the stimulation frequency (i.e.,
frequency and intensity. For sensory stimulation, STD was ob-
seeing pathways has differential effects on the MN output and
membrane potential after the stimulation cessation (Fig. 4
A, filled and open arrows) and a metabotropic component (the
fast appearance of sustained depolarization between pulses (Fig. 5B, middle arrow) and after
cessation of stimulation (Fig. 5B, right arrow). The intracellu-
lar recordings showed that mephenesin eliminated the inter-
pulse depolarization and restored the AHP (Fig. 5A, see the red
trace), confirming that these effects result from the activation of
interneuronal networks. At the system level, mephenesin
differtial effects on sensory vs. descending inputs. For
the sensory input (Fig. 5, C and E), there was a small decrease
in the coAP amplitude from the first stimulus pulse, which is
consistent with the findings that this input being dominated by
monosynaptic excitation via muscle spindle Ia afferents (Ben-
ett et al. 2004; Eccles et al. 1957; Jiang et al. 2009; Li et al.
2004; Shapovalov and Kurchavyi 1974). For subsequent pulses, there appeared to be a greater decrease, i.e., a de-
velopment of greater STD (see the red trace and gray bars in Fig.
5, C and E, respectively). This suggests that the Ia synapses
mediating the monosynaptic input undergo substantial STD
(note that the mephenesin presumably blocks the stimulus-
locked multisynaptic excitation and inhibition).

In descending stimulation, mephenesin sharply decreased
the coAP amplitudes for all pulses (in 7 of 7 ventral roots
tested; see the red trace and gray bars in Fig. 5, D and F,
respectively), suggesting that significant poly-EPSPs exist in
the descending input and that much of the descending EPSP is
mediated by a di- or polysynaptic pathways. Washing meph-
nesin out restored amplitudes toward their normal states (see
the blue trace and black bars in Fig. 5, D and F, respectively).
These effects of mephenesin on MN responses and system
plasticity emphasize the importance of local network excita-
tion. This excitation is sustained and may have both an ioni-
tropic component (the fast appearance of sustained depolariza-
tion between spikes upon stimulation initiation; Fig. 4, A and
C, filled and open arrows) and a metabotropic component (the
AHP reduction; Fig. 5A, arrows) and slow recovery of resting
membrane potential after the stimulation cessation (Fig. 4C;
see the prolonged depolarization in membrane potential).

Summary of Experimental Data

Our intracellular measurements and ventral root recordings
showed that electrical stimulation of the sensory and descend-
ning pathways has differential effects on the MN output and
system plasticity, both of which depend on stimulation fre-
quency and intensity. For sensory stimulation, STD was ob-
erved that was proportional to the stimulation frequency (i.e.,
the higher the stimulation frequency, the greater the depres-
sion). In contrast, bimodal system plasticity was observed for
descending stimulation: high stimulation frequencies (>25 Hz)
produced STF, whereas low stimulation frequencies (<25 Hz)
produced modest STD. Moreover, the pharmacological studies
revealed mild effects of stimulus-locked network inhibition but
strong effects of asynchronous network excitation.

Computer Simulations Analyze the Transformation Process
from Synaptic to System Plasticity

To examine how plasticity at the sensory and descending
synapses interact with MN intrinsic properties to produce the
overall system plasticity, we developed a multiscale, high-
fidelity computer model of the MN pool with excitatory and
inhibitory drives to MNs. The MN pool consisted of 50 model
neurons, each with a full representation of its anatomical and
electrical properties (Fig. 6, A and B; see APPENDIX for details)
(Elbasiouny et al. 2010; Elbasiouny et al. 2006, 2005). Excit-
atory and inhibitory synapses were distributed uniformly
throughout the dendritic tree of each neuron. The MNs varied
in their sizes and electrical properties and have been activated
in the order of their size from small to large neurons, as
required by experimental data in both humans and animals
(Henneman and Mendell 1981) (Fig. 6, C and D). We used the
pharmacological data (i.e., STR, PTX, and mephenesin admin-
istration, data in Figs. 3 and 4) to adjust various model
parameters (see MATERIALS AND METHODS). We focused initially
on understanding the responses at 25 Hz at 2xT intensity at
which sensory stimulation showed STF whereas descending
stimulation showed STF (Fig. 7). The goal of the model was to
reverse-engineer the coAP firings recorded from the ventral
roots (i.e., system output) from the system subcomponents (i.e.,
MN's and excitatory and inhibitory synapses) to isolate synaptic
plasticity contributions to overall system plasticity over the five
pulses.

First, the model indicated that the number of MNs in the
pool activated by sensory stimulation was larger than that
activated by descending stimulation; consistent with higher
sensory than descending coAP amplitudes. Figure 7, A–C,
illustrates the simulation analysis using sensory stimulation.
The x-axis in each graph is pulse number for the five pulse
stimulus trains. The analysis started at the synaptic level (Fig.
7, A, D, and G), with the behavior of the synaptic conductances
on the dendrites and soma of each neuron, and then progressed
through somatic depolarization to recruitment (Fig. 7, B, E,
and H) generating the system output via the coAPs measured in the
ventral roots (Fig. 7, C, F, and I). Step 1 was to identify the
behavior of the monosynaptic excitatory conductances ($G_{\text{exc}}$).
To do so, the amplitudes of these conductances (which were
equal on all MNs at all dendritic and somatic locations) were
adjusted to generate coAPs that matched the data from Eq. 6.
The excitatory conductances exhibited a marked depression,
i.e., STD, over the five pulses relative to the first pulse (Fig. 7A,
red trace). Step 2 was to identify the amplitudes of inhibitory
conductances that were phase locked to each pulse in the
presence of excitatory synapses from step 1. This identification
was done by increasing the inhibitory conductance amplitudes
($G_{\text{inh}}$) until the simulated coAPs decreased from step 1 and
matched the experimental data obtained in the presence of
mephenesin (Fig. 7C, green trace; see Eq. 5). The inhibitory
conductances also exhibited significant STD compared with
that at the first pulse (Fig. 7A, blue trace). In step 3, the somatic PSPs generated by these synaptic conductances were calculated (Fig. 7B). The amplitude of EPSPs and inhibitory postsynaptic potentials (IPSPs) tracked the behavior of the excitatory and inhibitory conductances, respectively, with both exhibiting STD (Fig. 7B, red and blue traces, respectively, note the conversion to negative values to reflect the hyperpolarizing nature of the IPSPs). Because excitatory synapses have larger driving force than inhibitory synapses, EPSPs have larger amplitudes than IPSPs. The amount of inhibition (i.e., IPSP amplitude) was generally small at all pulses, consistent with the weak inhibition effects on system plasticity observed in our pharmacological data (Fig. 3). Step 4 was to identify the amount of membrane depolarization provided by network

![Diagram](image-url)

Fig. 5. The effect of mephenesin on intracellular recordings, in presence (A) and absence (B) of cell firing and on ventral root recordings (C–F). A: recording from a MN shows effect of mephenesin (1 mM) on MN APs during sensory stimulation of 25 Hz. Note that when spikes were evoked, mephenesin eliminated the afterdepolarization and restored the AHP (see the 3 arrows pointing to the red trace). B: when APs were blocked using QX-314 during sensory stimulation at 5 Hz, mephenesin blocked the poly-EPSPs (left arrow), eliminated the depolarization in membrane potential between pulses (middle arrow), and eliminated the sustained depolarization after stimulation cessation (right arrow). Examples on the effect of mephenesin (1 mM) on ventral root output during sensory (C) and descending (D) stimulation (2xT, 25 Hz). Mephenesin caused stronger STD in C and replaced STF with STD in D (compare the red and black traces). After washout, moderate STD and STF were restored in C and D, respectively. Summary of ventral root data for the effect of mephenesin during sensory (E) and descending (F) stimulation. For each pulse, the number of asterisks indicates the level of statistical significance between the amplitudes at the control, mephenesin, and wash-out conditions (using the nonparametric one-way Kruskal-Wallis test, *P < 0.05; **P < 0.01; n = 9 for E; n = 9 for F).
Fig. 6. The MN pool model. A: structure of the MN pool consisting of 50 neurons that range in intrinsic properties to represent S-, FR-, and FF-type MNs in the mouse. The letters E and I represent the excitatory and inhibitory synapses on each MN in the pool. B: model morphology of an individual MN in the pool. The dendritic morphology is based on a medial gastrocnemius MN (cell 43/5) in Cullheim et al. (1987). The red and blue dots in the right top and bottom represent the distribution of E and I synapses on the MN dendrites, respectively. C: example of MN firing in the pool model during 5-pulse (P1 to P5) electrical stimulation at 25 Hz when inhibition was blocked. Neurons fire APs when the synaptic input is strong enough to bring them to their firing thresholds, otherwise EPSPs are observed. D: raster plot showing the firing profiles of all MNs in the pool model for the condition in C. A vertical mark indicates the firing of an AP. The arrows indicate the neurons whose firing profiles are shown in C. At each pulse, the coAP amplitude measured at the ventral root is proportional to the number of MNs that fired an AP.
excitation between stimuli. This was done by eliminating the AHP (see MATERIALS AND METHODS) and varying the amount of membrane depolarization between pulses, while driving the MNs with excitatory and inhibitory conductances, until the simulated coAPs matched control experimental data obtained in absence of any blockers (Fig. 7C, black trace; see Eq. 3).

Importantly, the membrane depolarization via network excitation showed variable amplitude over pulses and its peaks reached up to 3 mV (Fig. 7B, orange trace), consistent with our EPSP recordings from MNs. The net effect of EPSP, IPSP, and network excitation amplitudes (in mV) at the soma is shown in the black trace of Fig. 7B, indicating that the largest component of soma depolarization comes from EPSPs amplitude. Also, at the first pulse network excitation mediated via polysynaptic pathways did not have noticeable effects (at the 1st pulse, the orange trace has zero amplitude in Fig. 7B and the difference between black and green traces is negligible in Fig. 7C), confirming that monosynaptic excitation has the largest effect on overall system behavior. In sum, despite the large disinhibition (Fig. 7A, blue trace) and large network excitation (Fig. 7B, orange trace), the motor output showed STD (Fig. 7C, black trace) similar to the excitatory synapses behavior (Fig. 7A, red trace), indicating that monosynaptic excitatory synapses have the largest impact on the sensory system plasticity (the 2nd pulse is an obvious example of that).

For the descending input, which showed STF in overall system output at the same frequency (Fig. 7F, black), both excitatory and inhibitory conductances exhibited no marked changes over the five pulses (Fig. 7D, red and blue traces). Also note that the descending excitatory and inhibitory conductances were much smaller than sensory conductances at the first pulse (compare the red and blue traces at P1 in Fig. 7A–D), consistent with the much smaller coAPs and EPSPs generated by the descending input. Conversion to descending EPSPs and IPSPs followed the behavior of their respective synapses and IPSP amplitudes were notably small at all pulses (Fig. 7E, red and blue traces). Interestingly, the membrane depolarization via network excitation showed variable amplitude with increasing peaks over pulses, yet smaller in magnitude (Fig. 7E, orange trace, never exceeded 2 mV) than sensory stimulation, in agreement with our EPSP recordings from MNs. Consequently, the net depolarization at soma potential showed STF that resulted from the smaller, increasing network excitation riding on top of larger, steady EPSPs and much weaker, steady IPSPs (Fig. 7E, black trace). Importantly, network excitation had strong effects at the first pulse, in contrast to sensory stimulation (Fig. 7E, at P1 the orange trace has a positive amplitude and the difference between the black and green traces is sizable in Fig. 7F), consistent with the stimulation of descending polysynaptic pathways. In sum,
despite the lack of facilitation of excitatory synapses and the increased facilitation via inhibitory synapses at later pulses, the motor output showed STF (black trace in Fig. 7F) similar to network excitation (the 5th pulse is a clear example of that). Although weaker in magnitude than monosynaptic excitation, it was network excitation that produced STF in system output, indicating its critical role in shaping descending system plasticity.

We also examined descending stimulation at 5 Hz, the frequency that showed STD in system output (Fig. 7I) black trace) and compared it with descending stimulation at 25 Hz, which showed STF (Fig. 7F, black). Both excitatory and inhibitory synapses showed no marked changes over the five stimuli (Fig. 7G, red and blue traces) that were faithfully tracked by their respective steady PSPs: strong EPSPs and weak IPSPs (Fig. 7H, red and blue traces). The membrane potential mediated via network excitation showed variable amplitude but with decreasing peaks and much smaller amplitudes over stimuli (never exceeded 1 mV) and still with large contribution at the first pulse (Fig. 7H, orange trace). Accordingly, the net depolarization at the soma showed STD that resulted from the smaller, decreasing network excitation riding on top of larger, steady EPSPs and much weaker, steady IPSPs (Fig. 7H, black trace). These results confirm that network excitation, although weak in amplitude, shapes the descending system plasticity.

Summary of Simulation Data

Taken collectively, the MN pool model allowed the breakdown of MN synaptic inputs into components, monosynaptic excitation, polysynaptic excitation, and polysynaptic inhibition, and assessed their separate contributions to system output. The simulation results show that in sensory stimulation: 1) monosynaptic excitation has the largest impact on the motor system plasticity with STD at all frequencies; and 2) disynaptic inhibition and polysynaptic excitation have little contributions to system plasticity. In descending stimulation, on the other hand: 1) network excitation, although much weaker in magnitude than monosynaptic excitation, plays a critical role in shaping descending system plasticity; 2) the profile and magnitude of network excitation mediated via polysynaptic pathways mediate the bimodal plasticity in motor output observed experimentally: i.e., STD at decreasing low network excitation magnitudes at low stimulation frequencies but STF at increasing high network excitation magnitudes at high stimulation frequencies; and 3) monosynaptic excitation (which has large magnitude) and disynaptic inhibition (which has small magnitude) have little contributions to system plasticity across all frequencies.

Verification of Simulation Data

To verify that the MN pool model with its excitatory and inhibitory drive represented the effects of sensory and descending stimulation reasonably well, we compared the change in the EPSP amplitude in the pool model to that obtained from intracellular MN recordings (Fig. 8). For group comparison, the EPSP amplitude at each pulse was normalized to that of the first pulse for the model and experimental data, similar to ventral root recordings. The data demonstrated that simulated (white bars) and recorded (black bars) EPSPs had comparable amplitude ratios at each stimulation pulse for sensory (Fig. 8A) and descending (Fig. 8B) stimulation, indicating that the pool model replicated reasonably the behavior of EPSPs measured experimentally.

Sensitivity Analysis

To test the robustness of our simulation results, we conducted sensitivity analysis on key model parameters that influence the simulation results the most. These parameters were network excitation, intrinsic properties of MNs in the pool model, as well as their synaptic inputs. In the first set of simulations, we changed the way we calculated the amount of membrane depolarization provided by network excitation between stimuli given that it is the factor that we had the least information on. Also, the way we used experimental ventral root recordings to compute the separate effect of monosynaptic excitatory synapses in Eq. 6 assumes arithmetic manipulation of firing data obtained in presence of pharmacological blockers, which could be inaccurate. Therefore, we tuned the pool model using a predetermined value of membrane depolarization for network excitation (varied between 0 and 3 mV based on our intracellular MN recordings), as opposed to being a variable to solve for as in the original simulations, and then varied the excitatory conductances until the pool model matched the ventral root recordings in presence of the inhibitory blockers. Afterwards, we varied the inhibitory conductances, in presence of excitatory conductances and network excitation from the previous step, until the pool model matched the control experimental data, in absence of any blockers. With this approach, we avoided any mathematical manipulation of the ventral root recordings and we matched model output directly to recorded experimental data; however, membrane potential via network excitation was then assumed to be of steady amplitude throughout stimulation. The results of the new simulations are shown in Fig. 9, A and B, for sensory and descending stimulation, respectively, at 25 Hz (membrane depolarization was 1.5 mV in Fig. 9).
Second, our intracellular recordings showed that the width of the EPSPs evoked by sensory and descending stimulation was wide and the EPSPs were comprised of multiple events that are likely elicited from activation of polysynaptic sources (see the small arrows in Fig. 4, A and B, insets). To test the dependence of our simulation results on the EPSP width, we increased the EPSP duration in our simulations by 250% (called wide EPSPs in Fig. 9, C and D). Overall, the sensitivity analysis of these parameters confirmed our conclusions. For sensory stimulation, the excitatory and inhibitory conductances showed comparable behaviors to the original simulations (compare red and blue traces in Fig. 9, A and C, to those in Fig. 7A) and the monosynaptic excitatory conductances had the largest influence on system plasticity despite the large disinhibition and the steady depolarization from network excitation. For descending stimulation, the excitatory and inhibitory conductances showed comparable, but scaled, behaviors to the original simulations resulting in steady weak inhibition but stronger excitation (from monosynaptic and network sources). The weak facilitation in monosynaptic excitatory synapses (~10% in red traces of Fig. 9, B and D) did not account fully to the STF in ventral root output (80% in black traces of Fig. 7F) and network excitation was still necessary and made the larger contribution (~70%) to system plasticity. We also tested the effect of the synaptic delay, which was set to 0.7 ms, between the activation of the monosynaptic excitatory synapses (Gexcit) and the disynaptic inhibitory synapses (Ginhb). Since inhibition was phase locked to the stimulus, we varied the duration of the synaptic delay between 0 and 1.5 ms; however, the profiles of excitatory and inhibitory conductances were comparable to those in Fig. 7 (data not shown).

Third, we changed the time-dependent properties of mouse MNs to match those of cat MNs. In individual MNs of the pool, this involved doubling the specific membrane capacitance to double the membrane time constant, decreasing the Ca\textsuperscript{2+} removal rate in the membrane Ca\textsuperscript{2+} dynamics by a factor of 4 to reduce the AHP kinetics, and hyperpolarizing the reversal potential of the inhibitory synapses by 5 mV. With those numerous changes in cell properties, the pool model replicated the behaviors of cat MNs closely as in Elbasiouny et al. (2005) and the profile of excitatory and inhibitory conductances was comparable to that in the mouse model in response to sensory and descending stimulation (data not shown). Taken collectively, this analysis shows that our simulation results are robust and consistent when numerous model parameters were varied considerably across a wide range.

DISCUSSION

In the present study, we have undertaken a cell-to-system approach for investigating the short-term plasticity in neural output of the adult spinal motor system in response to repetitive stimulation of two synaptic pathways (sensory and descending inputs). Short train stimulation of the sensory afferents with a wide range of stimulation frequencies produced consistent STD in the ventral root motor output. In contrast, stimulation of the ventromedial descending tracts produced bimodal plasticity consisting of STD at low stimulation frequencies (up to 10 Hz) and STF at moderate to high frequencies (25 Hz and above). Ventral root recordings showed that network excitation had strong effects on system plasticity. Supported by intracellular recordings, computer simulations were used to unravel the synaptic mechanisms (e.g., the behavior of excitatory and inhibitory synaptic conductances in the MNs) underlying our ventral root recordings to explain the differential frequency-dependent plasticity mediated in the sensory and descending pathways.
pathways. For the sensory input, robust STD emerged at the system level because monosynaptic excitatory synapses governed the system behavior. For descending input, network excitation had the largest influence on system behavior: strong network excitation at high stimulation frequencies produced STF, whereas weak network excitation at low stimulation frequencies produced STD.

Studies of neural or synaptic plasticity often use fixed stimulation intensity coupled to a restricted range of stimulation frequencies (Barriere et al. 2008; Hayashi et al. 2003; Lev-Tov and Pinco 1992; Pinco and Lev-Tov 1993). Many of these studies examine the resulting changes in amplitudes of EPSPs and IPSPs in the subthreshold range and use this information to predict the cell firing activity. In the present study, we attempted a more general analysis, examining a wide range of stimulation frequencies (0.05 to 100 Hz) and intensities (1xT to 10xT) for both inputs. The stimulation intensity was determined in terms of the current threshold required for evoking a motor firing behavior (i.e., coAP) at the ventral root. Accordingly, stimulation intensity of 1xT in our study corresponds to the functional motor threshold which elicits a muscle contraction. Our threshold intensity is, however, likely to be higher than that for the threshold of sensory afferent activation. This difference in stimulation intensity is important to consider when comparing our results to literature because our data showed that neural plasticity in the coAPs from the ventral root output depends on the stimulation intensity (Fig. 3, see next section).

Our results are generally in agreement with previous works in spinal MNs. Repetitive activation of sensory input caused STD in EPSPs that recovered completely within 100 s (Lev-Tov and Pinco 1992). When inhibitory synapses were blocked, EPSP amplitudes from excitatory synapses showed STD similar to previous reports (Lev-Tov and Pinco 1992; Pinco and Lev-Tov 1993). For the sensory input, the monosynaptic excitation was presumably generated by muscle spindle Ia afferents (Bennett et al. 2004; Jiang et al. 2009; Lev-Tov and Pinco 1992). Based on studies in hindlimb MNs (Jankowska 2001), there are several potential sources for the inhibition that was time-locked to each stimulus pulse. At low stimulus intensities, Ia reciprocal inhibition and Golgi tendon organ Ib inhibition could both contribute, while at higher levels, multiple cutaneous and muscle afferents could contribute (Jankowska 2001).

For the descending input, the electrode was positioned ventromedially on the white matter, and it likely activated several descending axons such as those from the vestibulospinal and reticulospinal systems (Watson and Harvey 2009) and local descending pathways (i.e., short and long propriospinal pathways). Although descending stimulation generated monosynaptic EPSPs in MNs, our mephenesin data showed that nonmonosynaptic pathways contributed significantly to generating the coAP (Fig. 5F, note the large difference between white and gray bars), (Alstermark and Ogawa 2004; Alstermark et al. 2004; Floeter and Lev-Tov 1993).

It is important to note that the magnitude of short-term plasticity is dependent on stimulation intensity. In the sensory system, STD was the largest at 1xT that was completely eliminated when inhibition was blocked (Fig. 3, A and C). This was not the case at higher intensities. This could be explained by the type of inhibitory inputs activated. At low intensities, Ia-reciprocal inhibition afferents, which have low activation threshold and mediate strong inhibitory inputs to MN, are activated, which probably contribute largely to STD and thus their blockade by PTX and STR eliminates STD. At high intensities, on the other hand, both low-threshold and high-threshold (e.g., cutaneous afferents), with the latter having mixed polysynaptic excitatory and inhibitory effects on MN (Powers and Binder 1985a,b), are activated, leading to lower STD levels that do not get completely blocked by inhibition blockade. In the descending system, STD was also larger at low than high stimulation intensities (Fig. 3B) probably due to higher network excitation from activation of descending inputs. Interestingly, the synaptic STD on descending pathway could be observed with stimulation frequency as low as 5 Hz in neonatal spinal MNs (Floeter and Lev-Tov 1993) while this frequency evoked both system and synaptic STD on the descending pathway in our study, indicating either an age-related changes in plasticity or a difference in the descending components. It should also be noted that our experiments on STP were done at room temperature. As synaptic STD in supraspinal nucleus (Moldavan and Allen 2010) was reported to decrease with increasing temperature, the STPs in this study may differ in magnitude than those at physiological temperatures.

The source of the network excitation is unclear and its magnitude represents the net excitation and inhibition mediated via polysynaptic pathways. There are several types of interneurons with excitatory and inhibitory actions on MNs, including those involved in the generation of locomotion and those relaying sensory input from muscle and cutaneous afferents (Jankowska 2001). The reduction of the AHP may be due to activation of COOH terminals on MNs, which have recently been shown to be muscarinic and may be involved in locomotion (Miles et al. 2007; Zagoraiou et al. 2009). Despite its small amplitude compared with monosynaptic excitation, our results showed that network excitation has an especially important role in shaping system plasticity in the descending input. Much larger facilitation was seen on the poly-EPSPs in neonatal rat MNs (Floeter and Lev-Tov 1993) and on disynapses in the same descending pathways (Floeter et al. 1993), favoring a maturation in plasticity, but not differences due to descending location.

The computer simulations employed in this investigation were essential in bridging plasticity at the synaptic level into plasticity at the whole system output. All models are approximations, but modeling of spinal MNs has advanced rapidly in the past 10 yr (Bui et al. 2006, 2008; Elbasiouny et al. 2006, 2005, 2010; Powers et al. 2012). The models employed here were highly realistic and spanned the whole range from single synaptic inputs on MN dendrites to the motor pool firing output and likely to provide a reasonably accurate estimate of the synaptic conductances. We did assume that all synaptic conductances were the same on all MNs. Of course, there will be a significant degree of randomness from synapse to synapse, but focusing on the average behavior seems reasonable. Previous studies (reviewed in Powers and Binder 2001) have demonstrated that many inputs are distributed nonuniformly with respect to MN threshold, such that some generate more synaptic current in high-threshold MNs (e.g., the vestibulospinal input) (Westcott et al. 1995) and at least one sensory input generates more in low-threshold MNs (the muscle spindle Ia input) (Heckman and Binder 1988); however, these variations...
were small. The inhibitory inputs studied thus far generate about equal synaptic current in all MNs. Since multiple inputs are active in the present study, it seems reasonable to assume net equal distributions. Moreover, distribution of input among the pool of MNs is unlikely to affect plasticity behavior, which is indicated by the percent change in synaptic weights relative to the first pulse. A major focus of the study of MNs has recently been on the strong PICs that are generated primarily in dendritic regions (Binder 2002; Heckman et al. 2008; Hultborn et al. 2004). PICs, however, are unlikely to make a significant contribution to plasticity studied here because they depend on the presence of serotonin and norepinephrine released by axons descending from the brainstem. In the isolated sacral cord preparation in presence of anesthesia and without exogenous administration of these neuromodulators, PICs are small (Harvey et al. 2006a). Thus our simulated MNs had small PICs comparable to MNs in their low-to-moderate neuromodulatory state. We also ran simulations on MNs in their high neuromodulatory state (i.e., with large PICs), and the results were qualitatively similar (data not shown). We used our experimental recordings obtained in the presence of pharmacological agents (PTX, STR, and mephenesin) to tune and constrain unknown parameters. Given the uncertainty in the estimation of the effect of network excitation, we calculated its effects in two contrasting approaches: The first approach (top-down type approach) separated the effects of network excitation from other synaptic inputs mathematically and used estimated firing data to solve for network excitation at each pulse. The second approach (bottom-up type approach), on the other hand, used a preset value of network excitation across all pulses, which was varied within the recorded experimental range, and matched direct firing data that included combined effects of network excitation and synaptic inputs. Both approaches produced similar excitatory and inhibitory synaptic behaviors and confirmed the essential differences between sensory and descending inputs' simulations predict. The functional role of the differences in short-term plasticity in the two input pathways studied here may relate to their very different origins, sensory vs. descending. The STD in the Ia input to MNs has long been appreciated (Collins et al. 1986; Davis et al. 1985), although in the adult this plasticity has been mainly studied in anesthetized preparations, which lack the network activity that played such a major role in the present study. Movement evokes a strong increase in firing in proprioceptive afferents as well as other sensory inputs and a reduction in this inflow may be a necessary to avoid excessive interference with the movements (Jacobs and Formal 1993; Stein and Capaday 1988). The STD in the sensory input seen here may contribute to this sensory control. The descending input exhibited bimodal STP in our results, with facilitation emerging at high frequencies. This facilitation may assist in generating stronger motor outputs as descending drive increases.

In conclusion, differential forms of system plasticity result from the stimulation of two presynaptic pathways that share a common output. Our experimental recordings and computational analysis at the cellular and system levels indicated that synaptic inputs and network excitation act together to shape the ventral root neural output during repetitive stimulation, making the relationship between synaptic (input) and system (output) plasticity nonlinear and dependent on the net excitation received by MNs.

APPENDIX

Model was implemented and simulations were run in the NEURON simulation environment (Hines and Carnevale 1997), version 7.2. A variable integration time step was used with error of 0.001%. The MN pool model consisted of 50 cells. The morphology of each cell has been based on that of alpha-MN [cell 43/5, Cullheim et al. (1987)]. The passive properties of individual cells have been varied to represent those of S, FR, and FF types (Table A1).

The active properties of individual cells were based on the model developed by Elbasiouny et al. (2005). The somatic active conductances included the fast Na\(^+\), delayed rectified K\(^+\), Ca\(^{2+}\)-activated K\(^+\), and N-type Ca\(^{2+}\) channels, in addition to calcium dynamics that regulate intracellular and extracellular Ca\(^{2+}\) concentrations. The initial segment and axon hillock included fast and persistent Na\(^+\) and delayed rectified K\(^+\) channels. The dendritic active conductances included the low voltage-activated L-type Ca\(^{2+}\) channels placed at dendritic distances between 300 and 850 \(\mu m\). The magnitude of PIC was the same in MN types (Lee and Heckman 1998). Given that our ventral root recordings and intracellular measurements were obtained from the sacral cord in vitro preparation in which the neuromodulatory state is low, the conductance of Cav1.3 channels was set to produce low PIC amplitude at the soma (5 nA under voltage clamp at \(-70\) mV) (Lee and Heckman 2000).

The ionic currents, \(I_{ion}\), of different channels were described by the following general expression:

\[
I_{ion} = g_{ion} \times (V_m - E_{ion}) \quad (A1)
\]

where \(g_{ion}\) is the varying conductance of the ion channel; \(E_{ion}\) is the maximum conductance of the ion channel listed in Table A1; \(m\) and \(h\) are the activation and inactivation state variables, respectively; \(n\) and \(l\) are the order of activation and inactivation, respectively.

For each membrane state variable (\(\eta\)), the time and voltage dependence is given by:

\[
d\eta/dt = \alpha_\eta (1 - \eta) - \beta_\eta \eta \\
\tau_\eta = 1/(\alpha_\eta + \beta_\eta) \quad (A4)
\]

Somatic Channels

Fast Na\(^+\) channels.

\[
I_{Na} = g_{Na} \times m^3 h \times (V_m - E_{Na}) \quad (A5)
\]

\[
E_{Na} = 50 \text{ mV} \quad (A6)
\]

\[
\alpha_m = \left[-0.4 \times (V_m + 49)\right]/\left[e^{-(V_m+49)/5} - 1\right] \quad (A7)
\]

Table A1. Active and passive parameters of MNs in the pool model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Min Value</th>
<th>Max Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatic (R_m), (\Omega\text{-cm}^2)</td>
<td>70</td>
<td>255</td>
</tr>
<tr>
<td>Dendritic (R_m), (\Omega\text{-cm}^2)</td>
<td>3,500</td>
<td>12,750</td>
</tr>
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<td>Input resistance, (M\Omega)</td>
<td>0.5</td>
<td>1.6</td>
</tr>
<tr>
<td>(C_m), (\mu F/cm^2)</td>
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<td></td>
</tr>
<tr>
<td>Somatic (g_{K}), (S/cm^2)</td>
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<td></td>
</tr>
<tr>
<td>Somatic (g_{Kd}), (S/cm^2)</td>
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<td></td>
</tr>
<tr>
<td>Somatic (g_{KCa}), (S/cm^2)</td>
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<td></td>
</tr>
<tr>
<td>Somatic (g_{KCN}), (S/cm^2)</td>
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<td></td>
</tr>
<tr>
<td>Initial segment/axon hillock (g_{Na}), (S/cm^2)</td>
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<td></td>
</tr>
<tr>
<td>Initial segment/axon hillock (g_{K}), (S/cm^2)</td>
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<td></td>
</tr>
<tr>
<td>Initial segment/axon hillock (g_{Kd}), (S/cm^2)</td>
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<td></td>
</tr>
<tr>
<td>Dendritic Cav1.3, (mS/cm^2)</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

MN, motoneurons.
\[\beta_m = \left[0.4 \times (V_m + 25)\right]/\left[e^{(V_m+25)/5} - 1\right] \]  
\[\tau_h = 30/[e^{(V_m+60)/15} + e^{-(V_m+60)/16}] \]  
\[h_m = 1/[e^{(V_m+38)/7} + 1] \]

**Delayed rectifier \(K^+\) channels.**
\[I_{Kdr} = g_{Kdr} \times n^4 \times (V_m - E_K) \]
\[E_K = -80 \text{ mV} \]
\[\tau_{n} = 5/[e^{(V_m+50)/40} + e^{-(V_m+50)/50}] \]
\[n_m = 1/[e^{(V_m+31)/15} + 1] \]

**Calcium dynamics.** The intracellular \(Ca^{2+}\) concentration (measured in mM) in the soma depends on the total compartmental \(Ca^{2+}\) current, \(I_{Ca}\), according to the following balance equation (Booth et al. 1997; McIntyre and Grill 2002).
\[d[Ca]/dt = f \times [-\alpha \times I_{Ca} - k_{ca} \times [Ca]] \]
\[f = 0.01 \quad \alpha = 1 \text{ mol/Cm/cm²} \quad k_{ca} = 8 \text{ ms}^{-1} \]
\[[Ca^{2+}]_0 = 2 \text{ mM} \quad [Ca^{2+}]_\infty = 0.0001 \text{ mM} \]

where \(f\) is the percent of free to bound \(Ca^{2+}\) (set to 0.01 based on report from Helmchen et al. 1996). The parameter \(\alpha\) converts the total \(I_{Ca}\) to \(Ca^{2+}\) concentration. \(k_{ca}\) is the \(Ca^{2+}\) removal rate, where \(Ca^{2+}\) is removed by uptake into internal stores or by pump extrusion (Booth et al. 1997). In the soma, the total \(Ca^{2+}\) current is mediated by the \(N\)-type \(Ca^{2+}\) channels. \([Ca^{2+}]_s\) is the extracellular \(Ca^{2+}\) concentration, and \([Ca^{2+}]_i\), \(n_{m,\infty}\) is the intracellular \(Ca^{2+}\) concentration at time = 0.

The reversal potential for \(Ca^{2+}\) was calculated from Nernst equation as follows:
\[E_{Ca} = \left[(RT)/Z(F)\right] \times \ln\left(\frac{[Ca^{2+}]_s}{[Ca^{2+}]_i}\right) \]
\[= \left[(1,000 \times R \times 309.15)/(2 \times F)\right] \times \ln\left(\frac{[Ca^{2+}]_s}{[Ca^{2+}]_i}\right) \]
\[R = 8.31441 \quad V \text{ C mol}^{-1}K^{-1} \quad T = 36^\circ C + 273.15 \]
\[Z = 2 \quad F = 96485.309 \text{ C mol}^{-1} \]

where \(R\) is the gas constant, \(T\) is the absolute temperature on the Kelvin scale, \(Z\) is the valence for \(Ca^{2+}\), and \(F\) is Faraday’s constant. The factor 1,000 is used to convert \(E_{Ca}\) to mV.

**\(Ca^{2+}\)-dependent \(K^+\) channels.** The calcium-dependent potassium channel \([K(Ca^{2+})]\) is activated according to the following Hill expression (Booth et al. 1997):
\[I_{K(Ca^{2+})} = g_{K}(Ca^{2+}) \times \left((Ca^{2+})/((Ca^{2+}) + K_d)\right) \times (V_m - E_K) \]
\[K_d = 0.0005 \text{ mM} \quad E_K = -80 \text{ mV} \]

where \([Ca^{2+}]_i\) is the intracellular calcium concentration, and \(K_d\) is the half-saturation level.

**\(N\)-type \(Ca^{2+}\) channels.**
\[I_{CaN} = g_{CaN} \times m^2 \times h \times (V_m - E_{Ca}) \]
\[m = 1/[e^{(V_m+25)/5} + 1] \]
\[h = 1/[e^{(V_m+38)/7} + 1] \]

**Persistent \(Na^+\) channels.**
\[I_{Nap} = g_{Nap} \times n^3 \times (V_m - E_{Na}) \]
\[E_{Na} = 50 \text{ mV} \]
\[n = 0.0353 \times V_m + 21.4 \]
\[E_{Na} = 50 \text{ mV} \]

**Delayed rectifier \(Na^+\) channels.**
\[I_{Nap} = g_{Nap} \times n^3 \times (V_m - E_{Na}) \]
\[E_{Na} = 50 \text{ mV} \]
\[n = 0.000883 \times V_m + 25.7 \]
\[E_{Na} = 50 \text{ mV} \]

**Dendritic Channels**

**\(Ca_{v1.3}\) channels.**
\[I_{CaL} = g_{CaL} \times I \times (V_m - E_{Ca}) \]
\[E_{CaL} = 60 \text{ mV} \]
\[I_{m} = 1/[e^{(V_m+38)/7} + 1] \]

To account for the fast kinetics of mouse MNs, the following changes have been made to the models of individual MNs in the pool: the specific membrane capacitance was halved, the \(Ca^{2+}\) removal rate in the membrane \(Ca^{2+}\) dynamics was increased by a factor of 4, and the reversal potential of inhibitory synapses was depolarized by 5 mV (i.e., −75 mV). These changes resulted in faster membrane time constant and AHP kinetics in agreement with mouse MN electrical data (Manuel et al. 2009).

**Synaptic Inputs**

Each cell in the MN pool received two sets of synapses: excitatory and inhibitory synapses. The spatial distribution of these synapses was uniform on the dendrites and their conductances were the same across all neurons in the pool. The synapses were modeled as time- and voltage-dependent current sources and their conductances were described by an alpha function (Jones and Bawa 1997; Rall 1967; Segovia et al. 1990) as followed:
\[I_{syn}(t) = g_{syn}(t) \times (V_m - E_{syn}) \]
\[g_{syn}(t) = g_{syn} \times \frac{t}{\tau_{syn}} \times e^{(1 - \frac{t}{\tau_{syn}})} \]

where \(I_{syn}\) is the synaptic current; \(g_{syn}\) is the synaptic conductance; \(V_m\) is the membrane potential; \(E_{syn}\) is the reversal potential of the.

**Table A2. Synaptic conductances of the sensory and descending synapses**

<table>
<thead>
<tr>
<th></th>
<th>Sensory</th>
<th>Descending</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 Hz</td>
<td>25 Hz</td>
<td>5 Hz</td>
</tr>
<tr>
<td>(G_{excitatory})</td>
<td>0.016</td>
<td>0.009</td>
</tr>
<tr>
<td>(G_{inhibitory})</td>
<td>0.014</td>
<td>0.0022</td>
</tr>
</tbody>
</table>

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synapses (0 mV for excitatory synapses and −80 mV for inhibitory synapses); $g_{syn}$ is the maximum synaptic conductance; and $t_{rise}$ is the time-to-peak (0.2 ms for excitatory synapses and 2 ms for inhibitory synapses).

For the sensory and descending simulations, the excitatory and inhibitory synapses were distributed uniformly throughout the dendritic tree of each MN. The maximum synaptic weights for the sensory and descending synapses at the first pulse were as in Table A2.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: M.C.J. and S.M.E. conception and design of research; M.C.J., S.M.E., and W.F.C. performed experiments and simulations; M.C.J., S.M.E., and W.F.C. analyzed data; M.C.J., S.M.E., and C.J.H. interpreted results of experiments and simulations; M.C.J. and S.M.E. prepared figures; M.C.J. and S.M.E. drafted manuscript; M.C.J., S.M.E., W.F.C., and C.J.H. edited and revised manuscript; M.C.J., S.M.E., and C.J.H. approved final version of manuscript.

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