Short-term modulation at synapses between neurons in laminae II–V of the rodent spinal dorsal horn

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Zhang W, Schneider SP. Short-term modulation at synapses between neurons in laminae II–V of the rodent spinal dorsal horn. J Neurophysiol 105: 2920–2930, 2011. First published April 13, 2011; doi:10.1152/jn.00684.2010.—Unitary excitatory (EPSP) and inhibitory (IPSP) post synaptic potentials (PSPs) were evoked between neurons in Rexed’s laminae (L)II–V of spinal slices from young hamsters (7–24 days old) at 27°C using paired whole cell recordings. Laminar differences in synaptic efficacy were observed: excitatory connections were more secure than inhibitory connections in LII and inhibitory linkages in LII were less reliable than those in LIII–V. A majority of connections displayed paired-pulse facilitation or depression. Depression was observed for both EPSPs and IPSPs, but facilitation was seen almost exclusively for IPSPs. There were no frequency-dependent shifts between facilitation and depression. Synaptic depression was associated with an increased failure rate and decreased PSP half-width for a majority of connections. However, there were no consistent changes in failure rate or PSP time course at facilitating connections. IPSPs evoked at high-failure synapses had consistently smaller amplitude and showed greater facilitation than low-failure connections. Facilitation at inhibitory connections was positively correlated with synaptic jitter and associated with a decrease in latency. At many connections, the paired-pulse ratio varied from trial to trial and depended on the amplitude of the first PSP; dependence was greater for inhibitory synapses than excitatory synapses. Paired-pulse ratios for connections onto neurons with rapidly adapting, “phasic” discharge to depolarizing current injection were significantly greater than for connections onto neurons with tonic discharge properties. These results are evidence of diversity in synaptic transmission between dorsal horn neurons, the nature of which may depend on the types of linkage, laminar location, and intrinsic firing properties of postsynaptic cells.

Integrative properties of a neural network are influenced by the efficacy and reliability of the connections between constituent cells. In the central nervous system (CNS), short-term changes in synaptic transmission on a time scale of tens or hundreds of milliseconds are known to occur during repetitive activity of a presynaptic neuron, during which the synaptic response in the postsynaptic neuron can be either depressed or facilitated (Zucker and Regehr 2002). Synaptic modulation that occurs within this time frame would be expected to influence spinal integration of input from primary sensory afferents encoding dynamic and steady-state information.

Despite the recent renaissance in our understanding of dorsal horn functional organization, very little is known about short-term modifiability of synaptic transmission between dorsal horn neurons. Short-term synaptic depression has been reported for excitatory synapses between Aδ sensory afferents and neurons in the substantia gelatinosa, a principle relay for spinal nociceptive information (Wan and Hu 2003). A study by Chéry and De Koninck (2000) showed that GABAergic inhibitory linkages between neurons in Rexed’s lamina (L)I also undergo short-term depression. However, GABAergic connections between ventral horn neurons have been reported to exhibit evidence of short-term facilitation (Tanabe and Kaneko 1996), suggesting there are multiple forms of short-term plasticity within the spinal cord circuitry.

To study the dynamics of short-term synaptic plasticity within intrinsic dorsal horn circuitry, we performed simultaneous, whole cell recordings from synaptically connected pairs of neurons throughout LII–V. Pairs of stimuli were given to the presynaptic cell at varying interstimulus intervals, and systematic observations were made of the evoked responses in the postsynaptic cell. In addition, our experiments permitted a comparison of transmission properties at synapses in the superficial and deep dorsal horn, which, until now, had not been studied in the same experimental preparation. Our observations suggest that over half of the connections made by dorsal horn neurons displayed short-term changes in efficacy consistent with synaptic depression or facilitation. We found that short-term synaptic modulation was different for excitatory and inhibitory connections and was related to the intrinsic discharge properties of the postsynaptic neuron. We further found differences in synaptic reliability at connections between neurons in superficial and deep dorsal horn laminae. Differential expression of properties underlying synaptic transmission at local connections between dorsal horn neurons may reflect a basic principle underlying integrative function in the dorsal horn.

The spinal dorsal horn constitutes a major relay for many types of somatosensory information, including pain. From anatomic data, it can be estimated that this area consists mainly of small interneurons with axons that establish complex local and intersegmental connections (Scheibel and Scheibel 1968; Schneider 1993; Schneider et al. 1995; Schneider 2003; Grudt and Perl 2002). The organization of this circuitry was largely uncharted until studies using high-resolution electrophysiological approaches suggested that these circuits consist of excitatory and inhibitory linkages that can be related to sensory afferent inputs, morphology, and discharge properties of the pre- and postsynaptic neurons (Lu and Perl 2003, 2005; Kato et al. 2007; Santos et al. 2007; Schneider 2008). The available evidence suggests that the connections between dorsal horn neurons are generally unidirectional and of relatively low reliability (Lu and Perl 2003, 2005; Schneider 2008).

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METHODS

Tissue preparation. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Michigan State University and were conducted under guidelines established by the National Institutes of Health. The detailed procedures used to obtain spinal cord slices have been previously described (Schneider 2003, 2008). Briefly, 7- to 24-day-old Syrian hamsters of both sexes (LVG strain) were deeply anesthetized with urethane (1.5 mg/g ip). After the vertebral column had been removed, a 3- to 4-mm block of lumbar spinal cord was dissected in an ice-cold solution containing (in mM) 120 NaCl, 2.5 KCl, 1.25 MgSO4, 1.25 NaH2PO4, 26 NaHCO3, and 10 glucose (pH 7.35–7.45, 285–300 mosM) equilibrated with 95% O2-5% CO2. Parasagittal slices of the spinal cord were cut at a thickness of 250–300 μm with a vibrating tissue slicer (Vibratome 3000) in the same chilled dissection solution, transferred to artificial cerebrospinal fluid (aCSF) containing (in mM) 120 NaCl, 2.5 KCl, 2.5 CaCl2, 1.25 MgSO4, 1.25 NaH2PO4, 26 NaHCO3, and 10 glucose (pH 7.35–7.45, 285–300 mosM) equilibrated with 95% O2-5% CO2 at room temperature, and incubated in the same solution for 1 h.

Paired electrophysiological recordings. For electrophysiological recordings, individual slices were held in a submersion recording chamber (1.5 ml) mounted on a BX51W fixed-stage, upright microscope (Olympus) and continuously superfused with aCSF (5 ml/min) maintained at 27°C. Patch-type recording pipettes (4–6 MΩ) were pulled from borosilicate glass capillary tubing (TW150F-4, World Precision Instruments, Sarasota, FL) and filled with an internal solution containing (in mM) 100 K-gluconate, 20 KCl, 4 Mg-ATP, 10 phosphocreatine, 0.3 Li-GTP, and 10 HEPEs (pH 7.3, 300 mosM/l). We omitted the Ca2+ chelator EGTA from the internal solution to avoid buffering intracellular Ca2+ and possible presynaptic effects on transmitter release (Ohana and Sakmann 1998).

Neuronal somata were visualized for electrophysiological recordings with differential contrast optics and videomicroscopy using infrared illumination and a ×40 water-immersion objective (Schneider 2008). Cells suitable for paired recordings were located within 30 μm of the slice surface and separated by no more than 150 μm (the optical viewing field). A tight-seal whole cell recording was initially established in voltage-clamp mode for each cell (holding potential: −60 mV) before switching to current-clamp recording (Schneider 2008). Signals were recorded by patch-clamp amplifiers (AxoPatch 1D, MDS Analytical Technologies, Toronto, ON, Canada) and low-pass filtered (DC–5 kHz). Digitized data were acquired and analyzed offline using pCLAMP 9 software (MDS Analytical Technologies). Series resistance (3–40 MΩ) was monitored throughout the recordings, and compensation was adjusted accordingly. Synaptic linkage between cells was tested by eliciting action potentials in one neuron with brief depolarizing current pulses (10 ms, 5-s intervals) while responses were recorded in the other cell. Pulses between 75 and 325 pA were sufficient to reliably evoke action potentials from all of the neurons in our sample. A synaptic connection was evidenced by a stimulus-locked fluctuation in membrane potential of the target neuron (Fig. 1, A and B). From past experience, we found that weak connections between dorsal horn neurons can be overlooked in voltage-clamp recordings of postsynaptic currents (Schneider 2008). Therefore, all analyses of neuronal connectivity were performed on voltage responses obtained in current-clamp mode. Recording pipettes were back filled with internal solution containing 2% biocytin in four experiments to label cells for anatomical identification (Schneider 2008).

Data acquisition and analysis. After connectivity had been established, the firing properties of pre- and postsynaptic cells were examined by injecting pulses of depolarizing current (3-s duration) through the recording pipettes. Neuronal firing patterns were categorized on the basis of discharge timing and spike-frequency adaptation, as previously described (Schneider 2003). For analyses of short-term dynamics of synaptic transmission, action potentials were activated in the presynaptic cell by applying pairs of suprathreshold depolarizing current pulses once every 5 s and varying the interpulse interval between 50 and 500 ms. Analyses of the postsynaptic responses were performed using software tools in pCLAMP 9.

Averages were computed from 25 to 50 individual traces, excluding those traces in which the first action potential failed to activate a postsynaptic response to reduce bias in the data caused by conduction failure in the presynaptic axon. Our rationale was that if action potentials activated by the conditioning pulse are unable to reach the terminal and elicit release, there is no possible postsynaptic action of the first stimulation that could modify the second response. Failures of transmission were determined subjectively by visually examining each trace (Fig. 1B). To avoid errors from temporal summation, measurements of the amplitude of the second postsynaptic potential (PSP) were made from the zero-adjusted baseline. A paired-pulse ratio was computed by dividing the amplitude of the second synaptic response by the amplitude of the first response (PSP2/PSP1). Other measurements were made of the time course of the postsynaptic responses. Latency was determined as the time from the peak of the presynaptic action potential to 5% of the PSP amplitude. Rise time was defined as the time required to increase from 10% to 90% of the peak amplitude. Response half-width was defined as the time between points on the trace at which the postsynaptic response was half of its maximum value. Time constants of synaptic response decay were estimated by fitting the response to a standard exponential of the following form: f(t) = ∑A × e−τ × C, where t is time, A is the amplitude, τ is the time constant, and C is the offset voltage.

Statistical analyses. Numerical data are presented as means ± SD unless otherwise noted. Statistical analyses were performed using InStat (version 3, GraphPad Software). Departure of the data from a Gaussian distribution was tested using the method of Kolmogorov and Smirnov. Statistical comparisons of the difference between means were computed with an unpaired t-test with the Welch correction for unequal variances (two-tailed probability). A one-sample t-test was used to determine if paired-pulse ratios differed significantly from a value of 1.0. P values of <0.05 were considered significant in all analyses.

RESULTS

Tight-seal whole cell recordings were obtained from over 360 pairs of neurons distributed throughout Rexed’s LII–V. The results described here are based on 53 cell pairs that demonstrated unidirectional, monosynaptic linkages and permitted the acquisition of sufficient data of adequate quality. Nineteen pairs were recorded in LII, another twenty-four pairs were recorded in LIII and LIV, and one pair was located in L.V. (Locations of 9 cell pairs relative to dorsal horn laminae could not be determined.)

Transmission properties at dorsal horn synapses. In 19 cell pairs, single action potentials in 1 neuron evoked short-latency (<2 ms), time-locked excitatory PSPs (EPSPs) in the other cell (Fig. 1A). For 34 pairs, presynaptic action potentials led to hyperpolarizing inhibitory PSPs (IPSPs) in the postsynaptic neuron (Fig. 1B), outnumbering excitatory connections almost 2 to 1. Under voltage clamp at −60 mV, excitatory postsynaptic currents were recorded at most excitatory connections, whereas inhibitory postsynaptic currents were usually recorded only at connections with large IPSPs (≥3–5 mV). EPSPs were blocked by the application of 10 μM CNQX (n = 5), indicating that the connections were glutamatergic. IPSPs were sub-
stantially reduced by the application of 10 μM bicuculline (64 ± 6%) and blocked by the subsequent addition of 1 μM strychnine (n = 4), consistent with the corelease of GABA and glycine at the same inhibitory endings (Todd and Sullivan 1990; Keller et al. 2001; Inquimbert et al. 2007; Schneider 2008). Categorization of firing properties revealed that about two-thirds (67%, 12/18) of excitatory neurons evidenced rapid adapting, phasic firing and another 22% (4/18) showed idly adapting, phasic firing and another 22% (4/18) showed.

Table 1. Properties of unitary postsynaptic potentials evoked between neurons in Rexed’s LII–V of the hamster spinal cord

<table>
<thead>
<tr>
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<th>EPSPs</th>
<th>IPSPs</th>
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<tbody>
<tr>
<td>Latency, ms</td>
<td>1.3 ± 1.7 18</td>
<td>1.3 ± 0.7 33</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>2.5 ± 2.2 18</td>
<td>−1.7 ± 1.4 32</td>
</tr>
<tr>
<td>Rise time, ms</td>
<td>5 ± 4 17</td>
<td>10 ± 7* 32</td>
</tr>
<tr>
<td>Decay time constant, ms</td>
<td>147 ± 417 17</td>
<td>165 ± 288 21</td>
</tr>
<tr>
<td>Failure, %</td>
<td>8 ± 13 19</td>
<td>28 ± 21* 34</td>
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n, No. of measurements. The average resting potential of postsynaptic neurons was −50 ± 7 (n = 52). LII–V, laminae II–V; EPSPs, excitatory postsynaptic potentials; IPSPs, inhibitory postsynaptic potentials. *Significantly different from EPSPs (P < 0.0001).

The properties of synaptic potentials activated between neurons in LII–V are shown in Table 1. There were no differences in the absolute amplitude of EPSPs and IPSPs, and response latencies were similar. EPSPs had significantly faster rise times and lower failure rates than IPSPs. There was no correlation between the rise time and amplitude of unitary EPSPs or IPSPs, implying that synaptic responses were not distorted by electrotonic filtering (Fig. 1C). Finally, resting membrane potential and amplitude, rise time, decay τ, and failure rate of unitary synaptic potentials did not vary with the age of animals used in this study (7–24 days old).

We found that synaptic transmission at LII–V connections, overall, was relatively reliable (failure rate: 21 ± 20%, n = 53, at 27°C). However, inhibitory connections had a higher failure rate on average than excitatory linkages (Table 1), a difference shown in histogram form in Fig. 1D. Based on distributions of failure probabilities for EPSPs and IPSPs in these plots, connections could be divided into two subgroups: “high failure” and “low failure.” We defined high-failure connections as having transmission failure rates of ≥20%. This type was representative of the majority of inhibitory synapses (66%, 21/32). Low-failure IPSPs had failure rates (5 ± 7%) no different from EPSPs and were seen at a third of inhibitory linkages (34%, 11/32). High-failure IPSPs had significantly smaller amplitudes (−1.2 ± 0.6 mV) than low-failure IPSPs (−2.7 ± 2.0 mV) but similar half-width and decay τ. The resting potential for neurons receiving high-failure inhibitory connections was −5 mV more depolarized that neurons receiv-

Fig. 1. Synaptic transmission between pairs of dorsal horn neurons. A and B: current-clamp recordings from presynaptic (Pre) and postsynaptic (Post) cells. Arrows indicate action potentials triggered by brief depolarizing current pulses applied to the presynaptic neuron. A: excitatory postsynaptic potentials (EPSPs) activated at an excitatory linkage [5 traces; membrane voltage (V_m) of the postsynaptic neuron: −53 mV]. Inset: dual whole cell recording configuration used in the experiments. B: inhibitory postsynaptic potentials (IPSPs) activated at an inhibitory linkage (7 traces; postsynaptic V_m: −54 mV). Arrowheads indicate the traces showing transmission failures. Bottom traces show averages of 25 successive PSPs. C: plots showing that there was no correlation between the rise time and amplitude of unitary EPSPs (r = −0.1930, P = 0.4579) and IPSPs (r = 0.1763, P = 0.3344) recorded at connections between dorsal horn neurons in this study. D: histograms comparing transmission failure for EPSPs and IPSPs recorded at 53 laminae (LII–V connections. The dashed lines indicate the division between “low-failure” (to the left) and “high-failure” (to the right) connections (see text for details). E: photomicrograph (×20) showing two neurons in Rexed’s LII (*) labeled with biocytin during a whole cell recording that exhibited an inhibitory linkage (10 days old). Neuronal somata and dendrites were thrown out of focus to highlight the fine axon branches (arrows).
ing low-failure connections (−46 ± 6 vs. −51 ± 5 mV, P = 0.02).

Table 2 shows comparisons between unitary synaptic potentials recorded from neuron pairs in the superficial and deep dorsal horn. In the present study, inhibitory connections outnumbered excitatory connections in LII (1.7:1) but were encountered in more equal proportions in LIII–V (1.3:1). We found that the reliability of excitatory connections in LII did not differ significantly from those in LIII–V (6% vs. 10% failure rate). However, failure rates for inhibitory connections in LII were almost double those in LIII–V (38% vs. 22%), although the difference did not quite reach statistical significance (P = 0.07). EPSPs evoked between LII cell pairs had significantly fewer failures and faster rise times than IPSPs, but no such differences were observed for EPSPs and IPSPs at connections between LIII–V cells. Overall, the failure rate for connections between LIII–V neurons averaged 16%.

Several attempts were made to stain recorded cell pairs with biocytin during recording, resulting in the successful recovery of one pair of synaptically connected cells after histochemical processing (Fig. 1E). The difficulty in recovering labeled cells in the present experiments limits conclusions about the cell types involved in the various connections. However, both labeled cells exhibited features typical of local axon interneurons, the most common type of interneuron present in the hamster dorsal horn (Schneider 1993, 1995, 2003, 2008). Both cells shown in Fig. 1E were relatively small (soma diameter: 11–14 μm), with three to four smooth dendrites extending 150–180 μm from the somata. Dendritic processes intermingled and extended rostrocaudally in LII with a few branches coursing ventrally into LIII. Each cell produced a thin, highly branched axon bearing en passant varicosities exceeding the dendritic arbors, up to 400 μm from the somata. Due to the difficulty in identifying the origin of potential synaptic endings, we made no attempt to determine the postsynaptic distribution of the anatomic contacts.

Short-term modulation of transmission at dorsal horn synapses. The amplitude of single spike-evoked PSPs at LII–V connections fluctuated randomly and displayed periodic response failures (Fig. 1, A and B). However, when the presynaptic neuron was made to fire repetitively, the amplitude of responses in the postsynaptic cell could decrease or increase during short trains of action potentials (Fig. 2). Some connections depressed (Fig. 2A and B), some facilitated (Fig. 2C), and for others the postsynaptic response showed little or no change (not shown). The time course of alteration in response amplitude during pulse trains could be approximated with single exponentials having τ values ranging from ~200 ms to >2 s (Fig. 2).

Short-term synaptic modulation was studied more systematically using a paired-pulse paradigm to activate action potentials over intervals ranging from 50 to 500 ms. The postsynaptic response to the second action potential (PSP2) could be larger or smaller than the response to the first action potential (PSP1). Examples of postsynaptic responses are shown in Fig. 3, A–C. Paired-pulse ratios (PSP2/PSP1) were calculated for each trial and averaged for a given cell pair, after omitting those trials having failures to the first action potential. Paired-pulse ratios for more than half of the cell pairs examined (55%, 29/53) were significantly different from 1.0 for intervals up to 500 ms: 36% (19/53) of connections were depressing (Fig. 3, A and B) and 19% (10/53) were facilitating (Fig. 3C). By this definition, 63% (12/19) of excitatory pairs and 50% (17/34) of inhibitory pairs demonstrated short-term plasticity. Depression was observed at both excitatory and inhibitory connections; however, facilitation was seen almost exclusively at inhibitory linkages. This is reflected in the distribution of paired-pulse ratios shown in Fig. 3D. We found no correlation (r = 0.07, P = 0.6) between paired-pulse ratio and animal age (Fig. 3E). Furthermore, there were no differences between paired-pulse ratios for LII and LIII–V cell pairs at any of the testing intervals (Table 2), suggesting that short-term synaptic plasticity is similar in the superficial and deep dorsal horn.

Short-term synaptic plasticity at excitatory and inhibitory connections was not always associated with alterations in the reliability and time course of synaptic responses. We found that depression was accompanied by a doubling of failure rate (15% to 36%) at 47% (9/19) of connections and a decrease in PSP half-width (average: 49%, range: 23–70%) at 68% (13/19) of connections. Of 10 facilitating connections, 4 connections showed a decrease in failure rate (range: 12–18%), 3 connections showed an increase (range: 6–22%), and there was no change in release probability at 3 other connections. Significant changes in the duration of the postsynaptic response did not occur along with facilitation. High-

Table 2. Comparison of unitary postsynaptic potentials at connections in the superficial (LII) versus deep (LIII–V) dorsal horn

<table>
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<tr>
<th></th>
<th>LII</th>
<th>LIII–V</th>
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<tr>
<td></td>
<td>Means ± SD</td>
<td>n</td>
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<tr>
<td><strong>EPSPs</strong></td>
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<tr>
<td>Failure, %</td>
<td>6 ± 9</td>
<td>7</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>3.5 ± 2.6</td>
<td>6</td>
</tr>
<tr>
<td>Rise time, ms</td>
<td>4 ± 2</td>
<td>6</td>
</tr>
<tr>
<td>Decay time constant, ms</td>
<td>341 ± 697</td>
<td>6</td>
</tr>
<tr>
<td>Paired-pulse ratio</td>
<td>0.74 ± 0.43</td>
<td>7</td>
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<tr>
<td><strong>IPSPs</strong></td>
<td></td>
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<tr>
<td>Failure, %</td>
<td>38 ± 24*</td>
<td>12</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>−1.4 ± 0.8</td>
<td>12</td>
</tr>
<tr>
<td>Rise time, ms</td>
<td>13 ± 11†</td>
<td>10</td>
</tr>
<tr>
<td>Decay time constant, ms</td>
<td>60 ± 27</td>
<td>6</td>
</tr>
<tr>
<td>Paired-pulse ratio</td>
<td>1.26 ± 0.61</td>
<td>12</td>
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*Significantly different from EPSPs at LII connections (P = 0.0009); †significantly different from EPSPs at LII connections (P = 0.03).
failure inhibitory cell pairs (failure rate: $\approx 20\%$) had significantly higher average paired-pulse ratios than low-failure inhibitory pairs ($1.4$ vs. $1.0$, $P = 0.005$), suggesting that facilitation predominates at inhibitory linkages with lower release probability. Moreover, paired-pulse ratios for inhibitory connections were positively correlated ($r = 0.5788$, $P = 0.0006$) with latency variability (jitter) of the synaptic response (Fig. 4A). We also found that facilitation at inhibitory connections was associated with a decrease in latency of the postsynaptic response ($\Delta \text{latency} = \text{latency}_{\text{PSP1}} - \text{latency}_{\text{PSP2}}$, where $\Delta \text{latency}$ is the change in latency), as indicated by the negative values for $\Delta \text{latency}$ in Fig. 4B ($r = -0.6121$, $P = 0.0003$). As shown in Fig. 4, excitable cell pairs did not exhibit a similar relationship between paired-pulse ratio and either synaptic jitter ($P = 0.8978$) or $\Delta \text{latency}$ ($P = 0.5977$).

Both paired-pulse facilitation and depression have been observed at the same synapses elsewhere in the CNS, depending on the amplitude of the first synaptic response (Debanne et al. 1996; Pavlides and Madison 1999; Taverna et al. 2004). When examining trial-to-trial variability in paired-pulse ratios, we made a similar observation for connections between dorsal horn neurons (Fig. 5, A–D), even at synapses that did not evidence significant facilitation or depression. At excitatory connections, paired-pulse ratios $> 1.0$ could be observed but were rather infrequent (Fig. 5, A and B); depression (paired-pulse ratios $< 1.0$) dominated for most trials, regardless of the size of the first response. At inhibitory linkages, paired-pulse ratios $> 1.0$ were more common when the first IPSP was small and could occur at connections dominated either by facilitation (Fig. 5C) or depression (Fig. 5D) for different interpulse delays. Paired-pulse ratios for excitatory and inhibitory connections are plotted against mean normalized values of the first PSP amplitude in Fig. 5, E and F, respectively. Together, these data suggest a dependence of paired-pulse dynamics on the amplitude of the first PSP for both excitatory and inhibitory linkages.

Estimating the time course of paired-pulse facilitation and depression. The temporal profile of short-term modifiability of LII–V connections was investigated by varying the time interval between presynaptic stimuli between 50 and 500 ms ($n = 26$ cell pairs). Paired-pulse facilitation at inhibitory connections (mean paired-pulse ratio: $1.61 \pm 0.08$) was significant at intervals of 50 and 100 ms for 8 of 10 cell pairs (Fig. 6, A and B). Only a single phase of synaptic enhancement could be resolved. Significant paired-pulse depression of IPSPs (mean paired-pulse ratio: $0.74 \pm 0.04$) was limited to intervals of 250 ms or less in five of six connections (Fig. 7A, 1 and 2). However, paired-pulse depression of EPSPs (mean: $0.62 \pm 0.03$) lasted twice as long (500 ms) as IPSPs at 6 of 10 excitatory linkages (Fig. 7B, 1 and 2). Notably, shifts from facilitation to depression were never observed at any connection by increasing the paired-pulse interval over the range used in these experiments. Moreover, there were no differences in the time course of facilitation and depression at connections between cell pairs recorded in LII and LIII–V.

Short-term synaptic plasticity and neuronal discharge properties. Since dorsal horn neurons display considerable diversity in the pattern of spike-frequency adaptation activated by membrane depolarization (Thomson et al. 1989; Ruscheweyh and Sandkühler 2002; Schneider 2003; Prescott and DeKoninck 2002), we considered the possibility that short-term plasticity at connections could be related to the intrinsic firing properties of either the pre- or postsynaptic cell, thereby influencing the transmission of sensory information through local dorsal horn circuits (Schneider 2005, 2008). This hypothesis was tested by examining the discharge properties of each neuron activated by pulses of constant depolarizing current (Schneider 2003). Examination of the results re-
revealed a significant relationship between the paired-pulse ratio and firing pattern of the postsynaptic neuron (Fig. 8). For every testing interval we studied, paired-pulse ratios at connections onto neurons with rapidly adapting, phasic firing were significantly greater than for connections onto cells that exhibited a continuous, tonic discharge during steady membrane depolarization. Mean paired-pulse ratios for connections onto phasic versus tonic cells at each time interval were 1.20 vs. 0.78 (50 ms), 1.18 vs. 0.83 (100 ms), 1.40 vs. 0.97 (250 ms), and 1.25 vs. 0.90 (500 ms) (Fig. 8B).

**DISCUSSION**

The experiments in the present study provide direct evidence of short-term plasticity at synapses between neurons in the spinal dorsal horn. Our main findings document substantial heterogeneity in activity-dependent modulation at these connections and indicate that synaptic plasticity between dorsal horn neurons can be related to the nature of their connections as well as intrinsic discharge properties of the postsynaptic cells.

Properties of synaptic connections between neurons in the superficial and deep dorsal horn. Previous studies have hinted that synaptic connections between neurons in the superficial dorsal horn may differ from those in the deep dorsal horn (Lu and Perl 2003, 2005; Schneider 2008), spinal regions that receive different afferent input and are thought to serve different sensory functions (Willis and Coggeshall 2004). The present experiments enabled us to further address this issue by making direct comparisons between synaptic linkages in both of these regions using the same preparation. Our observations suggest that the potency of fast excitatory and inhibitory transmission is comparable for connections throughout the dorsal horn but that, overall, excitatory connections are more reliable than inhibitory connections. Despite the apparent differences in reliability, the capacity of synaptic connections in LII and LIII–V for short-term plasticity appears to be similar.

The present findings, together with those of our earlier study (Schneider 2008), emphasize the importance of synaptic inhibition to integrative dorsal horn function, based on the prevalence of cell pairs linked by inhibitory connections. We should note one difference between the present experiments and those of our previous study. Failure rates for unitary PSPs between LIII–V neurons in the present study were considerably less (16% vs. 44%) than those we estimated previously for LIII and IV connections (Schneider 2008). In the present experiments, we obtained recordings from fewer cell pairs linked by inhibitory connections [ratio of number of inhibitory connections to excitatory connections (I/E): 1:3:1] than in our prior study (I/E: 2:2:1). The apparent difference in sampling bias between the two studies could underlie the disparity in synaptic reliability, since...
failure rates were generally higher for inhibitory connections than excitatory connections in both studies.

Our results suggest that inhibitory connections in LII may be less reliable than those in LIII–V, although some caution should be applied when drawing a conclusion for two reasons. First, we observed a great deal of variability in the failure rates of unitary IPSPs between connections, possibly influenced by our experimental conditions (see below). Second, Lu and Perl (2003) reported that 71% of LII inhibitory connections in adult rats (10/14) had failure rates of <20%. In the present study, we found that only 33% of connections in hamster LII (4/12) had failure rates in this range, suggesting a lower reliability than that reported by Lu and Perl. The difference cannot be accounted for by effects of lowered temperature on synaptic reliability, since both studies were performed using similar bath temperatures. More likely, the disparity is due to differences in the species and developmental stage.

For the purposes of discussion, we will consider the possibility that experimental factors may have influenced the reliability of transmission that we measured at inhibitory linkages. The composition of internal recording and external bathing solutions could have produced a Cl− potential at the soma near the resting potential for some neurons (approximately −48 mV, 27°C). This raises the possibility that a low driving force for GABAergic IPSPs may have contributed to the relatively high failure rate of some connections. Two findings suggest this possibility. First, the failure rate was correlated with the size of unitary IPSPs, with low-failure IPSPs being larger than high-failure IPSPs. Second, the membrane potential of neurons receiving high-failure connections was ~5 mV more depolarized than those receiving low-failure connections. On the other hand, there was no correlation between the IPSP amplitude and resting membrane potential recorded at the soma, which might be expected if the amplitude of unitary IPSPs was influenced by a lowered Cl− gradient. Furthermore, from previous studies, axons of LIII and LIV interneurons terminate predominantly on small, distal dendrites of neighboring cells (Schneider et al. 1995), where the Cl− potential could be much different from the Nernst potential (Jarolimek et al. 1999; Kuner and Augustine 2000). On the basis of the whole of our evidence, we do not think that an altered Cl− gradient underlies the difference in the reliability of inhibitory transmission between connections in LII and LIII–V, nor can it fully account for the

![Fig. 4. Variation in synaptic latency for dorsal horn connections associated with short-term synaptic plasticity (○, EPSPs; •, IPSPs) at the 100-ms interval. A: plot of the coefficient of variation (%CV) of response latency of the first PSP (synaptic jitter) as a function of the paired-pulse ratio. Data for inhibitory connections were fitted with a linear regression (r = 0.5788) with a slope that was significantly different from zero (P = 0.0006). B: plot of the change in latency (Δlatency = latency_{PSP1} − latency_{PSP2}) as a function of the paired-pulse ratio. The regression line is for inhibitory connections (r = −0.6121, P = 0.0003). The dashed line indicates Δlatency = 0.]

![Fig. 5. Plots of the paired-pulse ratio as a function of amplitude of the first PSP for synapses between dorsal horn neurons. A and B: examples from two cell pairs connected by excitatory linkages that exhibited significant synaptic depression (paired-pulse ratio < 1.0, P < 0.05). C and D: cell pairs connected by inhibitory linkages. The connection in C exhibited facilitation (paired-pulse ratio > 1.0, P < 0.05), and the connection in D showed depression. Examples are representative of the range over which amplitude of the first PSP varied for excitatory and inhibitory connections in this study. E and F: summary of paired-pulse ratios for excitatory (n = 18) and inhibitory (n = 33) connections. For each cell pair, PSP amplitudes were normalized to the largest event (maximum value set to 1.0). Paired-pulse ratios were pooled from all connections and grouped according to normalized PSP amplitude into 0.1-sized bins. Average paired-pulse ratios across cell pairs were calculated and plotted versus the normalized amplitude. Error bars indicate SEs. The interstimulus interval (100 ms) was the same for all data shown. The dashed lines indicate paired-pulse ratios = 1.0.]

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difference in reliability between of excitatory and inhibitory connections that we observed.

**Properties of short-term synaptic plasticity in the spinal dorsal horn.** The method we used to calculate paired-pulse ratios for unitary PSPs differed from that often used and was chosen on the basis of anatomic considerations. Axons of dorsal horn interneurons in this preparation are extremely fine and highly branched (Schneider 1992, 2008; Schneider et al. 1995), increasing the probability of branch point failure within dorsal horn circuitry. To avoid introducing this bias in our estimates, we excluded stimulus trials from analyses when the first action potential failed to activate a postsynaptic response. Our results using this approach suggest that short-term plasticity of intrinsic dorsal horn circuitry demonstrates both similarities and differences with other CNS areas. First, the majority of dorsal horn excitatory neurons exhibit short-term depression at their connections; facilitation is not characteristic of excitatory synapses in the dorsal horn, as it is in other regions of the CNS (e.g., Salin et al. 1996; Granseth et al. 2002; Angulo et al. 2003; Cabezas and Bun 2006). Second, different populations of inhibitory neurons in the dorsal horn can be distinguished based on transmission reliability and exhibit corresponding differences in short-term plasticity. Similar to inhibitory synapses in the hippocampus (Jiang et al. 2000), we found that facilitation occurred at low-failure inhibi-

![Fig. 6. Time course of paired-pulse facilitation at an inhibitory linkage.](image)

![Fig. 7. Time course of paired-pulse depression at inhibitory (A) and excitatory (B) linkages between representative cell pairs.](image)
short-term synaptic dynamics in the dorsal horn. It is well known that dorsal horn inhibitory connections, whereas depression predominated at high-failure connections. In this regard, we should note that our definition of a high-failure inhibitory connection (failure rate: ≥20%) was determined by the distribution of failure probabilities calculated from the data and is lower than a more arbitrary designation (≥50%) used by Jiang et al. (2000) in their study. Nonetheless, we interpret this result to suggest that inhibitory circuits within the dorsal horn are capable of a wider range of modulation than excitatory circuits. Third, inhibitory neurons in the dorsal horn did not evidence the interval-dependent shifts from facilitation to depression that have been documented for excitatory and inhibitory pathways in the striatum (Czubakyo and Plenz 2002), and neocortex (Thompson et al. 1993, 1997; Gil et al. 1997). Thus, synaptic transmission at connections between dorsal horn neurons appears to be dominated by either facilitation or depression and does not switch between these processes as the level of presynaptic activity changes. Finally, the time course of facilitation and depression at LII–V synapses is broadly similar to that reported for connections in neocortex (Thomson et al. 1993a, 1993b, 1997; Gil et al. 1997, 1999; Thomson and Bannister 1999; Angulo et al. 2003), hippocampus (Miles and Wong 1986; Debanne et al. 1996; Salin et al. 1996; Jiang et al. 2000), striatum (Taverna et al. 2004; Fitzgerald et al. 2001), and thalamus (Granseth et al. 2002). However, short-term dynamics at excitatory and inhibitory linkages within intrinsic dorsal horn circuitry appears to differ. Paired-pulse depression of EPSPs persisted about twice as long as IPSPs (500 vs. 250 ms), suggesting that the short-term plasticity of excitatory connections is capable of longer duration than inhibitory connections.

It should be noted that a developmental shift in short-term plasticity from depression to facilitation has been described for synapses in other CNS regions during the postnatal period (Choi and Lovingier 1997; Pouzat and Hestrin 1997; Reyes and Sakmann 1999; Angulo et al. 1999; Jüttner et al. 2001). We found that depression and facilitation at dorsal horn synapses was unrelated to the age of the animals from which tissue slices were obtained. The present experiments were performed between postnatal days 7 and 24, when significant changes are taking place in rodent dorsal horn organization. It is well known that dorsal horn interneurons in the rat undergo axonal and dendritic maturation during the first 2–3 wk of life (Bicknell and Beal 1984; Beal et al. 1988). This period also coincides with a major reorganization of dorsal root afferent inputs to the dorsal horn (Fitzgerald et al. 1994; Park et al. 1999). Furthermore, a study (Walsh et al. 2009) has shown that the intrinsic properties of superficial dorsal horn neurons undergo significant changes postnatally. Our results do not show evidence that any aspect of synaptic transmission was related to developmental age. However, we cannot rule out the possibility that differences in transmission between LII and LIII–V neurons and the diversity in short-term synaptic plasticity we documented reflect processes underlying the maturation of intrinsic dorsal horn circuitry.

Mechanisms underlying short-term plasticity at dorsal horn synapses. The mechanisms underlying the short-term plasticity at connections between neurons in LII–V are an obvious question. Presynaptic regulation of short-term dynamics involving changes in presynaptic Ca2+ levels in the presynaptic terminal and/or quantal content (e.g., Zucker and Regehr 2002) is suggested by the fact that paired-pulse ratios at many connections depended on the amplitude of the first PSP, similar to findings elsewhere in the CNS (e.g., Debanne et al. 1996; Pavlides and Madison 1999; Taverna et al. 2004). Our observation that a decrease in synaptic strength was associated with an increase in failure rate in about half of the excitatory and inhibitory connections is consistent with this possibility. One way this could occur is via the regulation of transmitter release through feedback activation of presynaptic receptors. Inhibitory transmission at dorsal horn synapses has been shown to be suppressed by activation of GABA_B (Chéry and De Koninck 2000; Choi et al. 2008; Romei et al. 2009) and μ-opioid (Grudt and Henderson 1998; Kerchner and Zhuo 2002) receptors. Moreover, some intrinsic glutamatergic and GABAergic axon terminals in the dorsal horn dorsal colocalize the opioid peptide enkephalin (Todd et al. 1992, 2003; Schneider and Walker 2007). A recent study by Zhang and Schneider (2008) provides direct evidence that EPSPs and IPSPs evoked between dorsal horn neurons can be suppressed by presynaptic actions of opioids released from the same synapses. It is also tempting to link presynaptic mechanisms with short-term facilitation at inhibitory linkages. Although a pre-
vious study (Oleskevich et al. 2000) suggested that whether a synapse is prone to facilitation or depression is dependent on release probability, the mechanisms underlying dorsal horn inhibitory circuitry may be more complex. On one hand, we found that facilitation predominates at inhibitory linkages with high failure rates and that depression is associated with connections having low failures, consistent with observations at hippocampal synapses (Dobrunz and Stevens 1997). Our observation that synaptic jitter and Δlatency correlated with paired-pulse ratio for inhibitory connections (Fig. 4) is consistent with the idea that presynaptic mechanisms underly facilitation at these connections. However, even though we found that paired-pulse facilitation at some inhibitory connections was associated with a decrease in transmission failures, the failure rate actually increased at other connections. This suggests that increases in release probability cannot fully account for facilitation at all of these connections, as has been reported for single excitatory hippocampal synapses in culture (Chen et al. 2004). As shown in Fig. 4, there was no correlation between the paired-pulse ratio and synaptic jitter or Δlatency at excitatory connections, suggesting that fundamentally different mechanisms may underly short-term plasticity in excitatory and inhibitory dorsal horn circuits. In addition, we noted that paired-pulse depression at excitatory and inhibitory connections was paralleled by a decrease in the duration of the postsynaptic response, suggesting a postsynaptic mechanism. However, there was no evidence for change in response duration at facilitating inhibitory synapses, possibly indicating that postsynaptic mechanisms exert less influence at these connections.

**Physiological significance.** The functional implications of the present findings are obvious questions. Taken together, the findings emphasize the importance of local inhibitory circuits to sensory integration in both the superficial and deep dorsal horn and focus attention on the differences in the reliability of excitatory and inhibitory connections in LII circuitry that may have a significant impact on the integration of nociceptive information. We show here that short-term plasticity is not uniform across synapses that comprise intrinsic neural circuitry in the spinal dorsal horn and that synaptic modulation can be quite different for excitatory and inhibitory neurons. These differences are likely to have important implications for processing sensory information. Our finding of depression at excitatory linkages between LII–V neurons joins a previous finding that excitatory connections between Aδ primary afferent fibers and substantia gelatinosa neurons exhibit short-term depression (Wan and Hu 2003). Consequently, it is possible that afferent input corresponding to certain sensory channels, including nociception and thermal sensation, may be subject to a “self-limiting” effect in which low-frequency information is relayed with higher fidelity than high-frequency information at a majority of connections. It seems reasonable to postulate that facilitating and depressing inhibitory linkages may further shape these responses. Facilitating inhibitory connections, along with depressing excitatory linkages, may serve as low-pass filters, limiting the transmission of high-frequency afferent information. Depressing inhibitory linkages may serve an opposite function, enhancing transmission of high-frequency information.

The results shown in Fig. 8 suggest that synaptic transmission at connections onto phasic firing neurons is more likely to facilitate with repeated activity than connections onto tonic cells. Our previous work showed that the vast majority (>90%) of phasic firing cells in LIII–V are local axon interneurons with connections restricted to one or two spinal segments (Schneider 2003). Furthermore, connections made by phasic cells appear to be more reliable than tonic firing neurons (Schneider 2008). Our results seem to suggest that facilitating-type connections (perhaps inhibitory) predominate onto phasic cells, whereas tonic cells receive a relatively greater proportion of depressing inputs that could arise from both excitatory and inhibitory neurons. These differences in short-term synaptic dynamics may help shape the responses of phasic and tonic dorsal horn neurons to static and time-varying sensory stimuli (Schneider 2005).

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**DISCLOSURES**

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

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