Primary Afferent Stimulation Differentially Potentiates Excitatory and Inhibitory Inputs to Spinal Lamina II Outer and Inner Neurons

Yu-Zhen Pan and Hui-Lin Pan
Department of Anesthesiology, The Pennsylvania State University College of Medicine, The Milton S. Hershey Medical Center, Hershey, Pennsylvania 17033-0850

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Pan, Yu-Zhen and Hui-Lin Pan. Primary afferent stimulation differentially potentiates excitatory and inhibitory inputs to spinal lamina II outer and inner neurons. J Neurophysiol 91: 2413–2421, 2004. First published January 28, 2004; 10.1152/jn.01242.2003. Spinal lamina II (substantia gelatinosa) neurons play an important role in processing of nociceptive information from primary afferent nerves. Anatomical studies suggest that neurons in the outer (lamina IIo) and inner (lamina IIi) zone of lamina II receive distinct afferent inputs. The functional significance of this preferential afferent termination in lamina II remains unclear. In this study, we examined the differential synaptic inputs to neurons in lamina IIo and IIi in response to primary afferent stimulation. Whole cell voltage-clamp recordings were performed on neurons in lamina IIo and IIi, and of the rat spinal cord slice under visual guidance. Capsaicin (1 μM) significantly increased the frequency of glutamatergic miniature excitatory postsynaptic currents (mEPSCs) in all 27 lamina IIo neurons and significantly increased the amplitude of mEPSCs in 12 of 27 lamina IIo neurons. However, capsaicin only significantly increased the frequency of mEPSCs in 9 of 22 (40.9%) lamina IIi neurons and increased the amplitude of mEPSCs in 6 of these 9 neurons. Furthermore, the peak amplitude of EPSCs, evoked by electrical stimulation of the attached dorsal root, in 40 lamina IIo neurons was significantly greater than that (160.5 ± 16.7 vs. 87.0 ± 10.4 (SE) pA) in 37 lamina IIi neurons. On the other hand, the peak amplitude of evoked inhibitory postsynaptic currents (IPSCs) in 40 lamina IIo neurons was significantly smaller than that (103.1 ± 11.6 vs. 258.4 ± 24.4 pA) in 37 lamina IIi neurons. In addition, the peak amplitudes of both EPSCs and IPSCs, evoked by direct stimulation of lamina IIo, were similar in lamina IIo and IIi neurons. This study provides new information that stimulation of primary afferents differentially potentiates synaptic inputs to neurons in lamina IIo and IIi. The quantitative difference in excitatory and inhibitory synaptic inputs to lamina IIo and IIi neurons may be important for integration of sensory information from primary afferent nerves.

INTRODUCTION

Lamina II (substantia gelatinosa) of the spinal cord dorsal horn is a critical site for the relay and processing of primary afferent information (Cervero and Iggo 1980; Light and Perl 1979a,b; Woodbury et al. 2000). Lamina II is an important region for modulation of nociceptive information and regulation of the firing activity of dorsal horn projection neurons (Cervero and Iggo 1980; Li et al. 2002; Lu and Perl 2003; Yoshimura and Nishi 1995). Recent neuroanatomical studies have shown that the isoleucin-B4-positive C-fiber afferents predominantly terminate at lamina IIo (Pan et al. 2003; Woodbury et al. 2000). Furthermore, nociceptive neurons are mostly found in lamina I and IIo and innocuous mechanoreceptive cells are mostly located in lamina II (Light and Willcockson 1999). However, the potential difference in excitatory and inhibitory synaptic inputs between lamina IIo and IIi neurons is not fully known.

Neurons in lamina IIo may have different functions from those in lamina IIi in processing of nociceptive transmission from primary afferents in the spinal dorsal horn. Lamina II contains a variety of cells with different shapes and neurite distributions (Cervero and Iggo 1980; Grudt and Perl 2002). It also has diverse molecules and substances that influence synaptic transmission and neuronal excitability. For example, many molecules involved in nociception, including protein kinase C, substance P, vesicular glutamate transporters, and P2X3 receptors, are differentially distributed in lamina IIo and IIi (Li et al. 2003; McLeod et al. 1998; Polgar et al. 1999; Vulchanova et al. 1998). Furthermore, the heterogeneity of lamina IIo and IIi neurons is an important issue because many electrophysiological studies of lamina II neurons in the spinal cord slice are often performed in a “blind” fashion with little consideration of the subdivision of lamina II. We have shown that activation of α2-adrenergic receptors inhibits glutamatergic synaptic inputs to lamina IIi, but not lamina IIo, neurons in rat spinal cord slices (Pan et al. 2002). Therefore in the present study, we specifically determined the potential difference in synaptic inputs to neurons located in lamina IIo and IIi altered by activation of primary afferent nerves.

METHODS

Spinal cord slice preparation

The spinal cord transverse slices (300 μm in thickness) with an attached dorsal root were prepared as described previously (Li et al. 2002; Pan et al. 2002). Briefly, the lumbarosacral segment of the spinal cord of Sprague-Dawley rats (5–6 wk old, Harlan, Indianapolis, IN) was rapidly removed using halothane anesthesia and cut in a preoxygenated ice-cold sucrose artificial cerebrospinal fluid (ACSF) using a
vibratome (Technical Product International, St. Louis, MO). Then the slices were preincubated in Krebs solution oxygenated with 95% O2-5% CO2 at 34°C for ≥1 h before being transferred to the recording chamber. The sucrose ACSF was composed as follows (in mM): 234 sucrose, 3.6 KCl, 1.2 MgCl₂, 2.5 CaCl₂, 1.2 NaH₂PO₄, 12.0 glucose, and 25.0 NaHCO₃. The Krebs solution contained (in mM) 117.0 NaCl, 3.6 KCl, 1.2 MgCl₂, 2.5 CaCl₂, 1.2 NaH₂PO₄, 11.0 glucose, and 25.0 NaHCO₃. All protocols were approved by the Animal Care and Use Committee of the Pennsylvania State University College of Medicine and conformed to the National Institutes of Health guidelines on the ethical use of animals.

Recordings of postsynaptic currents of neurons in lamina IIₒ and IIᵢ

Recordings of miniature and evoked excitatory and inhibitory postsynaptic currents were performed using the whole cell voltage-clamp techniques (Li et al. 2002; Pan et al. 2002). The spinal cord slice was placed in a glass-bottomed chamber (Warner Instruments, Hamden, CT) and fixed with a grid of parallel nylon threads supported by a U-shaped stainless steel weight. The slice was perfused at 5.0 ml/min at 34°C maintained by an in-line solution heater and a temperature controller (TC-324, Warner Instruments, Hamden, CT). The lamina II has a distinct translucent appearance and can be easily distinguished under the microscope (BX50W, Olympus, Japan). To identify lamina IIₒ and IIᵢ, the entire lamina II in one side of the spinal dorsal horn was first viewed under the microscope using a ×4 or ×10 objective lens. The image of the entire lamina II was captured and enhanced through a CCD camera and displayed on a video monitor. Because there is no clear demarcation between lamina IIₒ and IIᵢ, we arbitrarily divided the lamina II in the spinal cord into three equal divisions from the dorsal to ventral boundaries: outer, middle, and inner zones (Fig. 1). Then, neurons in lamina IIₒ or IIᵢ were focused and visualized using a ×60 water-immersion objective lens with combined infrared and differential interference contrast optics as described previously (Li et al. 2002; Pan et al. 2002). For the purpose of this study, all the recordings were restricted to neurons located in lamina IIₒ or IIᵢ.

The electrode for the whole cell recording was pulled (P-97, Sutter Instrument, Novato, CA) using borosilicate glass capillaries (1.2 mm OD, 0.86 mm ID; World Precision Instruments, Sarasota, FL). The resistance of the electrode was ~5 MΩ when filled with the intracellular solution containing (in mM) 135.0 potassium gluconate, 5 KCl, 2.0 MgCl₂, 0.5 CaCl₂, 5.0 HEPES, 5.0 ATP-Mg, and 0.5 Na-GTP, adjusted to pH 7.2–7.4 with 1 M of KOH (290–300 mosM).

Recordings of postsynaptic currents began ~5 min later after the whole cell access was established and the current reached a steady state. The miniature excitatory postsynaptic currents (mEPSCs) of lamina IIₒ and IIᵢ neurons were recorded using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) at a holding potential of ~70 mV (Li et al. 2002; Pan et al. 2002). Signals were filtered at 1–2 kHz, digitized at 10 kHz (DigiData 1320A, Axon Instruments), and recorded into a Pentium computer using the pCLAMP 8.01 program. All mEPSCs were recorded in the presence of tetrodotoxin (TTX, 1 μM), bicuculline (10 μM), and strychnine (5 μM).

To determine the difference of synaptic inputs between lamina IIₒ and IIᵢ neurons in response to activation of primary afferents, the evoked EPSCs and IPSCs (eEPSCs and eIPSCs) were induced by electrical stimulation (0.1 ms, 1.0 mA) of the attached dorsal root through a suction electrode connected to a stimulator (Grass Instruments, West Warwick, RI). At this stimulation intensity, both myelinated and unmyelinated afferent fibers are activated (Yang et al. 1999; Yoshimura and Nishi 1993, 1995). The conduction velocity of the stimulated fibers was not measured in this study because the attached dorsal root was very short in the thin spinal cord slice preparations. To examine the difference of localized synaptic inputs between lamina IIₒ and IIᵢ neurons, the eEPSCs and eIPSCs were also evoked by focal stimulation (0.1 ms, 1.0 mA) through a bipolar tungsten electrode placed in the middle zone of lamina II. The resistance of the electrode for recordings of the evoked postsynaptic currents was ~5 MΩ when filled with the intracellular solution containing (in mM) 110.0 Cs₂SO₄, 0.5 CaCl₂, 2.4 MgCl₂, 5.0 BAPTA, 10.0 HEPES, 5 Na₂ATP, 0.33 GTP-tris salt, 10.0 lidocaine-H₁ethyl bromide (QX314), and 5.0 TEA-Cl (pH 7.3; osmolality, 275–280 mosM), as described in previous studies (Li et al. 2002; Pan et al. 2002). TTX was not used for recordings of eEPSCs and eIPSCs. Based on the optimal reversal potentials of eEPSCs and eIPSCs using this pipette solution (Li et al. 2002; Pan et al. 2002), the eEPSCs and eIPSCs were recorded at a holding potential of ~10 and ~70 mV, respectively.

Experimental protocols

The access and the input resistance were continuously monitored throughout the recording period. Recordings were abandoned if the access resistance was >30 MΩ and the input resistance changed >15%. To determine the synaptic input from capsaicin-sensitive fibers to lamina IIₒ and IIᵢ neurons, the effect of 1 μM capsaicin on mEPSCs was tested (Yang et al. 1999). After recording the mEPSCs of neurons in lamina IIₒ or IIᵢ for 3 min as the control, 1 μM capsaicin was perfused into the slice for 1 min. Then the mEPSCs were recorded for another 3 min after capsaicin took effect. Also, in some lamina IIₒ and IIᵢ neurons, the effects of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM, a glutamate non-NMDA receptor antagonist) was tested after examination of the initial effect of capsaicin on mEPSCs.

In separate groups of cells, we examined the difference of the excitatory and inhibitory synaptic inputs between lamina IIₒ and IIᵢ neurons in response to primary afferent stimulation. Both IPSCs and EPSCs of lamina IIₒ and IIᵢ neurons were evoked by electrical stimulation of the dorsal root at the same intensity (0.1 ms, 1.0 mA). To further determine if lamina IIₒ and IIᵢ neurons receive different local inhibitory and excitatory synaptic inputs, EPSCs and IPSCs were evoked by focal electrical stimulation of lamina II in separate groups of neurons. The effect of 20 μM CNQX on eEPSCs was tested in some lamina IIₒ and IIᵢ neurons at the end of the protocol. Also, to determine the types of neurotransmitters mediating IPSCs, the effect of bicuculline (10 μM, a GABA_A receptor antagonist) on eIPSCs was tested in some lamina IIₒ and IIᵢ neurons. If bicuculline did not completely block the eIPSCs, strychnine (5 μM, a glycine receptor antagonist) was then applied in the presence of bicuculline (Li et al. 2002; Pan et al. 2002).

TTX and QX-314 were purchased from Alomone Lab (Jerusalem, Israel). CNQX, bicuculline methiodide, strychnine, and capsaicin were obtained from Sigma (St. Louis, MO). Drugs were dissolved in Krebs solution and perfused into the slice chamber using syringe pumps.
Data analysis

Data are presented as means ± SE. The mEPSCs were analyzed off-line with a peak detection program (MiniAnalysis, Synaptosoft, Leonia, NJ). The cumulative probability of the amplitude and interevent interval was compared by the Komogorov-Smirnov test, which estimates the probability that two cumulative distributions are similar. Analyses of the peak amplitude of eEPSCs and eIPSCs were performed using Clampfit (Axon Instruments). The effects of capsaicin on the amplitude and frequency of mEPSCs were determined by paired 2-tailed Student’s t-test. Neurons were considered to be responsive to capsaicin if the mEPSCs were altered >20%. The amplitude of eIPSCs and eEPSCs was determined by the unpaired 2-tailed t-test. Neurons were considered to be responsive to capsaicin if the mEPSCs were altered >20%. The estimates the probability that two cumulative distributions are similar.

Results

Effect of capsaicin on mEPSCs of neurons in lamina II_o and II_i

Spontaneous mEPSCs were studied in a total of 27 lamina II_o neurons and 22 lamina II_i neurons. In the presence of 1 μM TTX, 10 μM bicuculline, and 5 μM strychnine, the frequency of mEPSCs varied from 0.3 to 14.8 Hz (5.1 ± 1.4 Hz) and the amplitude of mEPSCs ranged from 16.0 to 26.9 pA (20.7 ± 1.1 pA) in the 27 lamina II_o neurons. In the 22 lamina II_i neurons studied, the frequency of mEPSCs varied from 0.2 to 15.9 Hz (5.4 ± 1.4 Hz), and the amplitude of mEPSCs ranged from 10.9 to 30.5 pA (21.8 ± 1.4 pA).

Application of 1 μM capsaicin significantly increased the frequency of mEPSCs in all 27 lamina II_o neurons from 5.1 ± 1.4 to 13.4 ± 1.8 Hz (P < 0.05, Figs. 2 and 3A). The cumulative probability analysis of mEPSCs revealed that the distribution pattern of the interevent interval of mEPSCs was significantly shifted toward the left in response to capsaicin (Fig. 2). Capsaicin did not affect the amplitude (19.9 ± 1.1 vs. 19.8 ± 1.2 pA, Figs. 2A and 3B) of mEPSCs in 15 of these 27 (55.6%) lamina II_o neurons. However, in the remaining 12 (12/27, 44.4%) lamina II_o neurons, capsaicin increased the amplitude of mEPSCs from 21.7 ± 2.1 to 28.8 ± 2.9 pA (P < 0.05, Figs. 2B and 3B) and shifted the distribution pattern of the amplitude of mEPSCs toward the right (Fig. 2B). Application of 20 μM CNQX abolished mEPSCs and the effect of capsaicin in all 12 lamina II_o neurons tested (Fig. 2A).

In 13 of 22 (59.1%) lamina II_i neurons, application of 1 μM capsaicin did not significantly alter the frequency (5.3 ± 1.4 vs. 5.4 ± 1.5 Hz) and amplitude (22.1 ± 2.4 vs. 21.4 ± 2.2 pA) of mEPSCs (Figs. 4A and 5A). However, capsaicin significantly increased the frequency of mEPSCs in the remaining 9 (9/22, 40.9%) lamina II_i neurons from 5.4 ± 1.4 to 13.4 ± 2.5 Hz (P < 0.05, Figs. 4B and 5B). The distribution pattern of the interevent interval of mEPSCs was significantly shifted toward the left by capsaicin (Fig. 4B). Furthermore, capsaicin significantly increased the amplitude of mEPSCs in six of these nine neurons from 20.3 ± 2.3 to 28.6 ± 3.1 pA (P < 0.05, Figs. 4B and 5B). However, capsaicin did not significantly affect the amplitude of mEPSCs in the remaining three neurons (from 22.6 ± 2.1 to 21.1 ± 2.2 pA, Fig. 5B). Application of 20 μM CNQX abolished mEPSCs and the effect of capsaicin in all 10 lamina II_i neurons tested (Fig. 4A).

To determine the differential glutamatergic synaptic inputs from primary afferents to neurons in lamina II_o and II_i, the eEPSCs were elicited by electrical stimulation of the dorsal root at the same stimulating intensity. In 40 lamina II_o neurons, the mean peak amplitude of eEPSCs was 160.5 ± 16.7 pA, which was significantly greater than that (87.0 ± 10.4 pA) in 37 lamina II_i neurons (Fig. 6). CNQX (20 μM) completely blocked the eEPSCs in 12 lamina II_o and 13 lamina II_i cells tested (Fig. 6A). The eEPSCs were further analyzed according to the criteria used for identification of the mono- and polysynaptic inputs (Li et al. 2002). The eEPSCs were considered as monosynaptic if the latency was constant after electrical stimulation and no conduction failure occurred at a high (20 Hz) stimulation frequency (Fig. 7A). The eEPSCs in 62.5% (25/40) lamina II_o neurons met the criteria for monosynaptic input. In comparison, the eEPSCs were monosynaptic in 40.5% (15/37) lamina II_i neurons examined. The peak amplitude of both monosynaptic (204.3 ± 21.9 pA, n = 25) and polysynaptic

FIG. 2. A: effect of 1 μM capsaicin on the frequency of mEPSCs in a lamina II_o neuron. a: original tracings showing spontaneous mEPSCs during control, application of 1 μM capsaicin, and 20 μM CNQX. Cumulative plot analysis of mEPSCs of the same neuron showing the distribution of the interevent interval (b) and the peak amplitude (c) during control and capsaicin application. B: effect of 1 μM capsaicin on the frequency and amplitude of mEPSCs in a different lamina II_o neuron. a: raw tracings showing spontaneous mEPSCs during control, application 1 μM of capsaicin, and 20 μM CNQX. Cumulative plot analysis of mEPSCs of the same neuron showing the distribution of the interevent interval (b) and the peak amplitude (c) during control and capsaicin application.

Difference of EPSCs evoked from the dorsal root between lamina II_o and II_i neurons

To determine the differential glutamatergic synaptic inputs from primary afferents to neurons in lamina II_o and II_i, the eEPSCs were elicited by electrical stimulation of the dorsal root at the same stimulating intensity. In 40 lamina II_o neurons, the mean peak amplitude of eEPSCs was 160.5 ± 16.7 pA, which was significantly greater than that (87.0 ± 10.4 pA) in 37 lamina II_i neurons (Fig. 6). CNQX (20 μM) completely blocked the eEPSCs in 12 lamina II_o and 13 lamina II_i cells tested (Fig. 6A). The eEPSCs were further analyzed according to the criteria used for identification of the mono- and polysynaptic inputs (Li et al. 2002). The eEPSCs were considered as monosynaptic if the latency was constant after electrical stimulation and no conduction failure occurred at a high (20 Hz) stimulation frequency (Fig. 7A). The eEPSCs in 62.5% (25/40) lamina II_o neurons met the criteria for monosynaptic input. In comparison, the eEPSCs were monosynaptic in 40.5% (15/37) lamina II_i neurons examined. The peak amplitude of both monosynaptic (204.3 ± 21.9 pA, n = 25) and polysynaptic
(126.9 ± 22.5 pA, n = 15) eEPSCs in lamina IIo neurons was significantly larger than those (129.4 ± 19.0 pA, n = 15; 71.4 ± 8.5 pA, n = 22) in lamina IIi neurons (Fig. 7B).

**Focal stimulation-evoked EPSCs and IPSCs in lamina IIo and IIi neurons**

In separate groups of cells, we determined the potential difference of local excitatory and inhibitory synaptic inputs elicited by electrical stimulation of lamina II, between lamina IIo and IIi neurons. The focal stimulation-evoked IPSCs and EPSCs were recorded at the same intensity. The peak amplitudes of both eEPSCs (105.4 ± 10.7 vs. 92.5 ± 15.5 pA) and eIPSCs (231.2 ± 68.4 vs. 253.8 ± 75.6 pA) were not significantly different between 10 lamina IIo and 7 lamina IIi neurons (Fig. 9). Furthermore, 20 μM CNQX abolished eEPSCs, and 20 μM bicuculline plus 5 μM strychnine eliminated the eIPSCs in all cells examined.
In this study, we determined the differential excitatory and inhibitory synaptic inputs to neurons in lamina IIo and IIi of the spinal dorsal horn in response to primary afferent stimulation. We found that capsaicin significantly increased glutamatergic mEPSC frequency in all lamina IIo neurons examined. On the other hand, capsaicin only increased mEPSC frequency in 40.9% lamina IIi neurons tested. Furthermore, the peak amplitude of eEPSCs in lamina IIo neurons was significantly greater than that of lamina IIi neurons when the primary afferents were electrically stimulated at the same intensity. By contrast, the peak amplitude of eIPSCs in lamina IIo neurons was significantly smaller than that in lamina IIi neurons. However, the amplitude of both eEPSCs and eIPSCs, elicited by focal stimulation of lamina II, was not significantly different between lamina IIo and IIi neurons. Collectively, our study provides strong evidence that lamina IIo neurons receive a greater degree of excitatory inputs, whereas lamina IIi neurons receive a greater degree of inhibitory inputs. These data provide further evidence that neurons in lamina IIo and IIi may be functionally heterogenous and play a different role in processing of sensory information from primary afferents.

Spinal cord lamina II has been implicated as the primary integration site for processing of nociceptive information. Lamina II neurons receive inputs from Aδ- and C-fiber nociceptors as well as innocuous thermal receptors and mechanoreceptors (Light and Perl 1979b; Sugiura et al. 1986, 1993). Because these thin fibers are often involved in nociception, lamina II is presumed to be a critical center in pain mechanisms. However, these primary afferents projecting to lamina II also convey information about innocuous thermal and mechanical events (Light and Perl 1979b; Sugiura et al. 1986). Glutamate is the most important excitatory neurotransmitter in the spinal cord dorsal horn (Li et al. 2002, 2003; Pan et al. 2002; Yoshimura and Jessell 1989; Yoshimura and Nishi 1993). There are three possible origins of the glutamatergic inputs to lamina II neurons: primary afferents, interneurons, and descending fibers. The major source of glutamatergic inputs to lamina II neurons is from the primary afferents, especially nociceptive C fibers (Li et al. 2002; Pan et al. 2002; Schneider and Perl 1988; Yoshimura and Jessell 1989; Yoshimura and Nishi 1993). Capsaicin, a natural ingredient in hot peppers, has been widely used to excite a group of small-diameter primary sensory neurons and C-fiber terminals (Caterina et al. 1997, 2000; Yang et al. 1998, 1999). The vanilloid receptors (VR1) are predominately located on primary C-fiber afferents (Ma 2001; Pan et al. 2003). Capsaicin-sensitive primary afferents and VR1 receptors play an important role in nociceptive transmission (Caterina et al. 1997, 2000; Tominaga et al. 1998). In the present study, we used capsaicin as a pharmacological tool to determine the differential glutamatergic inputs from nociceptive afferents.
ceptive C-fiber afferents to neurons in lamina IIo and IIi. We found that capsaicin significantly increased the frequency of mEPSCs in all lamina IIo neurons tested. This suggests that lamina IIo neurons primarily receive glutamatergic inputs from nociceptive primary afferents and play an important role in processing of nociception. This observation is consistent with the neuroanatomical study that unmyelinated C-fiber afferents mainly project to lamina IIo (Light and Perl 1979b; Pan et al. 2003; Ralston and Ralston 1979; Woodbury et al. 2000). By contrast, we found that capsaicin did not affect the frequency of mEPSCs in ~59% of lamina IIi neurons. Thus some capsaicin-sensitive primary afferents also synapse with lamina IIi neurons. It should be noted that although capsaicin responses are more prevalent in lamina IIo than in IIi neurons, anatomical studies have shown a higher density of VR1-immunoreactive terminals in lamina IIi than in IIo (Guo et al. 1999; Valtschanoff et al. 2001). The exact reasons for this difference in degree are not fully known. One possible reason might be the different arbitrary methods used to divide the lamina II subdivisions. Also, it has been shown that there is a postnatal developmental shift of VR1 terminals in the superficial dorsal horn (Guo et al. 2001). Thus another possibility is that different age groups of rats are used in these studies. Whereas young (5–6 wk old) rats were used in the current study, adult mature rats were used in the immunocytochemistry experiments (Guo et al. 1999; Valtschanoff et al. 2001).

In addition to its effect on the frequency of mEPSCs, capsaicin significantly increased the amplitude of mEPSCs in some lamina IIo (12/27, 44.4%) and IIi (6/22, 27.3%) neurons. To our knowledge, this is the first study demonstrating that capsaicin increased both the frequency and amplitude of mEPSC of lamina II neurons. Although the finding that capsaicin increased the frequency, but not the amplitude,
Therefore the mEPSCs in many laminae II_o and II_i neurons clearly suggests a presynaptic action of capsaicin on VR_1 receptors (Yang et al. 1998, 1999), the mechanisms of the effect of capsaicin on the mEPSC amplitude are not clear. Based on the quantal hypothesis, these electrophysiological data appear to suggest a postsynaptic action of capsaicin in some lamina II neurons. Indeed, it has been shown that the VR_1 immunoreactivity is present in a few lamina II neurons and their dendrites (Valtschanoff et al. 2001). However, we found that the effect of capsaicin on the frequency and amplitude of mEPSCs in lamina II_o and II_i was completely eliminated by CNQX, a non-NMDA antagonist. Therefore the increased amplitude of mEPSCs is probably due to excessive presynaptic release of glutamate triggered by activation of VR_1 receptors. In this regard, many small-diameter primary afferent neurons possess TTX-resistant Na^+ channels and VR_1 receptors (Akopian et al. 1996; Blair and Bean 2002; Pan et al. 2003). It is possible that capsaicin depolarizes dorsal root afferents and leads to generation of TTX-resistant action potentials, which evoke the release of a large amount of glutamate-containing vesicles from the capsaicin-sensitive afferent terminals. This possibility is strongly supported by the demonstration that application of capsaicin can depolarize the dorsal root ganglia and dorsal root fibers through TTX-resistant Na^+ channels (Jefitinija 1994; Williams and Zieglansberger 1982). Therefore the mEPSC amplitude increase by capsaicin is likely caused by activation of VR_1 receptors and TTX-resistant Na^+ channels present on dorsal root fibers. Additionally, activation of VR_1 receptors produces an inward current carried by nonselective cations with a high permeability for divalent cations such as Ca^{2+} (Caterina et al. 1997; Liu and Simon 1994). It is possible that Ca^{2+} influx directly through the ionophore of VR_1 receptors may also contribute to the amount of glutamate release from the primary afferents.

In the present study, we further determined the excitatory glutamatergic inputs from primary afferents to lamina II_o and II_i neurons by electrical stimulation of the attached dorsal root fiber in spinal cord slices. We found that, using the same stimulation intensity, the peak amplitude of eEPSCs in lamina II_o neurons was significantly larger than that in lamina II_i neurons. Previous studies have shown that primary C- and Aδ-fiber afferents terminate in lamina II through direct (monosynaptic) and indirect (polysynaptic) projections (Cervero and Iggo 1980; Schneider and Perl 1988; Yoshimura and Jessell 1989). In this study, both mono- and polysynaptic EPSCs were recorded in lamina II_o and II_i neurons in response to activation of primary afferents. Importantly, the peak amplitudes of both mono- and polysynaptic EPSCs in lamina II_o neurons were significantly greater than those in lamina II_i. This finding is consistent with the capsaicin data and provides further evidence that lamina II_o neurons receive more glutamatergic inputs from primary afferents than lamina II_i neurons. Interestingly, we observed that the amplitudes of both eEPSCs and eIPSCs elicited from focal stimulation of lamina II were not significantly different between lamina II_o and II_i neurons. This observation suggests that the intrinsic synaptic inputs to lamina II_o and II_i neurons are similar. Because stimulation of capsaicin-sensitive nociceptive afferents uniformly potentiates the glutamatergic inputs to lamina II_o neurons, it is reasonable to suggest that this subdivision should be focused in spinal cord slice studies concerning the initial nociceptive inputs from nociceptors. However, this difference is largely quantitative and the glutamatergic input in some lamina II_o neurons was also potentiated by capsaicin. Also, it should be acknowledged that the method used to separate lamina II_o from lamina II_i is arbitrary and the cells in the middle zone of lamina II were not examined in this study.

GABA and glycine are the major inhibitory neurotransmitters that mediate the IPSCs in the spinal dorsal horn and play an important role in modulation of nociceptive transmission in lamina II neurons (Li et al. 2002; Lu and Perl 2003; Yoshimura and Nishi 1995). It has been shown that lamina II interneurons make monosynaptic contacts with other intrinsic interneurons receiving primary afferent inputs (Lu and Perl 2003; Yoshimura and Nishi 1995). Consequently, primary afferent stimulation can evoke IPSCs through indirect activation of lamina II inhibitory interneurons. We observed that some evoked IPSCs in lamina II_o and II_i neurons were eliminated by bicuculline and other IPSCs were abolished by bicuculline plus strychnine. Immunocytochemical studies have suggested that 45% GABAergic neurons contain glycine in spinal lamina II (Todd and Sullivan 1990; Todd et al. 1996). In the present study, the IPSCs were mediated by GABA in 44.0% lamina II_o neurons. By comparison, the IPSCs were GABAergic in 26.9% lamina II_i neurons. Thus although more lamina II_o neurons receive GABAergic inhibitory synaptic inputs, most lamina II_i neurons are modulated by both GABAergic and glycinerigic
inhibitory inputs. We found in this study that when the primary afferents were stimulated at the same intensity, the peak amplitudes of both GABAergic and mixed GABAergic/glycinergic eIPSCs in lamina II neurons were significantly smaller than those in lamina II neurons. These data suggest that lamina II neurons receive more inhibitory synaptic inputs than lamina II neurons and are probably subjected to more inhibitory modulation when the primary afferents are activated. On the other hand, because the lamina II neurons receive a greater degree of excitatory inputs than lamina II neurons, it is possible that lamina II neurons are more excitable than lamina II neurons and are important for the initial processing of information from the primary afferents. Some of the lamina II neurons may potentially receive inputs from low-threshold myelinated fibers including α-hair afferents (Light and Perl 1979b) and Aβ-hair follicle afferent fibers (Woolf 1987). Furthermore, Light and Willcockson have shown that the lamina II cells respond predominately to low-threshold inputs in vivo (Light and Willcockson 1999). Data from the present study are in general agreement with the previous findings that lamina II neurons appear to receive inputs from nociceptors, and many lamina IIi cells probably receive nonnociceptive primary afferent inputs. However, the precise roles and mechanisms of lamina II neurons in nociceptive integration are far from clear and need to be elucidated in future studies.

In summary, this electrophysiological study provides new information that spinal dorsal horn neurons in lamina II receive more excitatory and less inhibitory synaptic inputs than those in lamina II in response to activation of primary afferents. This difference in synaptic inputs to lamina II and IIi likely plays an important role in transmission and modulation of nociceptive information from the primary afferents. This information about the functional heterogeneity of lamina II neurons may be important for our understanding of the physiological function and the mechanisms of lamina II subdivision in the relay and processing of different sensory modalities.

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