Properties of Mouse Spinal Lamina I GABAergic Interneurons

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Dougherty, Kimberly J., Michael A. Sawchuk, and Shawn Hochman. Properties of mouse spinal lamina I GABAergic interneurons. J Neurophysiol 94: 3221–3227, 2005. First published July 13, 2005; doi:10.1152/jn.00184.2005. Lamina I is a sensory relay region containing projection cells and local interneurons involved in thermal and nociceptive signaling. These neurons differ in morphology, sensory response modality, and firing characteristics. We examined intrinsic properties of mouse lamina I GABAergic neurons expressing enhanced green fluorescent protein (EGFP). GABAergic neuron identity was confirmed by a high correspondence between GABA immunolabeling and EGFP fluorescence. Morphologies of these EGFP/GABA− cells were multipolar (65%), fusiform (31%), and pyramidal (4%). In whole cell recordings, cells fired a single spike (44%), tonically (35%), or an initial burst (21%) in response to current steps, representing a subset of reported lamina I firing properties. Membrane properties of tonic and initial burst cells were indistinguishable and these neurons may represent one functional population because, in individual neurons, their firing patterns could interconvert. Single spike cells were less excitable with lower membrane resistivity and higher rheobase. Most fusiform cells (64%) fired tonically while most multipolar cells (56%) fired single spikes. In summary, lamina I inhibitory interneurons are functionally divisible into at least two major groups both of which presumably function to limit excitatory transmission.

INTRODUCTION

GABA is the major inhibitory transmitter in the CNS. In the spinal cord, GABAergic interneurons are concentrated in the superficial laminae (I–III) where they reduce excitability by both pre- and postsynaptic inhibition. Axo-axonic GABAergic synapses onto primary afferent terminals produce presynaptic inhibition (Alvarez et al. 1992; Rudomin and Schmidt 1999; Schmidt et al. 1998) while postsynaptically, GABAergic neurons reduce the excitability of both projection neurons (Alvarez et al. 1992) and interneurons (Jankowska 1992).

Glutamic acid decarboxylase (GAD) is the rate-limiting enzyme in GABA synthesis. There are two isoforms of GAD expressed in the mature CNS—GAD65 and GAD67. A neuron is GABAergic if it contains either or both enzymes (Soghoianian and Martin 1998). Both enzymes are produced in cell bodies in all regions of the spinal cord except lamina IX (Barber et al. 1982; Ma et al. 1994). Almost all GAD+ boutons in the spinal gray matter are labeled with both 65 and 67 isoforms (Mackie et al. 2003). GABAergic neurons have been identified in transgenic mice expressing enhanced green fluorescent protein (EGFP) under the control of a GAD67 regulatory element (Heinke et al. 2004; Oliva et al. 2000). In hippocampus and neocortex, whereas EGFP expression was observed only in a subpopulation of somatostatin-containing interneurons, 99% of EGFP+ neurons were GAD67+ and hence GABAergic (Oliva et al. 2000). Recently, GABAergic neurons have been targeted in lamina II in these mice and found to comprise a larger fraction (35%; 54% in colchicine-treated) of the total identified GABAergic neurons and were a diverse population both neurochemically and physiologically (Heinke et al. 2004).

Lamina I is the most superficial lamina of the sensory dorsal horn (laminae I–VI) and receives input mainly from nociceptive mecanoreceptors and thermal afferents (Christensen and Perl 1970). Lamina I is made up of ascending tract neurons and local excitatory and inhibitory interneurons. Unlike lamina II, which contains mostly propriospinal and local interneurons (see Willis and Coggeshall 1991), lamina I, containing a relatively large number of ascending tract neurons, is one of the main sensory channels that transmits thermal, nociceptive, and other interoceptive stimuli to higher structures (Craig et al. 2001; Gauriau and Bernard 2004; Spike et al. 2003). Local inhibitory GABAergic interneurons make up ~25% of the neuronal population (Polgar et al. 2003; Todd and McKenzie 2002) and serve to limit afferent information by both pre- and postsynaptic inhibition mechanisms (Alvarez et al. 1992).

Cells in this region have been divided into several classes based on morphological and physiological properties. Morphologically, cells were divided into three main classes—fusiform (with longitudinal and ventral dendritic arbors or exclusively longitudinal arbors), pyramidal, multipolar (with many or few dendritic branches), and flattened (Han et al. 1998; Lima and Coimbra 1986; Prescott and De Koninck 2002). Dendritic extension of all three cell types is greatest in the rostrocaudal axis (Chery et al. 2000; Lima and Coimbra 1986). Lamina I neurons in rat can also be divided into categories based on firing properties. Five different subtypes were distinguished: tonic, phasic, initial burst, delayed onset, and single spike (Prescott and De Koninck 2002; Ruscheweyh and Sandkühler 2002). Additionally, lamina I projection neurons were classified as either gap- or bursting-firing neurons (Ruscheweyh et al. 2004). Prescott and De Koninck (2002) correlated firing with cell morphology. For example, most fusiform cells fire tonically, whereas multipolar cells fire with either delayed onset or a single spike after intracellular current injection. In cat, lamina I neurons have also been classified based on the types of sensory stimuli that they respond to (i.e., modality). These properties also correlate with morphology—fusiform cells are nociceptive specific (noxious), pyramidal cells are thermoreceptive specific (warm and cool), and multipolar cells are either polymodal nociceptive (noxious and thermal) or noc-

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ceptive specific (Han et al. 1998). However, this relationship may not hold true in rodent (Todd et al. 2002, 2005).

While lamina I ascending tract neurons have been studied extensively (i.e., Andrew and Craig 2002; Craig and Andrew 2002; Ruscheweyh et al. 2004), currently no studies have characterized lamina I neurons based on transmitter phenotype (but see Torsney and MacDermott 2004). We used transgenic mice containing EGFP under the control of a GAD67 regulatory element (Oliva et al. 2000) to identify spinal GABAergic neurons (cf. Heinke et al. 2004). The aims were twofold. The first was to characterize the morphology of GAD67-EGFP neurons and their overlap with GABA immunolabeling in lamina I. The second goal was to record from lamina I EGFP+ cells and characterize their properties in relation to prior observations in similarly located unidentified cell types (Prescott and De Koninck 2002; Ruscheweyh and Sandkühler 2002). We show that EGFP is a reliable marker of GABAergic phenotype in lamina I and that these GABAergic neurons are divisible into at least two groups representing a subset of the firing properties observed for neurons in this lamina.

Some of these data have been presented in abstract form (Dougherty et al. 2003, 2004).

METHODS

All experimental procedures complied with the National Institutes of Health guidelines for animal care and the Emory Institutional Animal Care and Use Committee. Homozygotic GAD67-EGFP mice obtained from Jackson Laboratory (Bar Harbor, ME) were used in all experiments. Lamina I was identified between the dorsal white matter and the relatively translucent substantia gelatinosa. No cell >20 μm from the edge of the white matter was considered (Chery et al. 2000). Some lamina I neurons may have been excluded from consideration because lamina I is thicker in the central part of the cord.

Immunohistochemistry

Mice at postnatal day (P) 14 were anesthetized with urethan (2 mg/kg ip), perfused with 1:3 vol/body wt ice-cold 0.9% NaCl, 0.1% NaN3, 1 unit/ml heparin, followed by equal volume/body weight of 4% paraformaldehyde or modified Lana’s fixative (4% paraformaldehyde, 0.2% picric acid, 0.16 M PO3); pH 6.9. All spinal cords were isolated and postfixed 1 h, cryoprotected in 10% sucrose, 0.1 M PO4, pH 7.4 until sectioned in 10 μm thick slices on a cryostat (Leitz 1720).

Ten nonconsecutive sections (100 μm apart) from lumbar segments 1–3 of two mice were used for EGFP/GABA and EGFP/NeuN comparison. All incubations and washes for immunohistological processing were performed in 0.1M PO4-buffered saline containing 0.3% triton X-100 (PBS-T). Tissue was washed overnight in PBS-T at 4°C followed by incubation in primary antibody for 48–72 h (either rabbit anti-GABA, 1:500 [Chemicon, Temecula, CA] or mouse anti-NeuN 1:500 [Sternerberger Monoclonals, Lutherville, MD]). Slides were then washed three times for 30 min and incubated in Cy3 anti-rabbit (GABA) or anti-mouse (NeuN) conjugated secondary antibody at 1:250 (Jackson ImmunoResearch, West Grove, PA). Lamina I was defined as the first layer of cells in the dorsal spinal gray. Lamina I cells were counted in nonconsecutive transverse and parasagittal sections (100 μm apart) for the GABA/EGFP and in transverse sections for NeuN/EGFP using the Neurolucida image analysis system (MicroBrightField, Williston, VT). All cell counts can only be regarded as estimates since stereological techniques were not used. Images of transverse sections (Fig. 1, A–C) were overlaid in CorelDraw 12.0 (Ottawa, Ontario).

Electrophysiology

Mice (P4–19, mean = 12) were anesthetized with urethan (2 mg/kg ip) and decapitated, and the spinal cord was carefully dissected out of

FIG. 1. Comparison of enhanced green fluorescent protein (EGFP) fluorescence to GABA immunolabeling in lamina I neurons in P14 glutamic acid decarboxylase 67 (GAD67)-EGFP mice. A: EGFP+ cells are evident throughout the dorsal horn. B: somatic GABA labeling is restricted to the superficial laminae; however, GABA− processes are seen throughout the dorsal horn. C: overlay of A and B shows that most of the EGFP+ cells in lamina I are also GABA+ (colored yellow; filled arrows), but there are EGFP− cells that are not GABA− (stick arrow) and GABA− cells that are not EGFP+ (open arrow). Transverse spinal slice (10 μm) is from lumbar cord. Scale bar is 50 μm.

For cell morphology, cells in lamina I were counted in L1–6 parasagittal sections in two mice at P14. A total of 1,496 cells were classified by morphology. Criteria for discerning fusiform, pyramidal, and multipolar cell types were soma shape and number of primary processes (Prescott and De Koninck 2002). Because cells were examined in two dimensions, it is possible that primary process were missed. However, morphology is most evident in parasagittal and horizontal sections because most primary processes extend in the rostral-caudal plane. The flattened morphology can only be identified in the horizontal plane; however, flattened cells are not GABAergic (Lima et al. 1993).
the body cavity and placed in a cooled (<4°C) artificial cerebrospinal fluid (ACSF) containing (in mM): 250 sucrose, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 25 glucose, 1.25 NaH₂PO₄, and 26 NaHCO₃ at a pH of 7.4. The ACSF was continuously oxygenated with 95% O₂-5% CO₂.

Position of the cell in lamina I was verified using differential-interference contrast optics (DIC) at ×40 to show that the cell was in or adjacent to the white matter in that focal plane (Chen and Gu 2005). Then the electrode was lowered into the slice, and the cell was targeted for whole cell patch-clamp recordings using DIC. Both voltage- and current-clamp data were acquired on computer with the pCLAMP acquisition software Clampex (v 9.0; Axon Instruments). Immediately after rupture of the cell membrane (in voltage clamp at −80 mV), the current-clamp recording configuration was used to determine resting membrane potential. Series resistance was subtracted. Most experiments were conducted in the current-clamp mode, although in a few cases, voltage-clamp recordings were made. Cells were brought to −80 mV by injecting bias current through the headstage. Then, a series of 1 s hyperpolarizing and depolarizing current steps were undertaken. Liquid junction potentials were not corrected for. Firing type was determined by the response to current steps at and above threshold (Prescott and De Koninck 2002; Ruscheweyh and Sandkühler 2002). Resistance was calculated from the average of steady-state responses to 1 s long hyperpolarizing voltage steps. Exponential curve fit of responses to hyperpolarizing current steps was used to calculate membrane time constant (τₘ). Rheobase was the minimum current step magnitude required to recruit an action potential. Voltage threshold was determined at rheobase by measuring the membrane potential at the inflection point at which the action potential was initiated (e.g., Fedirchuk and Dai 2004). The membrane potential value at threshold was largely insensitive to the holding potential in the range of holding potential values tested (−60 to −90 mV; r = 0.12). Maximal firing frequency was calculated as the reciprocal of the interspike interval between the first two spikes at the highest current step. Capacitance was calculated by dividing τₘ by resistance (τₘ = RC).

A total of 244 lamina I EGFP⁺ cells were recorded from. For analysis of firing properties, only cells with resting membrane potentials more negative than −50 (n = 133) were included. For analysis of cellular properties based on morphology, cells with membrane potentials more negative than −40 (n = 57) were considered because the additional cells had similar properties to those in the former group.

Statistical comparisons between firing properties were made using one-way ANOVAs (InStat, GraphPad Software, San Diego, CA). Membrane properties of the two morphological cell types were statistically compared using a Student’s t-test and reported as means ± SE.

RESULTS

EGFP⁺ cells represent the majority of lamina I GABAergic neurons

The number of cells expressing endogenous EGFP was compared with the number of cells immunolabeled for GABA in the same section in lamina I of lumbar cord at P14 (Fig. 1). Both EGFP fluorescence and GABA immunolabeling were observed predominantly in the dorsal horn. An overlay of EGFP and GABA labeling shows that most but not all EGFP⁺ cells were also GABA⁺ in the dorsal horn (Fig. 1C). In lamina I, most of the EGFP⁺ cells are GABA⁺ (73%), whereas the percentage of GABA⁺ cells expressing EGFP is 68%. EGFP⁺ cells do not contribute to a fixed percentage of the population of lamina I neurons. Thus, although most lamina I cells are EGFP⁺ and GABA⁺, some cells appear to express only EGFP or GABA.

Lamina I EGFP⁺ cell morphology was determined by the shape of the soma and number of primary dendrites seen in parasagittal sections as described by Prescott and DeKoninck (2002). This was also examined at P14. The percentages reported here are averages from two mice. Multipolar cells have round somas and several primary dendrites. These constituted 65% of the EGFP expressing neurons (Fig. 2A) and resembled type IIA cells described by Lima and Coimbra (1986). Fusiform cells have a flattened soma and two primary dendrites and accounted for 31% of EGFP⁺ cells (Fig. 2B). EGFP⁺ pyramidal shaped cells, with three primary dendrites, were rare (4%). Dendrites could frequently be followed for long distances by endogenous EGFP fluorescence (Fig. 2C).

Membrane properties of EGFP⁺ neurons

Whole cell recordings were made from visually identified EGFP⁺ neurons (n = 244) in lamina I. Due to stringent inclusion criteria (see METHODS), 133 cells were further analyzed. In response to depolarizing current steps from a holding potential of −80 mV, these neurons displayed one of three different firing patterns (Fig. 3A). The highest incidence of cells responded with a single spike to current steps, comprising 44% of sampled neurons. Cells that fired tonically throughout the current step represented 35% of the population, whereas
21% of cells fired with several spikes at current step onset (termed initial burst). Only one EGFP\(^+\) neuron responded with delayed firing during current step application and will not be considered further in this study.

Passive and active membrane properties were measured for the populations of cells with resting membrane potentials more negative than −50 mV and divided into three groups based on the aforementioned differences in firing properties (Table 1). Resting membrane potentials did not differ between the three groups. Tonic and initial burst cells were statistically indistinguishable for all passive membrane and threshold properties measured. It is possible that these cells represent one group with modifiable firing properties. First, some cells that fired tonically also instead fired an initial burst with larger amplitude current steps (\(n = 19\); Fig. 3B). Second, other cells classified as tonic based on initial recordings converted into initial burst cells later in the recording (\(n = 10\); Fig. 3C1), and the reverse conversion was also seen (\(n = 4\); Fig. 3C2). In contrast, this never occurred in cells firing single spikes.

Cells firing single spikes differed significantly from the other two groups in several membrane properties (Table 1). Rheobase was higher in single spike cells (135 pA) than tonic cells (65 pA) and initial burst cells (71 pA), suggesting that single spike neurons are less excitable. Single spiking cells also had lower resistance and shorter \(\tau_m\) values than that of both tonic and initial burst cells (424 compared with 721 and 618 M\(\Omega\) and 16 compared with 30 and 28 ms, respectively). Interestingly, there was a strong inverse correlation between \(\tau_m\) and rheobase (Fig. 4A; \(r = -0.68\)) indicating that \(\tau_m\) contributed significantly to the value of rheobase. Longer \(\tau_m\) values for tonic and initial burst cells support a greater capacity for synaptic integration. Capacitance, maximum firing frequency, and spike height were not significantly different between groups. Membrane time constant (\(\tau_m\)) was well correlated with membrane resistance (Fig. 4A, \(r = 0.39\)) and cell capacitance values (\(r = 0.53\)), and there was an inverse correlation between cell capacitance and resistance (\(r = -0.42\)). Threshold voltage was similar between cell populations suggesting that there are no differences in the voltage-dependent activation of Na\(^+\) spikes in these cell populations.

Firing properties were compared with cell morphology in 25 identified fusiform cells and 32 identified multipolar cells. Most fusiform cells fired tonically (64%) with a minority displaying initial burst (24%) and single spike activity (12%). In contrast, the majority of multipolar cells fired a single spike (56%) with a minority displaying tonic (31%) and initial burst firing (13%). Multipolar and fusiform cells also had differences in cellular membrane properties (Table 2). As compared with multipolar cells, fusiform cells had a longer \(\tau_m\) (30 vs. 19 ms). Fusiform cells also had larger cell capacitance (50 vs. 34 pF) and lower rheobase (74 vs. 110 pA), suggesting that fusiform cells are larger and more excitable than multipolar cells. We then compared the distribution of membrane properties in each morphological cell type separated as single-spike, tonic and initial burst firing populations (Fig. 4B). Single-spke cells in both morphological types had higher rheobase, lower resistances, and shorter \(\tau_m\). Thus the differences in membrane properties between morphological types are at least partly due to the higher incidence of single spike neurons in multipolar

![Firing properties displayed by lamina I EGFP\(^+\) cells. In response to 1 s long current steps, EGFP\(^+\) lamina I neurons fired tonically (A1, \(n = 46\)) with a single spike (A2, \(n = 59\)) with an initial burst (A3, \(n = 28\)) or after a delay (not shown, \(n = 1\)). B: several cells classified as tonic fired with an initial burst in response to larger-amplitude current steps (\(n = 19\)). C: during the course of recordings, some cells that initially fired tonically subsequently switched to firing an initial burst (C1, \(n = 10\)) in response to current steps of the same magnitude. Conversely, some cells firing with an initial burst switched to tonic firing (C2, \(n = 4\)) later during the recordings. All scale bars are 20 mV and 100 ms (as shown in A).](http://jn.physiology.org/Downloaded from http://jn.physiology.org/)
cells and a higher incidence of tonic and initial burst neurons in fusiform cells.

**DISCUSSION**

We used transgenic mice expressing EGFP under the control of a GAD67 regulatory element (Oliva et al. 2000) to characterize morphological and physiological properties of lamina I GABAergic interneurons. The view taken was that an understanding of the response properties of local inhibitory interneurons would provide insight into mechanisms controlling excitability at this nodal point in sensory processing. The primary finding of the present work is that lamina I inhibitory interneurons are functionally divisible into at least 2 major groups.

**Morphology of GAD67-EGFP neurons and overlap with GABA immunolabeling in lamina I**

The present study demonstrates that transgenic mice expressing EGFP fluorescence under the control of a GAD67 regulatory element can be used to identify some GABAergic neurons in lamina I. We noted that 73% of EGFP cells were also GABA+. Additionally, EGFP+ cells make up 19% of the population of lamina I neurons. Because ~25% of lamina I is GABAergic (Polgar et al. 2003), approximately three out of four GABAergic neurons can be visualized by the endogenous EGFP. This further confirms the reliability of EGFP as a reporter of the GABAergic phenotype (Heinke et al. 2004; Oliva et al. 2000). However, because ~25% of GABA+ cells are not EGFP+, it is possible that EGFP is not reporting a subpopulation of GABAergic inhibitory interneurons and that these unlabeled interneurons represent another functional neuron subpopulation. This is unlikely for the following reasons.

**TABLE 2. Comparison of intrinsic membrane properties within the two predominant morphological classes**

<table>
<thead>
<tr>
<th>Membrane property</th>
<th>Fusiform (25)</th>
<th>Multipolar (32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane potential, mV</td>
<td>−58.8 ± 2.2</td>
<td>−59.9 ± 1.9</td>
</tr>
<tr>
<td>Resistance, MΩ</td>
<td>723 ± 82</td>
<td>697 ± 69</td>
</tr>
<tr>
<td>$\tau_m$, ms</td>
<td>30.0 ± 3.4</td>
<td>18.7 ± 2.6*</td>
</tr>
<tr>
<td>Capacitance, pF</td>
<td>47.8 ± 6.3</td>
<td>33.9 ± 3.1*</td>
</tr>
<tr>
<td>Rheobase, pA</td>
<td>74 ± 12</td>
<td>110 ± 12*</td>
</tr>
<tr>
<td>Max firing frequency, s⁻¹</td>
<td>41.2 ± 3.5</td>
<td>39.6 ± 4.6</td>
</tr>
<tr>
<td>Spike height, mV</td>
<td>56.1 ± 2.9</td>
<td>50.0 ± 2.2</td>
</tr>
<tr>
<td>Voltage threshold, mV</td>
<td>−48.5 ± 1.7</td>
<td>−43.5 ± 1.5*</td>
</tr>
</tbody>
</table>

*P < 0.05 via t-test.
First, the percentage of double-labeled cells increases after blocking axonal transport (Dougherty et al. 2004; Heinke et al. 2004), suggesting that, in many neurons, GABA and/or EGFP is trafficking out of somata. Second, neuron identity relies on fluorescence and several neurons may be below detection threshold. Third, we have observed a considerable heterogeneity of peptide content in these neurons (Dougherty et al. 2004), indicating that EGFP expression is found in neurochemically diverse populations of GABAergic neurons. Finally, EGFP+ neurons were mostly fusiform (31%) and multipolar (65%) and an earlier study suggested that populations of multipolar and fusiform cells in lamina I are GABAergic, whereas flattened and pyramidal cells are not GABAergic (Lima et al. 1993).

It is possible that the number of fusiform cells was overestimated in morphological counts because cells were counted in parasagittal sections. Morphology is best determined in the parasagittal plane in the lateral half of the cord and in the horizontal plane in the medial half. Because lamina I is difficult to delineate in the horizontal plane, the parasagittal plane was used (as in Prescott and De Koninck 2002). In thin sections (10 μm), it is possible that cells of other morphologies appear fusiform, particularly those in the medial part of the cord. However, only 31% of cells were fusiform in this plane which seems reasonable when compared with the Lima et al. (1993) study where 43% of GABAergic neurons were shown to be fusiform. Morphological classifications and comparisons are complicated due to inconsistencies between groups. Lima and Coimbra (1986) have classified lamina I neurons into four main groups—fusiform, multipolar, flattened, and pyramidal. Studies which correlated morphology with either modality or firing (Han et al. 1998; Prescott and De Koninck 2002), did not include a distinct flattened morphology but included flattened cells in the multipolar category. Additionally, Han et al. (1998) targeted larger cells, presumably mostly projection neurons, so cells called multipolar were likely flattened because multipolar cells are probably local interneurons.

Electrophysiological characterization of lamina I GAD67-EGFP+ neurons

Measured passive and threshold properties of EGFP+ neurons are comparable to previous estimates from unidentified lamina I neurons (Graham et al. 2004; Prescott and De Koninck 2002; Ruscheweyh and Sandkühler 2002). Because recordings were undertaken at room temperature, results cannot be directly compared with in vivo studies. With respect to firing properties, Prescott and DeKoninck (2002) identified four firing properties in unidentified adult rat lamina I neurons—tonic, phasic, delayed, and single spike. Ruscheweyh and Sandkühler (2002) found all four of these properties along with initial burst in unidentified neurons in lamina I of younger rats (P18–28). Presently, three of these firing properties were found in lamina I GABAergic interneurons in the young mouse—tonic, single spike, and initial burst. Interestingly, Ruscheweyh et al. (2004) observed two firing patterns in identified lamina I projection neurons (called gap and bursting firing) that were never seen in our EGFP+ neurons, demonstrating that firing properties divide, at least partly, into functional classes.

While we treated the population of neurons that fired tonically or with an initial burst as separate populations based on previous work (Ruscheweyh and Sandkühler 2002), several observations suggest that these neurons may constitute one functional class with modifiable firing properties. First, passive membrane and threshold properties were indistinguishable between these populations. Second, for a range of current steps both firing types could be observed in 28% of these neurons (Fig. 3D). Third, initial burst and tonic firing properties were often bidirectionally interconvertible in individual neurons during the course of a recording (Fig. 3, E and F). Last, modulatory transmitters can reversibly convert firing properties between these two types (Garraway and Hochman 2001a). Because there is evidence of continued monoaminergic modulation of dorsal horn neurons in acute slice preparations (Garraway and Hochman 2001b), such shifts are possible.

In comparison, the passive and threshold properties of single spike cells differed considerably from the aforementioned cells. They were less excitable (higher rheobase) and had lower resistance and membrane time constant (τm) values. Because these values were well correlated in individual neurons, it is clear that a lower membrane resistivity contributes to reduced cell excitability.

Correlation of morphology and firing properties

Fusiform and multipolar morphologies were the predominant cell types of lamina I EGFP+ neurons. Because cells of all three firing properties were found in both morphological cell types, morphology and intrinsic membrane properties are shaped at least partly by independent mechanisms. Nonetheless, the majority of fusiform cells were tonic or initial burst (88%), and the majority of multipolar cells were single spike (56%). These differences in incidence and their associated differences in membrane properties led multipolar cells to have a comparatively higher rheobase and shorter τm values. These properties, and the smaller capacitance of multipolar cells, are consistent with the results obtained by Prescott and DeKoninck (2002), indicating that fusiform cells are larger and more excitable than multipolar cells.

The cells termed phasic by Prescott and DeKoninck (2002) are analogous to the initial burst cells in our study and in the work of Ruscheweyh and Sandkühler (2002). With this in mind, the incidence of the three major firing types in fusiform neurons here is very similar to that reported by Prescott and DeKoninck (2002). Because not all fusiform neurons are GABAergic (Lima et al. 1993), the implication is that firing properties for this cell morphology are not distinguished based on transmitter phenotype and excitatory interneurons with the same morphology express a similar range in firing properties. Comparing our work to Prescott and DeKoninck (2002), multipolar cells in both GABAergic neurons and lamina I cells in general are mostly single spike (56% and 58%, respectively) with initial burst/phasic cells making up a small percentage of the multipolar population (13% and 5%, respectively). However, we never observed delayed firing, whereas Prescott and DeKoninck (2002) never observed tonic firing in multipolar neurons. We cannot explain these striking differences but offer several possible explanations. First, it is possible that the cells expressing delayed firing were flattened cells that were not differentiated from multipolar cells. Flattened cells are not GABAergic (Lima et al. 1993) and so would not have been targeted based on EGFP expression in the current study. Second, Prescott and...
DeKoninck (2002) studied adult rat, and we studied the young mouse, so it is possible that firing properties differ between species or developmentally (but see Hochman et al. 1997). Last, it is possible that delayed, tonic, and initial burst cells are one neuron population whose firing properties are sculpted by neuromodulation, as observed in other studies (e.g., Garraway and Hochman 2001a) and that unknown factors bias different studies toward different neuromodulatory "states". 

We conclude that the firing properties of the lamina I inhibitory apparatus are functionally divisible into at least two major groups representing a subset of the firing properties observed for neurons in this lamina.

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