Renshaw cells (RCs) receive excitatory inputs from motoneurons to which then they inhibit. The gain of this spinal recurrent inhibitory circuit is modulated by inhibitory synapses on RCs. Inhibitory synapses on RCs mature postnatally, developing unusually large postsynaptic gephyrin clusters that colocalize glycine and GABA<sub>A</sub> receptors. We hypothesized that these features potentiate inhibitory currents in RCs. Thus, we analyzed glycinergic and GABAergic “inhibitory” miniature postsynaptic currents (mPSCs) in neonatal (postnatal day 1 (P1) to P5) and mature (P9–P15) RCs and compared them to other ventral interneurons (non-RCs). Recorded neurons were Neurobiotin filled and identified as RCs or non-RCs using post hoc immunohistochemical criteria. Glycinergic, GABAergic, and mixed glycine/GABA mPSCs matured differently in RCs and non-RCs. In RCs, glycinergic and GABA<sub>A</sub> mPSC peak amplitudes increased 230 and 45%, respectively, from P1–P5 to P9–P15, whereas in non-RCs, glycinergic peak amplitudes changed little and GABA<sub>A</sub> amplitudes decreased. GABA<sub>A</sub> mPSCs were slower in RCs (P1–P5, \( \tau = 58 \text{ ms} \); P9–P15, \( \tau = 43 \text{ ms} \)) compared with non-RCs (P1–P5, \( \tau = 27 \text{ ms} \); P9–P15, \( \tau = 14 \text{ ms} \)). Thus, fast glycinergic currents dominated “mixed” mPSC peak amplitudes in mature RCs, and GABA<sub>A</sub> currents dominated their long decays. In non-RCs, GABAergic and mixed events had shorter durations, and their frequencies decreased with development. Functional maturation of inhibitory synapses on RCs correlates well with increased glycine receptor recruitment to large gephyrin patches, colocalization with α3/α5-containing GABA<sub>A</sub> receptors, and maintenance of GABA/glycine corelease. As a result, charge transfer in GABA, glycine, or mixed mPSCs was larger in mature RCs than in non-RCs, suggesting RCs receive potent inhibitory synapses.

**Key words:** development; subunit composition; corelease; gephyrin; GABA; synapse; motor
to gephyrin and containing colocalized GABA$_\lambda$ and glycine receptors (Alvarez et al., 1997; Geiman et al., 2002). These receptors are postsynaptic to boutons expressing markers, suggesting significant GABA/glycine corelease. We hypothesized that these structural and neurochemical properties increase synaptic inhibition by simultaneously enhancing peak amplitudes and time courses of postsynaptic currents (Geiman et al., 2002). These characteristics are likely adapted to RC function because, unlike other interneurons, they receive motor axon excitatory inputs of large amplitude and long duration that evoke high-frequency burst firing (Renshaw, 1946; Eccles et al., 1954). Synaptic structure on RCs matures postnatally in conjunction with ventral horn motor output and locomotion (Geiman et al., 2000). To investigate functional features characteristic of inhibitory synapses on RCs, we analyzed the amplitude and time courses of spontaneous miniature inhibitory currents and their GABAergic and glycineric components during postnatal development and compared them to other ventral interneurons of similar ages.

Materials and Methods

**Tissue preparation and immunohistochemical processing.** The distribution, density, and immunocytochemical properties of RCs were analyzed in spinal cord sections obtained from early [postnatal day 1 (P1), P5] and late (P10, P15) postnatal rat spinal cords. This information was then used to target our recordings to a specific area of the spinal cord containing high densities of RCs and to classify recorded cells as “Renshaw” (RC) or “non-Renshaw” (non-RC) using post hoc immunohistochemistry. For histological processing, a total of 12 pups (three rats in each age group) were used. Rats were anesthetized with an overdose of sodium pentobarbital (>90 mg/kg, i.p.) and perfused transcardially with cold 0.1M phosphate buffer (0.9% NaCl, 3.4 mM KCl, and 6 mM NaHCO$_3$, pH 7.3), followed by fixative (4% paraformaldehyde in 0.1 M phosphate buffer). Spinal cords were removed, postfixed for 1 h at room temperature, and cryoprotected at 4°C in 0.1 M phosphate buffer with 15% sucrose. Twenty-micrometer-thick transverse sections were obtained on a cryostat from L4–L5 spinal cord segments and collected on slides. Some sections were dual immunolabeled for neuronal nuclear protein (NeuN) [mouse monoclonal antibody (mAb); Chemicon, Temecula, CA], a generalized neuronal marker, and for calbindin [rabbit polyclonal antibody (pAb); Swant, Bellinzona, Switzerland], an RC marker in the ventral horn of the developing spinal cord (Geiman et al., 2000). In other sections, these markers were combined with immunostaining for the vesicular acetylcholine transporter (VACHT) (goat pAb; PharMingen, San Diego, CA). Different dual or triple immunolabelings were used to map the distribution of RCs to estimate their density in ventral horn regions targeted for recording (see Fig. 1A, B) and to analyze the competence of criteria for distinguishing RCs from neighboring non-RC interneurons in neonatal rat spinal cord based on the presence of abundant “cholinergic contacts” (see Fig. 1C–F).

The sections were washed in 0.01 M phosphate buffer containing 0.9% saline and 0.1% Triton X-100 (PBS/TX) and blocked with normal horse serum (NHS) (1:10 in PBS/TX). For dual (calbindin/NeuN or calbindin/ VACHT) or triple (calbindin/NeuN/VACHT) color immunofluorescence, the sections were incubated overnight at 4°C with different combinations of primary antibodies against calbindin D28K (rabbit pAb; dilution, 1:2000), NeuN (mouse mAb; 1:1000), or VACHT (goat pAb; 1:1000). Immunofluorescence was visualized with donkey anti-rabbit, anti-mouse, or anti-goat antibodies (1:50 dilutions) coupled with α-methylcoumarin (AMCA), cyanine 3 (Cy3), Cy5, or FITC (Jackson ImmunoResearch, West Grove, PA). All primary and secondary antibodies were diluted in PBS/TX. Finally, after washing the excess of antibodies with PBS, the sections were coverslipped with Vectashield (Vector Laboratories, Burlingame, CA).

**Confocal microscopy and image analysis.** Dual Cy3 and FITC immunofluorescence and triple Cy3, Cy5, and AMCA immunofluorescence were visualized with a Fluoview FX laser-scanning confocal microscope (Olympus, Tokyo, Japan), except that AMCA fluorescence was recorded with normal epifluorescence and a digital camera (Spot2; Diagnostic Instruments, Sterling Heights, MI). Triple color immunolabelings including FITC, Cy3, and Cy5 fluorochromes were imaged in a TCS confocal system (Leica, Nussloch, Germany). The proportions of calbindin-immunoreactive (IR) profiles to all small-sized NeuN-IR profiles in ventral LVII and LIX were estimated in low-magnification confocal images (10×) using ImagePro-Plus software (version 3.0.01; Media Cybernetics, Silver Spring, MD). The border between LIX and LVII was delineated by the distribution of motoneuron cell bodies (large NeuN-IR profiles). To accommodate for the progressive spinal cord growth and to minimize sampling variations, counting in ventral LVII was performed using a rectangular window, the linear dimensions of which were increased in steps of 25 μm for each successive older age group (200–275 μm width and 100–175 μm height from P1 to P15) (see Fig. 1A, B). The box was placed in ventral LVII with the lateral border aligned to the medial margin of the LIX lateral motoneuron pool and the ventral border with the ventral funiculus gray–white matter boundary. The box in LIX was placed as a lateral extension in LIX of boxes drawn in LVII at each age. Sections from 12 animals (3 per age group), with at least three sections per animal (i.e., six ventral horns), were included in the analysis. To reduce errors because of assumptions about similarities in cell body size, shape, or orientation of ventral interneurons, only nucleoplasm-containing IR profiles were counted. Errors caused by differential splitting of cells at the surfaces were avoided by counting only nucleoli at least 3 μm within the tissue sections. These precautions were taken to assure unbiased sampling of profiles from RCs versus other neuronal subtypes.

To reconstruct VACHT coverage on calbindin-IR or NeuN-IR cells, optical section stacks (step size, 0.5 μm) were obtained throughout the cell body and proximal dendrites of the neurons. Dual immunofluorescence was captured in two-channel mode with a 60× oil immersion objective (numerical aperture, 1.4) digitally zoomed 1.5×. Cross talk between channels was avoided as in previous reports (Geiman et al., 2002). Images used for figure composition were filtered (high-Gauss filter, Image Pro-Plus 4.0; Media Cybernetics) and adjusted for contrast, brightness, and dynamic resolution for best quality presentation without changing or altering the information content in the images.

**Slice preparation.** We recorded ventral horn interneurons from spinal cord slices obtained from 1- to 5- or 9- to 15-d-old postnatal rat pups. All animals were anesthetized (50 mg/kg pentobarbital) and decapitated, and their spinal cords were removed quickly. Dissection was in ice-cold (4°C) sucrose artificial CSF (S-acsf) bubbled with 95% O$_2$ and 5% CO$_2$. The S-acsf composition was as follows (in m M): 26 NaHCO$_3$, 10 glucose, 3 KCl, 1.25 NaH$_2$PO$_4$, 2 MgCl$_2$, 2.4 CaCl$_2$, and 218 sucrose. Transverse slices from the L4–L5 segments (300–400 μm thick) were cut with a Vibratome (50–100 mm thick sections) or a Leica (VT1000S) vibratome. Slices were transferred to normal oxygenated aCSF (in which sucrose was removed and 130 m NaCl and 2 mM CaCl$_2$ were added) and incubated for 1 h at 36°C and then allowed to stabilize at room temperature (22–25°C) for at least 30 min before electrophysiological recordings. Slices were transferred into the recording chamber and perfused continuously (at a rate of 4 ml/min) with oxygenated normal aCSF.

**Whole-cell recordings.** Whole-cell voltage-clamp recordings were obtained from somata of visually identified ventral horn interneurons (<250 μm diameter) using an Olympus BX 50WI microscope equipped with infrared-differential interference contrast (DIC), a 40× water immersion objective, and a CCD camera (CCD-Iris; Sony, Tokyo, Japan). Preferential recording areas were ventral LVII and LIX regions containing the highest densities of immunocytochemically identified RCs (see Fig. 2A, B). Patch electrodes (2–5 MΩ resistance) contained the following (in m M): 120 CaCl$_2$, 4 NaCl, 4 MgCl$_2$, 1 Cl$_2$Ca, 10 HEPES, 0.2 EGTA, 3 Mg-ATP, and 0.3 GTP-Tris. In all of the experiments, 0.4% Neurobiotin (Vector Laboratories) was added to the internal solution. Only recordings with access resistance between 5 and 20 MΩ were considered acceptable for analysis. The access resistance was checked throughout the experiments, and recording was abandoned if it changed >15%. Junction potentials (usually <5 mV) were corrected after inserting the pipette into the bath. Cells were voltage clamped at −75 mV. In our recording conditions, the reversal potential for chloride currents was close to 0 mV;
thus, GABAergic and glycineric currents were readily detected as inward currents well separated from baseline noise. Synaptic currents were recorded and low-pass filtered at 5 kHz with an Axopatch 200B amplifier (Axon Instruments, Union City, CA), Data were digitized at 10 kHz and acquired using Axograph (version 4.6; Axon Instruments). For each cell, we obtained two to three segments of 5 min continuous recordings of spontaneous activity under drug combinations that pharmacologically isolated the synaptic currents of interest.

To isolate miniature spontaneous synaptic currents of GABAergic or glycineric origin [miniature postsynaptic currents (mPSCs)], recordings were performed in the presence of tetrodotoxin (TTX) (1 μM; Tocris Cookson, Ballwin, MO), and α-tubocurarine chloride (10 or 30 μM; Sigma, St. Louis, MO) applied by bath perfusion. Glyceric or GABAergic mPSCs were subsequently isolated by adding either bicuculline methiodide (10 μM; Sigma) or strychnine hydrochloride (0.25 μM; Sigma) to the ACSF solution.

Data analysis. Off-line data analysis and curve fitting was performed with both pClamp 9.0 and MiniAnalysis (Synaptosoft, Decatur, GA). Events were detected by setting the threshold value for detection at three times the level of the root mean square noise (3 pA). Therefore, the detection threshold was 8–10 pA. We routinely scolded through detected events and visually rejected any superimposed or spuriously detected events. Peak mPSC amplitudes were measured at the absolute maximum of the currents, taking into account the noise at baseline and around the peak. Time to decay was calculated as the time from peak to 33% decay of peak amplitude. Rise times were measured between 10 and 90% of the peak. The charge transferred (in picocoulombs) was measured as the integrated area under averaged mPSCs.

Curves fitting to mPSC decay (from peak to end) was performed on averaged traces with simplex algorithm least squares exponential fitting provided by MiniAnalysis software and using single- or double-exponential equations of the form: 
\[ y = A_i + A e^{(-x/\tau_i)} \]
and 
\[ y = A_0 + A_i e^{(-x/\tau_i)} + A_2 e^{(-x/\tau_2)} \]
respectively. Decay phases of individual mPSCs recorded under TTX, CNQX, and α-tubocurarine chloride were fitted either by one or two exponential functions that usually resulted in events fitting into one of three classes: “fast decay,” “slow decay,” or mixed events (see Results). The averaged glycineric and GABAergic mPSCs in individual neurons were always best fitted by a biexponential decline. In contrast, individual and averaged mPSC decays of pharmacologically isolated glycineric or GABAergic events were best fitted by single monoequivalent curves. Although this procedure yielded good correlation fits (usually \( r > 0.95 \)) for averaged events and many individual mPSCs, it failed in a proportion of individual events in which curve fits clearly departed from trace points (\( r < 0.7 \)). For this reason, classification of events falling in fast-decaying, slow-decaying, and mixed categories in recordings containing mixed GABA, and glycineric currents was aided by visual inspection of individual events in which the program failed to provide good curve fitting. These events showed fluctuations in their decay phases usually because of superimposition of other events or random baseline oscillations. The proportion of events added by visual identification of inflections in their decays were, on average (of all cells analyzed), as follows: P1–P5 RCs, 14.8% of all events; P9–P15 RCs, 4.0% P1–P5 non-RCs, 13.4%; P9–P15 non-RCs, 1.8%. Not surprisingly, more events were added in younger cells displaying the longer decays (Tables 1–3). In a few young neurons with high frequencies of spontaneous

### Table 1. Properties of glycineric mPSCs

<table>
<thead>
<tr>
<th></th>
<th>Rise time (ms)</th>
<th>Amplitude (pA)</th>
<th>Decay time (ms)</th>
<th>( \tau_{025} ) (ms)</th>
<th>Frequency (Hz)</th>
<th>Q (pC)</th>
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<tbody>
<tr>
<td>RCs</td>
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<tr>
<td>P1–P5</td>
<td>(n = 6)</td>
<td>1.71 ± 0.23</td>
<td>37 ± 5</td>
<td>6.7 ± 0.9</td>
<td>6.7 ± 0.5</td>
<td>0.21 ± 0.06</td>
</tr>
<tr>
<td>P9–P15</td>
<td>(n = 6)</td>
<td>0.78 ± 0.06*</td>
<td>124 ± 10*</td>
<td>4.0 ± 0.3*</td>
<td>3.6 ± 0.4*</td>
<td>0.70 ± 0.21</td>
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<tr>
<td>Non-RCs</td>
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<tr>
<td>P1–P5</td>
<td>(n = 6)</td>
<td>1.79 ± 0.21</td>
<td>46 ± 8</td>
<td>7.6 ± 0.8</td>
<td>7.5 ± 0.8</td>
<td>0.47 ± 0.14</td>
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<tr>
<td>P9–P15</td>
<td>(n = 7)</td>
<td>0.96 ± 0.13*</td>
<td>60 ± 5*</td>
<td>4.2 ± 0.2*</td>
<td>3.8 ± 0.2*</td>
<td>1.69 ± 0.63*</td>
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Values are mean ± S.E. The asterisks indicate significant differences (\( p < 0.05 \); two-way ANOVA; Duncan’s multiple range test) between age groups; the * symbol indicates significant differences between RCs and non-RCs of the same age.
Results

Targeting and identification of recorded neurons as RCs

RCs are characterized by monosynaptic cholinergic inputs from motor axon collaterals, strong calbindin expression, large gephyrin clusters on their proximal somatodendritic membranes, and preferential location of lower lumbar segments in ventral LVII and ventral LIX (Eccles et al., 1954; Jankowska and Lindstrom, 1971; Fyffe, 1990; Arvidsson et al., 1992; Alvarez et al., 1997, 1999; Carr et al., 1998). Two previous studies in spinal cord slices used stimulation of motor axons as search stimulus to identify and record RCs (Oleskevich et al., 1999; Dourado and Sargent, 2002). In both studies, ventral root cholinergic monosynaptic inputs were identified in <10% of recorded neurons. This low yield could have resulted from targeting ventral horn regions with low densities of RCs and perhaps also from motor axon damage during slicing. To overcome these problems, we determined anatomically the optimal sampling sites in the neonatal spinal cord and tested several properties for RC post hoc histochemical identification.

Intense calbindin immunoreactivity is a distinctive feature of RCs (Arvidsson et al., 1992; Carr et al., 1998; Geiman et al., 2000). Ventral horn neurons with intense calbindin immunoreactivity are mostly located in ventral LIX and LVII (Fig. 1A,B), in a region corresponding to the adult RC area (Thomas and Wilson, 1965; Jankowska and Lindstrom, 1971; Fyffe, 1990). The proportion of calbindin-IR neurons to all NeuN-IR small-sized neurons increased during postnatal development from 30.1 ± 2.6% (P1–P5; mean ± SE) to 44.3 ± 3.6% (P10–P15) in ventral LVII and from 31.6 ± 3.8% to 57.3 ± 5.4% in ventral LIX (Fig. 1F) (see Materials and Methods for a description of the boxed regions of interest that were analyzed). At the same time, expansion of the neuropil reduced neuronal density from P1 to P15, particularly in ventral LVII (Fig. 1, compare C, D). In conclusion, sampling in the low cell density region shown in Figure 2A should result in a 30–60% chance of recording from an RC. This region appears darker with DIC optics because of the increased density of myelinated axons, likely motor axons, crossing this area.

This distinctive regional distribution is important for targeting recordings to RCs, but it is insufficient to identify this neuronal phenotype. Therefore, we investigated other features that could permit RC identification. Adult calbindin-IR RCs display high densities of cholinergic contacts on their dendrites (Alvarez et al., 1999). Similarly, neonatal calbindin-IR RCs received higher densities of VACH-T contacts than other NeuN-IR neurons (Fig. 1C–E). Two other features of adult RCs, large gephyrin clusters and calbindin immunoreactivity, were inadequate for RC characterization in the present study. Gephyrin-IR clusters on RCs undergo postnatal maturation (Geiman et al., 2000), and in neonates they are not different from gephyrin-IR clusters on other neurons (data not shown). On the other hand, calbindin immunoreactivity in recorded neurons was weaker and more difficult to detect than in non-recorded cells. Possibly, calbindin dilution into recording pipettes significantly lowers calbindin immunoreactivity in neurons recorded in whole-cell mode.

Neurons selected for recording were visualized with DIC optics in the target region. Thereafter, post hoc immunohistochemical confirmation of a high density of VACH-T-IR contacts on Neurobiotin-filled neurons was a necessary condition for RC classification (Fig. 2C,D); 27.5% (60 of 218) of intracellularly recorded and stained cells were classified as RCs in our target population. Only neurons with good quality recordings (>25 min of stable recordings at constant low series resistance) and

Figure 1. Distribution of immunohistochemically defined RCs in the ventral horn. A, B, Low-magnification confocal images showing the distribution of calbindin-IR (FITC; green) and NeuN-IR (Cy3; red) neurons in the spinal cord at P1 (A) and P15 (B). The RC region was identifiable at both ages in ventral LVIII and LIX and contains a relatively high density of calbindin-IR neurons. The boxes indicate the placement and dimensions of the areas used to estimate neuronal percentages in ventral LVIII and LIX. The percentage of calbindin-IR neurons to all other neurons was estimated inside these boxes. C, D, Triple immunofluorescence for calbindin (Cy5; blue), NeuN (FITC; green), and VAChT (Cy3; red) in the ventral horn of P1 and P15 rat spinal cords. Neuronal density in ventral LVIII was higher at P1. The distribution of calbindin-IR neurons is noted with arrowheads at P1 (C) and with arrows at P15 (D). VAChT immunoreactivity was strong in C–E. The density of VAChT-IR contacts (Cy3; red) was higher on dendrites (arrowheads) and somata of calbindin-IR neurons (Cy5; blue) compared with NeuN-IR (non-calbindin-IR; FITC; green) neurons. F, Percentages of calbindin-IR neurons (RCs) to all NeuN-IR neurons of small size (i.e., interneurons; large-sized NeuN-IR motoneurons were excluded from the counts) gradually increase with age in ventral LVIII (gray circles) and LIX (filled circles). Scale bars: (in B) A, B, 200 μm; (in D) C, D, 100 μm; E, 20 μm.
intracellular staining and in sections with successful VAChT immunolabeling were analyzed. VAChT immunolabeling of C terminals on neighboring motoneurons (Alvarez et al., 1999) was used as internal control of immunolabeling quality. The locations of neurons analyzed are shown in Figure 2B. We analyzed 20 neurons identified as RCs at each age (P1–P5 and P9–P15). All RCs were preferentially located within the ventralmost 200 μm of LVII and LIX. RC axons were directed toward ipsilateral motor pools, and sometimes varicosities were observed in LIX. The dendrites of RCs formed small arborizations, mostly restricted to the RC area. Non-RC interneurons were more dispersed in the ventral horn, occasionally distant from the motoneuron pools, and their morphological features were very variable. Axons recovered from non-RCs projected toward ipsilateral LIX motoneuron pools, crossed the midline into the contralateral spinal cord, or coursed toward the lateral funiculus. Some dendritic arborizations in this cell group were large and widespread, whereas others had restricted distributions. Thus, non-RCs likely comprise several neuronal subtypes. We analyzed 21 non-RCs at P1–P5 and 31 at P9–P15.

**Differential maturation patterns of GABAergic/glycinergic mPSCs in ventral horn interneurons**

We first compared all “inhibitory” mPSCs isolated with TTX, CNQX, and D-tubocurarine in RCs (n = 7 at P1–P5 and n = 7 at P9–P15) and non-RCs (n = 9 at P1–P5 and n = 8 at P9–P15). These mPSC events are mixtures of GABAergic and glycinergic events, hereafter called mPSCs-GABA/Gly, and were blocked by and glycinergic events, hereafter called mPSC events are mixtures of GABAergic

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**Figure 2.** Immunohistochemical identification and recording from ventral horn interneurons. A, Representative image of a P12 rat spinal cord slice through the lower lumbar region. A patch pipette is visible in the image and points to the area targeted for recordings containing the highest densities of RCs. This area appears slightly darker under infrared-DIC optics because of its high content in myelinated motor axons. B, Schematic maps showing the location of interneurons recorded at P1–P5 (left) and P9–P15 (right). Red dots, RCs; blue dots, non-RCs. The dashed lines delineate lateral and medial motoneuron pools (LIX). C, D, Superimposition of confocal optical sections reconstructing VAChT-IR coverage (FITC; green) on two Neurobiotin-filled (Cy3; red) ventral horn interneurons at P4 and P5 and respectively classified as a non-RC (C) and an RC (D). The bottom left insets are single optical sections indicating localization of each neuron (Cy3; black and white images) relative to LIX and midline (ml; dotted line). The top right insets are higher-magnification images of boxed dendrites in C and D. Note the dense coverage by VAChT-IR varicosities on the RC dendrite shown in D. E, F, GABA and glycine mPSCs recorded in the cells shown in C and D. Neurons were voltage clamped at −75 mV, and mPSCs were isolated with TTX (1 μM), CNQX (10 μM), and D-tubocurarine (30 μM). The reversal potential for Cl⁻–mediated currents was close to 0 mV. Neurons exhibited fast-decaying (filled circles), slow-decaying (open circles), and dual-component (asterisks) mIPSCs (top traces). Slow and mixed events had longer durations in RCs. Glycinergic or GABAergic mIPSCs were subsequently isolated by adding bicuculline (10 μM; E, F, middle traces) or strychnine (0.25 μM) (see Fig. 5) to the aCSF. Isolated glycinergic currents were blocked with strychnine (example shown in F), and isolated GABAergic currents were abolished with bicuculline. Scale bars: A, B, 200 μm; (in D) C, D, 20 μm; C, D, left inset, 100 μm; C, D, color inset, 5 μm.

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Peak amplitudes of mPSCs\textsubscript{GABA/Gly} were also differentially regulated during postnatal development in each neuronal type. The mean peak mPSC\textsubscript{GABA/Gly} amplitude increased by 121.3% [100 × value(P9–P15) – value(P1–P5)/value(P1–P5)] in the RC group (p < 0.05; two-way ANOVA; Duncan’s test). In contrast, the average peak amplitude increase in non-RCs was moderate and did not reach statistical significance (61.2%; p = 0.07). Cumulative probability plots of peak mPSC\textsubscript{GABA/Gly} amplitudes shifted to the right with development in both RCs and non-RCs. The magnitude of change was much greater for RCs (Fig. 3G,H), and peak amplitude distribution histograms in P9–P15 RCs showed strong skews toward high-amplitude events.

In addition, we observed developmental changes in frequency, but these are best reported below, following descriptions of individual and mixed GABA/glycine unitary events.

Glycinergic mPSC development
To analyze the specific neurotransmitter components of these inhibitory synaptic currents, we first isolated pharmacologically glycinergic mPSCs (called mPSCs\textsubscript{Gly}) by blocking glutamatergic (CNQX), nicotinic (\textalpha{-}tubocurarine), and GABAA (bicuculline) receptors. At early postnatal times, mPSCs\textsubscript{Gly} were similar in both cell types (Fig. 4A,B, top traces). Mean peak amplitude, 10–90% rise time, decay time, and time constant (\textgamma{Gly}) were not significantly different between both cell types (p ≥ 0.05; two-way ANOVA; Duncan’s test) (Table 1). During development, mPSCs\textsubscript{Gly} amplitudes became faster in both cell types (Fig. 4A–F; C,D, insets; Table 1). Rise time versus decay time plots (Fig. 4C,D) and distribution histograms (Fig. 4E,F) revealed a shortening in the range of decays, the mean values of which for RCs and non-RCs were, respectively, 1.69 and 1.82 times faster in P1–P5 neurons (Table 1). Common kinetic changes in pharmacologically isolated mPSCs\textsubscript{Gly} probably reflect similar maturation of postsynaptic glycine receptor functional properties in both cell types. In contrast, mPSCs\textsubscript{Gly} amplitudes matured differently in RCs and non-RCs (Fig. 4G,H; Table 1). P9–P15 RCs exhibited a large increase (230.4%) in mean mPSCs\textsubscript{Gly} peak amplitude, relative to P1–P5 RCs. In contrast, the smaller increase (31%) in mean peak amplitude in non-RCs did not reach statistical significance (p = 0.2; two-way ANOVA; Duncan’s test). Consequently, non-RC mPSCs\textsubscript{Gly} amplitude distribution histograms of different ages essentially overlapped, and only a very small shift was noted.
in their cumulative probability functions (Fig. 4G). In contrast, amplitude distributions from P9–P15 RC mPSCsGABA were mostly skewed toward higher values, and their probability functions significantly shifted to the right (Fig. 4H). Thus, whereas mPSCsGABA with amplitudes >100 pA were infrequent in RCs at early ages (4.5% of all events) and in non-RCs at early and late postnatal ages (11.0 and 14.8%, respectively), events >100 pA comprised nearly 50% of all mPSCsGABA in P9–P15 RCs.

The frequency of pharmacologically isolated glycinergic events increased more than twofold from early to late postnatal ages in both RCs and non-RCs (see Fig. 6A3, Table 1). Event frequencies were always higher in non-RCs of all ages. This observation parallels morphological findings indicating the presence of 5–15 small independent active zones per synapse on non-RCs compared with fewer numbers of larger active zones in inhibitory synapses contacting adult RCs (Alvarez et al., 1997).

**GABAergic mPSC development**

In the ventral spinal cord, spontaneous miniature GABAergic mPSCs (mPSCsGABA) are generally downregulated in frequency and amplitude, and their duration is shortened during postnatal development (Gao et al., 2001). However, significant numbers of bicuculline-sensitive mPSCs with remarkably slow kinetics persist in mature RCs (Fig. 3). To analyze their postnatal development, mPSCsGABA were pharmacologically isolated in the presence of 0.25 μM strychnine added to the CNQX, t-tubocurarine, and TTX mixture (Fig. 5A, B). Pharmacologically isolated GABAergic mPSCs recorded from non-RCs (n = 8) and RCs (n = 6) at P1–P5 were similar in 10–90% rise time and peak amplitude (p > 0.05; two-way ANOVA; Duncan’s test) (Fig. 5G, H; Table 2). However, mean decay time and τGABA of mPSCsGABA in neonatal RCs was 2.1 times longer than in non-RCs (p < 0.05; two-way ANOVA; Duncan’s test) (Fig. 5A–F, Table 2).

Differences in pharmacologically isolated mPSCsGABA between both cell types increased with development (Fig. 5A–H). The average decay times and τGABA of mPSCsGABA were shortened in P9–P15 non-RCs, becoming nearly threefold faster than those measured in RCs of similar age (Table 2). Rise time versus decay time plots, distribution histograms, or cumulative probability curves were compressed or shifted toward shorter values in P9–P15 non-RCs. In contrast, their ranges essentially overlapped in RCs from both ages (Fig. 5C–F). Nevertheless, the averaged τGABA suggested an acceleration of mPSCsGABA decay kinetics in RCs with development (p < 0.05; two-way ANOVA; Duncan’s test) (Table 2). Mean peak amplitudes significantly increased by 45.1% in RCs (p < 0.05; two-way ANOVA; Duncan’s test), while...
Postnatal development of glycine–GABA mixed mPSCs

The characteristics of inhibitory currents in RCs and non-RCs are also influenced by the amount of cotransmission between GABA and glycine. We analyzed cotransmission in both cell types by studying the characteristics of dual-component mPSCs from recordings obtained in the absence of bicuculline and strychnine, as those shown in Figures 2 and 3. According to the characteristics of pharmacologically isolated glycinerergic and GABAAergic mPSCs, we characterized three types of events based on their decay kinetics and developmental maturation (Fig. 7A): fast (≤15 ms at P1–P5 and ≤10 ms at P9–P15), slow (>15 ms at P1–P5 and >10 ms at P9–P15), and mixed (with an inflection separating fast and slow components). Decay parameters were estimated from decay phases best fitted to monoexponential or biexponential curves (r > 0.9 in ~80–95% fits in different cell groups). However, a population of mPSCs with long decays sometimes displayed superimposed events or other noise fluctuations that prevented good fits (r < 0.7). To avoid underestimation of the number of events in each category, clear mPSCs with random fluctuations in their decays were classified as fast, slow, or mixed by visual inspection and were added to the populations of mPSCs classified by curve fitting. Visually classified events are a small proportion of all of the mPSCs analyzed in each cell group (P1–P5 RCs, 14.8% of all events; P9–P15 RCs, 4.0%; P1–P5 non-RCs, 13.4%; P9–P15, non-RCs, 1.8%). Decay time constants of averaged single fast and slow mPSCs selected in this way were very similar to pharmacologically isolated glycinerergic and GABAAergic currents in each neuronal subgroup (p = 0.1; two-way ANOVA). Moreover, the decay constants of fast (τf) and slow (τs) components of biexponentially decaying events coincided with those obtained from single “fast” and “slow” mPSCs (Fig. 7A, Table 3).

The relative percentage of fast events increased significantly from early to late postnatal ages (Fig. 7B, C) in non-RCs (from 41.9 to 77.5%) and RCs (from 26.7 to 56.3%), in parallel to the developmental increase in the frequency of glycinerergic mPSCs in both cell types (Fig. 6A, 3).

Higher percentage values of fast mPSCs in non-RCs relative to RCs result from greater numbers of mPSCs in non-RCs at all ages. Amplitude distribution histograms and cumulative probability plots of GABAergic mPSCs significantly shifted in older ages toward larger amplitudes in RCs but not in non-RCs. Bin width, 5 pA.

Together, these results suggest significant differences in the functional development of inhibitory synaptic currents in RCs compared with non-RC interneurons (Fig. 6).
events was significantly reduced with age in non-RCs, whereas it remained unchanged in RCs. Finally, the percentage of mixed mPSCs decreased in both neuronal types, although to a larger extent in non-RCs (Fig. 7B, C). The reduction in the number of mixed events in RCs probably derives from the increased frequency of pure fast glycine/Cergic events. Finally, the downregulation of GABAergic currents in non-RCs contributes to reduce further the incidence of dual mPSCs in these neurons compared with RCs.

To further analyze the contribution of fast (likely glycine/Cergic) and slow (likely GABAergic) components to mixed events in RCs and non-RCs, we estimated the relative contribution to absolute peak amplitudes in mixed mPSCs of the fast and slow components (Af, As) (Fig. 7D, Table 3). Af and As were calculated from the parameters of decay curve fittings using the biexponential formula $y = A_0 + A_f e^{-x/\tau_f} + A_s e^{-x/\tau_s}$. As indicated by the Af/As ratios in Figure 7D, the relative contribution of the fast component was similar in RCs and non-RCs at early postnatal ages, accounting for ~60% of the total peak amplitude (Table 3). The relative contribution of fast versus slow components was similar in non-RCs of different ages, whereas Af/As ratios were significantly increased in RCs at later postnatal ages (Fig. 7D) ($p < 0.05$; two-way ANOVA; Duncan’s test). In conclusion, GABAergic mPSCs and GABAergic components in mixed mPSCs were maintained during development to a larger extent in RCs compared with non-RCs. Thus, GABAergic currents are responsible for the retention in RCs of mixed events with slow decays during development, but their contribution to peak amplitudes is reduced in older ages because the large increase in the fast glycine/Cergic currents.

**Charge transfer of GABA/glycine mPSCs in mature ventral horn interneurons**

Although GABAergic components might contribute to peak amplitude less than glycine/Cergic components, their long time courses suggest strong contributions to the total inhibitory synaptic current. We calculated the total charge transferred by glycine/Cergic and GABAergic components in single and dual mPSCs in RCs and non-RCs recorded from P9–P15 animals. Absolute charge transfer associated with glycine/Cergic, GABAergic, or mixed currents recorded from RCs was always larger than for non-RCs (Fig. 8, filled circles (RC), open circles (non-RCs); Tables 1–3). Both peak amplitudes and time courses contributed to increase charge transfer, but the relative contributions of each parameter was different for GABA or glycine mPSCs. Correlations between charge and peak amplitude or decay constant of glycine/Cergic, GABAergic, or dual mPSCs suggest that absolute charge transfer is better related with peak amplitude, but not with decay time, in fast glycine/Cergic mPSCs and with both decay time and peak amplitude in slow GABAergic mPSCs (Fig. 8A1–B2). As a result, in mixed mPSCs, both peak amplitude (dominated by the fast glycine/Cergic current) and time course (dominated by the second decay from the slower GABAergic currents) are both well correlated with peak amplitude (Fig. 8C1–C3).

**Discussion**

Inhibitory synapses on mature RCs are characterized by larger peak amplitudes, slower decays, and higher percentages of GABAergic currents compared with other ventral interneurons. In combination, these characteristics increase inhibitory charge transfer in RCs compared with non-RCs. Given the high density of inhibitory synapses on RCs (Harrington et al., 1994), we conclude that during postnatal development, inhibitory synaptic in.
and anchoring more postsynaptic receptors and increase gephyrin clusters set RCs apart from other neurons (Alvarez et al., 1998; Singer et al., 1998; Smith et al., 2000; Nabekura et al., 1998). Development of specific structural and molecular properties can be explained by the development of specific structural and molecular properties of inhibitory synapses on RCs and appear well adapted to RC function.

**Maturation of postsynaptic inhibitory current peak amplitudes**

The most striking feature of inhibitory synapses on RCs was the large increase in glycinergic mPSC peak amplitudes with development. The magnitude of this postnatal increase is larger than in non-RCs (although the proportion of mixed events decreased), and Af/As ratios increased in RCs. Data are expressed as mean exponential fitted curves (dashed lines) are superimposed on single events or dual-component mPSCs, respectively. Mixed mPSCs were non-RC (top traces) and one RC (bottom traces) at P1–P5 (gray traces) and P9–P15 (black traces). Monoexponential or biexponential decay time constants (green, blue, and red symbols) were similar to the decay time constants of single fast- and slow-decaying events, respectively. Data are expressed as mean percentage \( F \) (for the fast component) and \( S \) (for the slow component). The Af/As ratio was initially similar in RCs and non-RCs. With development, it remained constant in non-RCs (although the proportion of mixed events decreased), and Af/As ratios increased in RCs. Data are expressed as mean \( F \) and \( S \). The asterisk indicates significant differences with \( p < 0.05 \) (two-way ANOVA; Duncan’s test).


puts on RCs become greatly potentiated compared with other ventral interneurons. These functional features can be explained by the development of specific structural and molecular properties of inhibitory synapses on RCs and appear well adapted to RC function.

**Maturation of postsynaptic inhibitory current time course**

The long time courses of GABA \(_{A}\) postsynaptic currents are major contributors to total charge transfer underlying inhibitory currents in RCs and are well explained by the expression of \( \alpha_3/\alpha_5 \)-containing GABA \(_{A}\) receptors (Geiman et al., 2002). Recombinant \( \alpha_3 \)-containing GABA \(_{A}\) receptors are the least sensitive to GABA (Sigel et al., 1990; Verdoorn, 1994), resulting in slower opening, deactivation, inactivation, and postsynaptic current lengthening postsynaptic currents. Indeed, average gephyrin cluster sizes were correlated with average glycinergic mPSC peak amplitudes (Lim et al., 1999; Van Zundert et al., 2004). Modification of postsynaptic receptor properties could also contribute to increased peak amplitude. However, glycinergic receptor properties mature similarly in RC and non-RCs. Similar studies on cranial motoneurons also concluded that receptor accumulation is the major contributor to postnatal increases in average glycinergic mPSC peak amplitude (Singer and Berger, 1999). Thus, the best explanation for the large increase in glycinergic current amplitudes in RCs is higher recruitment of glycine receptors to large postsynaptic gephyrin clusters.

Gephyrin has been also related to postsynaptic GABA \(_{A}\) receptor clustering (Craig et al., 1996; Essrich et al., 1998; Sassoe-Pognetto and Fritschy, 2000); however, GABAergic mPSC peak amplitudes were enhanced to a smaller degree in developing RCs. GABAergic mPSC amplitudes can also increase through accumulation of postsynaptic GABA \(_{A}\) receptors (Nusser et al., 1997, 1998), but a recent report found only weak correlation with gephyrin clustering (Levi et al., 2004). Gephyrin interactions with glycine and GABA \(_{A}\) receptors suggest a tighter relationship between gephyrin clustering and glycine receptors than with GABA \(_{A}\) receptors (Meyer et al., 1995; Meier et al., 2000, 2001; Dahan et al., 2003; Hanus et al., 2004). Gephyrin disruption causes complete declustering of glycine receptors (Kirsch et al., 1993; Feng et al., 1998) and loss of GABA \(_{A}\) receptors (Essrich et al., 1998; Kneussel et al., 1999), but significant gephyrin-independent GABA \(_{A}\) receptor clusters remain (Fischer et al., 2000; Kneussel et al., 2001; Levi et al., 2004). Moreover, during early synapse formation, postsynaptic GABA \(_{A}\) receptors cluster postsynaptically before gephyrin (Dumoulin et al., 2000; Danglot et al., 2003; Levi et al., 2004). Thus, although gephyrin contributes to stabilize GABA \(_{A}\) receptors at postsynaptic densities (Danglot et al., 2003), it is not always necessary for GABA \(_{A}\) receptor postsynaptic recruitment and clustering. In addition, GABA \(_{A}\) receptors are best stabilized by a gephyrin splice isoform containing the C5 cassette (Meier and Granry, 2004); therefore, postsynaptic GABA \(_{A}\) receptor numbers and currents might be best related to the relative abundance of specific gephyrin isoforms than to overall gephyrin content.

**Maturation of postsynaptic inhibitory current time course**

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Table 3. Properties of mixed mPSCs

<table>
<thead>
<tr>
<th>Mixed mPSC parameters</th>
<th>n</th>
<th>( \Delta A ) (percentage of peak)</th>
<th>( \Delta A ) (percentage of peak)</th>
<th>( \tau_c ) (ms)</th>
<th>( \tau_c ) (ms)</th>
<th>Frequency (Hz)</th>
<th>Q (pC)</th>
</tr>
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<tbody>
<tr>
<td>RCs</td>
<td></td>
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<tr>
<td>P1–P5 (n = 11)</td>
<td>11</td>
<td>( 58 \pm 4 )</td>
<td>( 42 \pm 4 )</td>
<td>( 5.8 \pm 0.3 )</td>
<td>( 65.5 \pm 5.1 )</td>
<td>( 0.32 \pm 0.08 )</td>
<td>( 1.72 \pm 0.19 )</td>
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<tr>
<td>P9–P15 (n = 12)</td>
<td>12</td>
<td>( 69 \pm 2^* )</td>
<td>( 31 \pm 2^* )</td>
<td>( 3.5 \pm 0.2^* )</td>
<td>( 42.6 \pm 1.8^* )</td>
<td>( 0.24 \pm 0.04 )</td>
<td>( 1.95 \pm 0.17 )</td>
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<tr>
<td>Non-RCs</td>
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<tr>
<td>P1–P5 (n = 10)</td>
<td>10</td>
<td>( 60 \pm 3 )</td>
<td>( 40 \pm 3 )</td>
<td>( 6.2 \pm 0.3 )</td>
<td>( 33.2 \pm 2.3^* )</td>
<td>( 0.30 \pm 0.05 )</td>
<td>( 1.02 \pm 0.11^* )</td>
</tr>
<tr>
<td>P9–P15 (n = 10)</td>
<td>10</td>
<td>( 61 \pm 1^* )</td>
<td>( 39 \pm 1^* )</td>
<td>( 3.9 \pm 0.2^* )</td>
<td>( 15.8 \pm 0.9^* )</td>
<td>( 0.19 \pm 0.04 )</td>
<td>( 0.64 \pm 0.23^* )</td>
</tr>
</tbody>
</table>

Values are mean \( \pm S.E. \). The asterisks indicate significant differences (p < 0.05; two-way ANOVA, Duncan’s multiple range test) between age groups; the § symbols indicate significant differences between RCs and non-RCs of the same age.

(Gingrich et al., 1995; Serafini et al., 1998). Decay time constant and total charge transfer in RC GABAergic mPSCs was strikingly similar to those reported in reticular thalamic (Rt) neurons (Browne et al., 2001), another neuron that expresses \( \alpha_3/\alpha_5 \) GABA\(_A\) receptors. Interestingly, Rt neurons and RCs have common functional features: both provide recurrent negative feedback and respond to excitatory synaptic inputs with high-frequency burst firing. The properties of \( \alpha_3/\alpha_5 \)-containing GABA\(_A\) receptors might be well adapted to the common integrative properties exhibited by these cells.

Slow-decaying GABA\(_A\) currents were good predictors of whether or not recorded neurons would display anatomical features of RCs. In addition, GABAergic currents decayed 25% faster in mature RCs compared with immature RCs. A parallel speeding of GABA\(_A\) currents was found for the faster decays of non-RCs. These changes could suggest developmentally regulated alterations in GABA\(_A\) receptor subunit structure affecting channel kinetics. Alternatively, they could be explained by alterations in receptor localization (Chery and De Koninck, 1999), \( \beta \) subunit phosphorylation (Hinkle and MacDonald, 2003), or GABA reuptake maturation (Draguhn and Heinemann, 1996) (but see Chery and De Koninck, 1999; Nusser and Mody, 2002; Overstreet and Westbrook, 2003). Our immunocytochemical data suggest that GABA\(_A\) receptors in RCs are highly clustered at synapses in adults and neonates (Geiman et al., 2002; T. Culberston and F. J. Alvarez, unpublished observations), indicating that major developmental changes in receptor localization in RCs are unlikely. Our data also suggests that GABA\(_A\) receptors in RCs contain \( \beta_3 \) subunits (Geiman et al., 2002). Protein kinase A-dependent phosphorylation of \( \beta_3 \) subunits slows down GABA\(_A\) receptor deactivation and prolongs postsynaptic currents (Hinkle and MacDonald, 2003). In addition, neurosteroid prolongation of GABAergic currents is potent on \( \beta_2/3 \)-containing GABA\(_A\) receptors (Wingrove et al., 1994). In the spinal cord, neurosteroid modulation weakens with age (Keller et al., 2004), and this decline is perhaps partly responsible for the acceleration of GABAergic currents.

A reduction in glycinergic current decay time was also observed in both RCs and non-RCs during development. A switch from neonatal \( \alpha_2 \)- to adult \( \alpha_1 \)-containing glycinic receptors occurs during the first two postnatal weeks in the rat spinal cord (Akagi and Miledi, 1988; Becker et al., 1988; Malosio et al., 1991) and correlates with a decrease in glycine receptor mean open time and PSC decays (Takahashi et al., 1992; Singer et al., 1998). Decay parameters measured at P1–P5 were faster than those reported in neurons with predominant expression of \( \alpha_2 \) glycine receptors and similar to neurons at intermediate developmental stages expressing both \( \alpha_1 \) and \( \alpha_2 \) glycine receptors (Singer et al., 1998; Van Zundert et al., 2004). Previously, we detected significant amounts of \( \alpha_1 \) glycine receptors in RCs just after birth (Geiman et al., 2000). Therefore, glycinergic decay acceleration in RCs is best explained by progressive downregulation of neonatal \( \alpha_2 \) subunits.

### Maturation of cotransmission

GABA and glycine corelease is generally demonstrated by spontaneous mPSCs displaying biphasic decays with partial time constants similar to those of GABAergic and glycinergic mPSCs. Because mPSCs are interpreted as the postsynaptic action of neurotransmitter released from single vesicles, the presence of...
mixed mPSCs suggests GABA and glycine corelease from individu-
al vesicles (Jonas et al., 1998), a possibility also supported by the
use of a common vesicular transporter (Sagne et al., 1997;
Chaudry et al., 1998) (but see Katsurabayashi et al., 2004). Biaph-
sic mPSCs indicative of corelease have been shown at synapses in
the spinal cord (Jonas et al., 1998; Chery and De Koninck, 1999;
Gao et al., 2001; Keller et al., 2001), cerebellum (Dumoulin et al.,
2001), and brainstem (O’Brien and Berger, 1999; Russier et al.,
2002; Nabekura et al., 2004). A postnatal decrease in the level of
cotransmission was reported for many neurons, and it is usually
associated with downregulation of GABAergic currents and a
switch to mainly glycine-mediated inhibitory synaptic mecha-
nisms (Kotak et al., 1998; Gao et al., 2001; Keller et al., 2001;
Nabekura et al., 2004). Most ventral interneurons show a similar
developmental pattern; however, RCs were different because of
the remarkable maintenance of GABAergic and mixed currents.
Correspondingly, morphological data indicate that 60–75% of
inhibitory terminals on adult RCs coexpress presynaptic and
postsynaptic markers suggesting mixed neurotransmission, a
percentage higher than in other ventral horn synapses (Geiman et
al., 2002). The mechanisms that regulate the amount of cotra-
sm mission are unknown (van den Pol, 2004), but the choice has
important consequences on the timing and strength of synaptic
inhibition. High levels of cotransmission over RCs enhance in-
hibitory current strength by permitting large peak amplitudes
while maintaining very slow time courses. Russier et al. (2002)
showed that inhibitory synapses decreased motoneuron firing
more efficiently when GABAergic and glycinergic transmission
occurred simultaneously compared with experimental situations
in which one or other component was blocked. Cotransmission
also allowed inhibition with complementary time courses, tran-
sient by glycine and sustained by GABA.

Inhibitory modulation of RC function

The functional properties of inhibitory synaptic currents in RCs
increase synaptic inhibition in amplitude and duration and de-
velop in parallel to the spinal circuitry underlying locomotion. In
the adult spinal cord, RC-mediated recurrent inhibition modu-
lates the dynamic behavior of motoneuron firing by acting as a
gain regulator of their discharge and recruitment (Windhorst,
1996; Uchiyama et al., 2003; Hultborn et al., 2004). The fine
organization of recurrent inhibition on different motoneuron
types and pools allows RCs to influence motoneuron firing vari-
rability, force gain, and motor discharge synchronization in hom-
onymous and synergistic motoneuron pools and muscles. RCs
also facilitate activity in antagonists through regulation of recip-

cr al Ia inhibitory interneurons (Hultborn et al., 1979). Thus,
despite the relative simplicity of the recurrent inhibitory loop,
RCs exert a variety of complex functions during motor activity.
Not surprisingly recurrent inhibition is finely modulated during dif-
erent motor behaviors. For example, the level of recurrent inhibi-
tion changes during voluntary movements according to the strength
of muscle contraction and motor task (Hultborn and Pierrot-
Deselligny, 1979; Katz and Pierrot-Deselligny, 1999; Iles et al.,
2000). This modulation implies the existence of effective mecha-
nisms to decouple RC firing from motor axon inputs. However, RCs
receive long-lasting cholinergic EPSPs from motor axons and dis-
play unusual low thresholds for action potential generation. These
peculiarities result in the characteristic tendency of RCs to discharge
bursts of spikes in response to incoming motor axon volleys, even
when only one action potential travels down a single motor axon (for
review, see Windhorst, 1990). Modulation of RC activity therefore
requires complementary well-matched inhibition. Inhibitory syn-
apses on RCs are strategically located more proximal to the cell body
than cholinergic synapses (Alvarez et al., 1999) and their synaptic
structure (Alvarez et al., 1997) and molecular/neurochemical orga-
nization (Geiman et al., 2002) seem to correlate with the large am-
litude and relatively long time courses of inhibitory currents de-
cscribed here.

In conclusion, the functional, structural, and molecular char-
acteristics of inhibitory synapses on RCs appear well adapted to
counteract robust and long-lasting motor axon-driven excitatory
inputs in RCs and allow effective modulation of RC activity and
recurrent inhibition of motoneurons.

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