Single Granule Cells Excite Golgi Cells and Evoke Feedback Inhibition in the Cochlear Nucleus

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In cerebellum-like circuits, synapses from thousands of granule cells converge onto principal cells. This fact, combined with theoretical considerations, has led to the concept that granule cells encode afferent input as a population and that spiking in individual granule cells is relatively unimportant. However, granule cells also provide excitatory input to Golgi cells, each of which provide inhibition to hundreds of granule cells. We investigated whether spiking in individual granule cells could recruit Golgi cells and thereby trigger widespread inhibition in slices of mouse cochlear nucleus. Using paired whole-cell patch-clamp recordings, trains of action potentials at 100 Hz in single granule cells was sufficient to evoke spikes in Golgi cells in ~40% of paired granule-to-Golgi cell recordings. High-frequency spiking in single granule cells evoked IPSCs in ~5% of neighboring granule cells, indicating that bursts of activity in single granule cells can recruit feedback inhibition from Golgi cells. Moreover, IPSPs mediated by single Golgi cell action potentials paused granule cell firing, suggesting that inhibitory events recruited by activity in single granule cells were able to control granule cell firing. These results suggest a previously unappreciated relationship between population coding and bursting in single granule cells by which spiking in a small number of granule cells may have an impact on the activity of a much larger number of granule cells.

Key words: auditory; cerebellum; inhibition; microcircuits

Introduction

Cerebellar cortex and cerebellum-like circuits contain an abundance of granule cells. Granule cells make excitatory synapses onto principal cells in these circuits that are too weak to individually impact principal cell firing (Barbour, 1993; Brunel et al., 2004; Roberts and Trussell, 2010). Furthermore, theoretical studies emphasizing the role of principal cells as pattern learning devices highlight the importance of population coding by granule cells (Marr, 1969; Albus, 1971; Liu and Regehr, 2014). For these reasons, granule cells are typically thought to encode mossy fiber input as a population, with individual granule cells being dispensable for the overall function of the circuit (Arenz et al., 2009; Galliano et al., 2013b). However, granule cells make excitatory synapses with Golgi cells, inhibitory interneurons that feedback onto granule cells (Dugué et al., 2005; Balakrishnan et al., 2009). Golgi cells also receive excitatory input from mossy fibers (Kanichay and Silver, 2008; Cesana et al., 2013; see Fig. 1A for

circuit diagram), but the granule-to-Golgi cell synapses are typically considered too weak to excite Golgi cells (Dieudonné, 1998; Xu and Edgley, 2008; Prsa et al., 2009). However, recent evidence suggests that the ascending axons of granule cells makes synapses onto Golgi cells that are nearly as strong as, and many times more numerous than, mossy fiber synapses onto Golgi cells (Cesana et al., 2013). Granule cell synapses onto Golgi cells are also known to undergo potent short-term synaptic facilitation (Beierlein et al., 2007), raising the possibility that bursts of spikes in individual granule cells may provide suprathreshold excitation to Golgi cells. Due to the divergence of Golgi cell axons to hundreds of granule cells (Eccles et al., 1967), spiking in single granule cells may evoke inhibition in a large population of granule cells.

We used paired recordings to address these questions. However, paired recordings are only feasible in brain areas where connection probabilities between cells are sufficiently high to gather an interpretable dataset. Indeed, in the relatively compact granule-Golgi cell network of the cerebellum-like regions of the mouse cochlear nucleus (Oertel and Young, 2004), we now report a Golgi-to-granule connection probability of 38% and a granule-to-Golgi connection probability of 33%, 1.5–3 times the corresponding values reported in the cerebellum (Crowley et al., 2009; Cesana et al., 2013). In connected granule-to-Golgi cell pairs, bursts of 10 action potentials at 100 Hz evoked long-latency Golgi cell action potentials in ~40% of granule-to-Golgi pairs. To test whether spiking in granule cells could evoke IPSCs, a burst of spikes was evoked in one granule cell, which resulted in IPSCs in the same cell or in a simultaneously recorded granule cell in 5–6% of recordings. Paired Golgi-to-granule recordings showed that single unitary IPSPs could pause granule cell firing. Compu-

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tational modeling suggested that the duration of inhibition of granule cell spiking increased with the number of bursting granule cells. Together, these results suggest that spiking of individual granule cells can recruit Golgi cells to deliver inhibition to a large number of granule cells.

Materials and Methods

Animals. Postnatal day 16 (P16)–P24 wild-type (C57Bl/6) or *IG17* homozygous or heterozygous transgenic mice were used for electrophysiological experiments. The *IG17* line expresses GFP fused to the human interleukin-2 receptor α subunit under control of the promoter for metabotropic glutamate receptor subtype 2 (mGluR2) gene (Watanabe et al., 1998; Watanabe and Nakanishi, 2003). Golgi cells are the only inhibitory cell type in cochlear nucleus expressing GFP in *IG17* mice (Irie et al., 2006). For immunostaining (see Fig. 1B,C), *IG17*^{+/−} mice were bred to *GABA_AR- α 6-Cre*^{+/−} mice (Fünfschilling and Reichardt, 2002) to generate *IG17*^{+/−}/*GABA_AR- α 6-Cre*^{+/−} mice. Granule cells are the only cochlear nucleus cells expressing Cre recombinase in *GABA_AR- α 6-Cre*^{+/−} mice (Fünfschilling and Reichardt, 2002). *IG17*^{+/−}/*GABA_AR- α 6-Cre*^{+/−} mice were then crossed to *Ai9*^{+/+} mice (Madisen et al., 2010) to generate *IG17*^{+/−}/*GABA_AR- α 6-Cre*^{+/−}/*Ai9*^{+/+} mice. One *IG17*^{+/−}/*GABA_AR- α 6-Cre*^{+/−}/*Ai9*^{+/+} P24 mouse was used for immunostaining. Male or female mice were used in all experiments. All experimental procedures involving animals were approved by the Oregon Health and Science University Institutional Animal Care and Use Committee.

Immunohistochemistry. One mouse was anesthetized with isoflurane and the brain was removed after transcardial perfusion with 6.7 mM PBS, pH 7.4, and subsequent perfusion with 4% paraformaldehyde in PBS (w/v). The brain was kept in 4% paraformaldehyde in PBS overnight. The brain was embedded in 4% agar in PBS (w/v) and sliced into 40 μ m coronal sections the following day using a vibratome (Leica VT1000S). The slices were then incubated for 1 h in a blocking solution consisting of 2% (w/v) goat serum, 0.3% (w/v) bovine serum albumin, and 0.2% (v/v) Triton X-100 in PBS. The slices were incubated overnight in blocking solution containing Alexa Fluor 488-conjugated anti-GFP antibody (10 μ g/ml; Invitrogen). The following day, slices were washed in PBS, mounted, and imaged using confocal microscopy.

Slice preparation. After anesthesia, mice were decapitated and coronal brain slices (300 μ m) containing cochlear nucleus were cut in either warm (34°C) standard artificial CSF (ACSF) or K-gluconate-based solution (Dugué et al., 2005; Dugué et al., 2009). Standard ACSF contained the following (in mM): 130 NaCl, 2.1 KCl, 1.2 KH₂PO₄, 3–6 HEPES, 1 MgSO₄, 1.7 CaCl₂, 10 glucose, and 20 NaHCO₃ (bubbled with 95% O₂/5% CO₂; ~305 mosm). The K-gluconate cutting solution contained the following (in mM): 130 K-gluconate, 15 KCl, 0.5–2 EGTA, 20 HEPES, and 25 glucose, ~320 mOsm, and pH adjusted to 7.4 with NaOH. Two to 5 μ M 3-[(R)-2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid [(R)-CPP] and/or 50 nM minocycline was routinely added to cutting solutions to increase slice viability (Rousseau et al., 2012). Slices were incubated in 34°C ACSF for 15–30 min after slicing and then stored at room temperature until recording. All recordings were performed in the standard ACSF solution.

Electrophysiological recording. Slices were transferred to a recording chamber on the stage of an upright microscope (Zeiss Examiner.D1) and perfused continuously with ACSF using a peristaltic pump (Gilson Minipulse 3). Bath temperature was maintained at 34–36°C by an inline heater (Warner Instrument TC-324B). Cells were visualized with a 40× objective lens with Dodt gradient contrast optics using a Sony XC-ST30 infrared camera. GFP-positive cells were visualized using epifluorescence optics and a custom-built LED excitation source.

Current and voltage-clamp recordings were made with a K-gluconate-based internal solution containing the following (in mM): 113 K-gluconate, 2.75 MgCl₂, 1.75 MgSO₄, 9 HEPES, 0.1 EGTA, 14 Tris₂-phosphocreatine, 4 Na₂-ATP, 0.3 Tris-GTP, osmolarity adjusted to ~295 mOsm with sucrose and pH adjusted to 7.25 with KOH. All reported membrane values recorded with the K-gluconate-based internal solution were corrected offline for a −10 mV junction potential. In Figure 5, E and F, a KCl-based solution was made by exchanging the

K-gluconate for KCl and used to record from granule cells. For IV curves (see Fig. 2B,C) and extracellular stimulation of Golgi axons (see Fig. 3F), voltage-clamp recordings were made with a CsCl-based internal solution composed of the following (in mM): 115 CsCl, 4.5 MgCl₂, 8 QX-314-Cl, 10 HEPES, 10 EGTA, 4 Na₂-ATP, and 0.5 Tris-GTP, osmolarity ~295 mOsm and pH adjusted to 7.25 with CsOH. The CsCl-based internal solution had a small junction potential (~2 mV) for which no correction was made. Spermine (100 μ M) was added to the CsCl-based internal for some experiments (see Fig. 2B,C). Patch pipettes were pulled from borosilicate glass (WPI) and open-tip resistances were 3–6 M Ω when filled with internal solution when recording from Golgi cells and 5–11 M Ω when recording from granule cells.

Data acquisition and analysis. Single and dual whole-cell patch-clamp recordings were made using a MultiClamp 700B amplifier using Clampex 9.2 (Molecular Devices). Granule cells were identified based on their small soma size ($\leq 10 \mu$ m), characteristic intrinsic properties (Balakrishnan and Trussell, 2008), and lack of GFP expression when using *IG17* mice. Golgi cells were identified based upon their GFP expression in *IG17* mice, multipolar appearance, medium- to large-sized somas ($\geq 15 \mu$ m), and intrinsic properties (Irie et al., 2006). Whole-cell access resistance was 6–25 M Ω in voltage-clamp recordings from Golgi cells and 12–35 M Ω in voltage-clamp recordings from granule cells. Access resistance was compensated by 70% online. Recordings were acquired at 10–50 kHz and low-passed filtered at 10 kHz using a Digidata 1322A (Molecular Devices).

For paired recordings in which the presynaptic cell was recorded in current clamp, action potentials were evoked in Golgi cells with a 1 ms, 1.2–1.8 nA current injection and in granule cells with a 1 ms, 0.6–0.9 nA current injection. In experiments determining whether single granule cells could evoke Golgi cell spikes in granule-Golgi cell pairs (see Fig. 5A,B), postsynaptic Golgi cells were held to potentials slightly hyperpolarized to the resting potential (-75.2 ± 1.0 mV, $n = 17$) to prevent spontaneous firing because the resting membrane potential of Golgi cells tended to gradually depolarize during prolonged whole-cell recordings (data not shown). When recording postsynaptic currents, Golgi cells were held at −60 to −70 mV and granule cells were held at either −40 or 0 mV. In single voltage-clamp recordings from granule cells examining feedback inhibition (see Fig. 5E,F), action currents were evoked by a 1 ms depolarization to 0 mV from a holding potential of −60 to −70 mV.

In analyzing kinetic data from postsynaptic events in paired recordings, postsynaptic events were aligned at onset using Axograph X and averaged. When analyzing the kinetics of EPSCs in paired recordings between granule and Golgi cells, the EPSC in response to the first granule cell action potential was analyzed whenever possible to avoid changes in EPSC kinetics related to short-term synaptic plasticity at granule cell synapses (Satake and Imoto, 2014). Synaptic latency was calculated by taking the difference between the time of the averaged peak of the presynaptic action potential and the time of the peak of the first derivative of the postsynaptic current (Crowley et al., 2009). EPSCs and IPSCs were fitted with either a monoexponential or biexponential decay function in Clampfit (Molecular Devices) as follows:

$$A_{fast} \times e^{\frac{-T}{\tau_{fast}}} + A_{slow} \times e^{\frac{-T}{\tau_{slow}}} \quad (1)$$

where $T = t - t_0$, and t_0 is the time to which the first point of the fit corresponds. The biexponential fit was considered the best if it reduced the sum of squared errors compared with the mono-exponential fit by more than half.

Chemicals. All drugs were obtained from Sigma-Aldrich except for minocycline-HCl and LY 354740, which were obtained from Tocris Bioscience. All drugs were bath-applied.

Computational modeling. A simplified computational model of the granule-Golgi system was constructed in Neuron (version 7.2; Carnevale and Hines, 2006). A single Golgi cell and 500 granule cells were simulated using published models of cerebellar granule cells and Golgi cells (Solinas et al., 2007; Simões de Souza and De Schutter, 2011). The spontaneous firing of the Golgi cell was silenced with a −25 pA current injection. All granule cells received input from four mossy fibers, which were modeled

Figure 1. The cochlear nucleus granule-Golgi cell network. **A**, Circuitry of granule cell domains in the cochlear nucleus. Excitatory granule cells (Gr; red circles) and inhibitory Golgi cells (Go; green multipolar cell) receive excitatory synaptic input from mossy fibers (blue half circles), giant excitatory terminals. Parallel fibers, the axons of granule cells, make synapses onto the Golgi cell and onto other parallel fiber target neurons ("PF Target"); these include superficial stellate, cartwheel, and fusiform cells in cochlear nucleus. The Golgi cell synapses onto granule cells. **B**, Confocal image of granule cells (TdTTomato⁺, red) and Golgi cells (GFP⁺, green) in a paraformaldehyde-fixed 40 μ m slice of cochlear nucleus. VCN, Ventral cochlear nucleus; CBL, cerebellar cortex; gl, granule cell region; dl, deep layer of DCN; ml, molecular layer of DCN. Cells within deep layer are largely unipolar brush cells (which also express GFP in the *IG17* mouse line; Irie et al., 2006), whereas Golgi cells distribute in the granule cell regions. **C**, Closer view of granule cells and Golgi cells in a granule cell region overlying the VCN. Go, Golgi cell; Gr, granule cell.

as synaptic conductances on the granule cell membrane. The mossy fiber-to-granule cell synapse was modeled using EPSC waveforms, NMDA receptor Mg^{2+} block, and short-term synaptic plasticity parameters from Schwartz et al. (2012). One to 10 granule cells received a burst of mossy fiber input that caused the cells to fire 10.2 ± 0.1 spikes at 109.1 ± 0.7 Hz. All nonbursting granule cells received an inhibitory input from the Golgi cell. A random number was selected from a uniform distribution to specify the number of bursting granule cells receiving feedback inhibition from the Golgi cell in a simulation run such that, on average, half of the bursting granule cells received feedback inhibition.

Only the bursting granule cells synapsed onto the Golgi cell and the synapse was placed onto the soma of the multicompartmental Golgi cell model (Solinas et al., 2007) in accordance with the fast rise times of EPSPs in paired granule-to-Golgi cell recordings (20–80% rise time: 0.67 ± 0.08 ms, $n = 10$). Experimental data from paired recordings was used to fit synaptic conductance waveforms and short-term synaptic plasticity. Synaptic conductance waveforms were modeled as being of the form:

$$G(t) = \text{weight} \times \frac{1}{a_{\text{norm}}} \times \left(1 - e^{-\frac{T}{\tau_{\text{rise}}}}\right)^n \times \left(d_1 e^{-\frac{T}{\tau_{d1}}} + d_2 e^{-\frac{T}{\tau_{d2}}}\right) \quad (2)$$

where *weight* is the synaptic strength, $T = t - t_{\text{event}}$, $T \geq 0$; a_{norm} is the peak amplitude of the waveform; τ_{rise} is the time constant of the rising phase; d_{1-2} are the weighted percentages that each of the slow decay time constants contribute to the decay; and τ_{d1-2} are decay time constants (Rothman and Silver, 2014). Granule-to-Golgi cell EPSCs and Golgi-to-granule cell IPSCs from paired recordings were fit to Equation 2. For the Golgi-to-granule cell inhibitory synapse, *weight* = 1.14 nS, $n = 3$, τ_{rise} = 0.27 ms, d_1 = 60.99%, τ_{d1} = 2.66 ms, d_2 = 39.01%, and τ_{d2} = 13.56 ms. For the granule-to-Golgi cell excitatory synapse, *weight* = 1.1 nS, $n = 2$, τ_{rise} = 0.17 ms, d_1 = 100%, and τ_{d1} = 0.66 ms.

Weight for the granule-to-Golgi cell synapse was set so that the average latency to the first spike for the model Golgi cell when stimulated with a single granule cell input at 100 Hz was 57.9 ms after the start of the granule cell spike train, similar to the mean latency to the first IPSC observed in dual and single granule cell recordings (58.7 ms; see Fig. 5C,E). Inhibitory synaptic weight was set to the corresponding conductance of the average IPSC in paired Golgi-to-granule cell recordings. In Figure 6D, the synaptic latency and weight of the Golgi-to-granule cell synapses were randomized by fitting experimentally observed distributions of synaptic weight and latency to probability density functions and assigning values probabilistically in simulations. Synaptic latencies were well fit by a normal distribution with a mean of 0.92 ms and an SD of 0.23 ms. Synaptic weights were fit with a log-normal distribution with a mean of 1.14 nS and an SD of 1.1 nS. The Varela et al. (1997) model of short-term synaptic plasticity was used to model Golgi-to-granule and granule-to-Golgi cell synapses. In this model, the peak amplitude of the i^{th}

postsynaptic current (PSC) resulting from the i^{th} presynaptic action potential is the product of the peak amplitude of the initial PSC (*weight*), a depression variable (*D*), and a facilitation variable (*F*) as follows:

$$PSC_i = \text{weight} \times D_i \times F_i \quad (3)$$

After each presynaptic action potential, *D* is multiplied by a constant factor *d*, where $d < 1$. *F* is increased by a constant factor *f*, where $f > 1$. Both *D* and *F* decay back to 1 according to recovery time constants τ_D and τ_F , respectively. For the Golgi-to-granule cell synapse, $d = 0.81$, $\tau_D = 132$ ms. For the granule-to-Golgi cell synapse, $d = 0.73$, $\tau_D = 60.9$ ms, $f = 1.99$, and $\tau_F = 38$ ms.

Simulations were run 20–100 times and the spike times of the nonbursting granule cells were binned into 1 or 2 ms bins for each run. The bin counts were averaged for the 50 ms preceding the first Golgi cell spike and a *t* test was used to compare the bin counts after the Golgi spike with the bin counts during the pre-

Golgi spike control period (Roberts and Trussell, 2010). The duration of inhibition was considered as the length of time after the onset of the IPSP for which the counts in contiguous bins were significantly different from the control period. For simulations in which 3–4 granule cells were bursting, there were 2 periods of inhibition separated by periods of 10 ms or more during which the bin count was not significantly different than the control period; in these cases, only the first period of inhibition was plotted in Figure 6D.

Statistics. All averages are reported as mean $\pm$ SEM.

Results

Golgi cells in the cochlear nucleus

To determine whether single granule cells can provide suprathreshold excitation to Golgi cells, it was necessary to perform paired recordings between granule and Golgi cells. Although such recordings have been made in the cerebellar cortex (Vervaeke et al., 2012; Cesana et al., 2013), they have never been reported in the cochlear nucleus. Golgi cells were identified for whole-cell recording based upon their expression of GFP in the *IG17* mouse line (Watanabe et al., 1998; Watanabe and Nakaniishi, 2003; Irie et al., 2006), in which GFP-tagged human interleukin-2 receptor α subunit is expressed under the control of the mGluR2 promoter.

The spatial relationship between Golgi and granule cells was examined using triply transgenic mice in which granule cells express tdTomato and Golgi cells express GFP (Fig. 1B,C; see Materials and Methods). In fixed thin slices from this mouse, granule cells appeared to cluster around Golgi cells with their somas within 10–40 μ m of the Golgi cell soma. Because connection probability typically decreases with distance (Levy and Reyes, 2012), we targeted these granule cells located near the Golgi cell soma for paired recordings between granule and Golgi cells.

Synaptic properties of unitary granule-to-Golgi cell inputs

Action potentials (APs) in granule cells triggered EPSCs in Golgi cells in 75 of 227 dual recordings, corresponding to a connection probability of 33%. Under voltage clamp at -60 mV, these EPSCs were fully blocked by the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) antagonist GYKI 53665 (20 μ M; Paternain et al., 1995; Fig. 2A; $98 \pm 1\%$ block, $n = 3$ pairs), indicating that EPSCs recorded near resting potentials were mediated exclusively by AMPARs. *N*-methyl-D-aspartic acid

receptor (NMDAR)-mediated EPSCs could be evoked by single granule cell APs at positive holding potentials, but the ratio of the NMDAR to the AMPAR EPSCs was low even at a holding potential of +60 mV (0.12 ± 0.03 ; $n = 9$ pairs).

AMPA-mediated EPSCs had rapid monoexponential or biexponential decay kinetics (Table 1). The current–voltage relationship of AMPAR-mediated EPSCs showed rectification when 100 μ M spermine was included in the Golgi cell intracellular solution (Fig. 2*B, C*), but not when it was omitted from the intracellular solution (Fig. 2*C*). The difference in the IV relations between the spermine-containing and spermine-free recording conditions was significant at a holding potential of +60 mV ($p < 0.05$, unpaired t test). These results indicate that EPSCs are mediated, at least partly, by Ca^{2+} -permeable AMPARs (Gardner et al., 2001; Liu and Cull-Candy, 2002), as in the cerebellum (but see Menuz et al., 2008; Cesana et al., 2013). The results also support the hypothesis that Ca^{2+} permeability of AMPARs is target dependent in cochlear nucleus because stellate cell target AMPARs are also Ca^{2+} permeable (Apostolides and Trussell, 2014) whereas cartwheel and fusiform cell target AMPARs are not (Gardner et al., 1999, 2001).

The granule-to-Golgi cell synapse showed prominent short-term facilitation in response to high-frequency trains of granule cell APs (5 APs, 90–100 Hz: EPSC5/EPSC1 ratio = 2.56 ± 0.27 , $n = 16$; Fig. 2*D, F*). Trains of granule cell APs at 10 Hz did not produce short-term facilitation of EPSCs (EPSC5/EPSC1 ratio = 1.10 ± 0.10 , $n = 6$). During the high-frequency train, failures to release were readily apparent and appeared to become less frequent for later stimuli in the train (Fig. 2*E*). To examine the change in release probability during facilitation, the probability of synaptic failures F was measured during the train and plotted as $1 - F$ (Fig. 2*F*). These data show that facilitation increased in exact proportion to the decline in synaptic failures when stimulating at high frequency, indicating that facilitation may be accounted for solely by an increase in release probability, as opposed to a postsynaptic effect.

Synaptic properties of unitary Golgi-to-granule cell inputs

We next examined the synaptic properties of unitary Golgi-to-granule cell inputs. Golgi cell spikes evoked postsynaptic responses in granule cells in 43 of 110 dual recordings, yielding a connection probability of 38%. Golgi cell spikes evoked IPSCs in granule cells that showed a variable degree of block by 5 μ M the GABA_A receptor antagonist SR 95531 (range 94% to –3% block; average of $50 \pm 18\%$ block, $n = 6$), indicating a variable contribution of GABA to synaptic transmission in these cell pairs. Figure 3*A* shows an example pair in which most of the IPSC was blocked by SR 95531. Figure 3*B* shows a different pair in which the IPSC was insensitive to SR 95531 but was abolished by 1 μ M of the glycine receptor antagonist strychnine. Therefore, our results indicate that Golgi cells inhibit granule cells by releasing GABA and/or glycine. Evoked IPSCs showed failure rates of 15% (Table

Figure 2. Properties of AMPAR-mediated EPSCs at the granule-to-Golgi cell synapse. *A*, EPSC evoked in Golgi cell (bottom) by AP in presynaptic granule cell (top) was blocked by 20 μ M GYKI 53665 (red trace). *B*, AMPAR-mediated EPSC isolated by bath application of R-CPP, SR 95531, and strychnine obtained with 100 μ M spermine included in the recording pipette. Golgi cell was held at -60 , -30 , 0 , 30 , and 60 mV. *C*, Population IV curve for AMPAR-mediated EPSCs with EPSC amplitudes normalized to the amplitude at -60 mV. Black circles are averages for pairs in which spermine was not included in the presynaptic intracellular solution ($n = 6$). Red triangles are averages for pairs for which 100 μ M spermine was included in the presynaptic recording pipette ($n = 7$). Asterisk indicates that normalized current amplitudes are significantly different between the two recording conditions at $+60$ mV ($p < 0.05$, unpaired t test). The IV curve with spermine shows rectification, indicating that at least some of the AMPARs at this synapse are Ca^{2+} permeable. *D*, 90 Hz AP train in the presynaptic granule cell (top) evoked EPSCs in the postsynaptic Golgi cell (bottom). The average of several trials is shown in black and the individual trials are shown in gray. *E*, Expanded view of first and fifth stimuli in data from *D*. Synaptic failures are readily apparent in the first set of responses. *F*, Facilitation of EPSC during granule cell AP trains at 90–100 Hz ($n = 16$). Circles show normalized peak current of the EPSC. On the same plot is EPSC probability (triangles, $1 - F$ = synaptic failure frequency), which matches exactly the average increase in EPSC amplitude ($n = 16$).

1), suggesting that Golgi cells mediate reliable inhibition of granule cells.

Indeed, when postsynaptic granule cells were made to fire through depolarizing current injection, a single AP in the presynaptic Golgi cell led to an IPSP and a cessation in granule cell firing (Fig. 3*C*). The granule cell interspike interval increased significantly from 17.0 ± 3.7 ms to 37.5 ± 7.8 ms after the Golgi cell AP ($n = 8$; paired t test, $p = 0.01$). Granule cell spikes from 8 pairs were sorted into 1 ms bins and summed together and are shown in Figure 3*D*, revealing that single Golgi cell APs reduced granule cell firing for ~ 25 ms. As expected for a reliable synapse with a low rate of synaptic failure, IPSCs evoked by trains of Golgi cell APs depressed (10 APs, 100 Hz: IPSC10/IPSC1 ratio = 0.33 ± 0.04 , $n = 10$; Fig. 3*E*), consistent with a high release probability at this synapse (Zucker and Regehr, 2002).

Golgi cells are the only source of inhibition onto granule cells

Inhibition of cochlear nucleus granule cells has not been extensively studied and it is not clear whether Golgi cells are the only source of inhibition onto granule cells (Alibardi, 2002; Alibardi, 2003). To determine whether Golgi cells are the sole source of inhibition to granule cells, as in the cerebellum (Eccles et al., 1967; Hamann et al., 2002), inhibitory inputs to granule cells were stimulated extracellularly in the presence of glutamate receptor antagonists. Because Golgi cells are the only inhibitory cells in the cochlear nucleus that express the mGluR2 or mGluR3 receptor (Jaarsma et al., 1998; Irie et al., 2006) and activation of mGluR2 receptors on Golgi cell axon terminals results in a reduction of release probability (Mitchell and Silver, 2000), it was rea-

Table 1. Synaptic properties of unitary Golgi-to-granule and granule-to-Golgi synapses

Connection (no. of pairs)	Synaptic strength (nS)	Success amplitude (nS)	Failure rate	Synaptic delay (ms)	Rise time (10–90%; ms)	τ_{fast} (ms)	τ_{slow} (ms)	% fast	Weighted τ (ms)
Granule-to-Golgi (21)	0.24 ± 0.04	0.77 ± 0.07	0.71 ± 0.03	0.89 ± 0.04	0.33 ± 0.02	0.39 ± 0.05	1.73 ± 0.33	71.55 ± 5.95	0.85 ± 0.045
Golgi-to-granule (29)	1.32 ± 0.21	1.44 ± 0.20	0.15 ± 0.03	0.98 ± 0.04	0.68 ± 0.05	6.03 ± 0.69	31.69 ± 3.46	65.61 ± 3.13	14.90 ± 1.38

For granule-to-Golgi synaptic parameters, there were 15 pairs in which EPSCs were best fit by a single exponential and 6 pairs in which EPSCs were best fit by a double exponential. τ_{fast} and τ_{slow} refer to fits for the pairs with bi-exponential decay, whereas *Weighted τ* includes mono-exponential fits and weighted bi-exponential fits.

Figure 3. Properties of Golgi-to-granule cell inhibitory synapses. **A**, IPSC evoked in the postsynaptic granule cell by presynaptic Golgi cell AP. Top, Individual traces (gray) are shown along with the average (black). Bottom, IPSCs were reduced in amplitude by bath application of 5 μM SR 95531 (individual traces in light red and average in darker red). **B**, IPSC evoked in a different paired recording between a presynaptic Golgi cell and granule cell. Top, Individual traces (gray) are shown along with the average (black). Bottom, SR 95531 (5 μM) failed to block the IPSC (individual traces in red and average in darker red), but subsequent addition of 1 μM strychnine fully blocked the IPSC (average in blue). **C**, Granule cell (bottom) was depolarized with current injection to evoke spiking, and a single AP was evoked in a presynaptic Golgi cell (top). Golgi cell AP evoked an IPSP in the granule cell that caused a pause in spiking. **D**, Histogram showing binned and summed granule cell spikes for 10 trials each in 8 pairs. Red vertical bar indicates the time of the peak of the Golgi cell AP (at 0 ms). **E**, A 100 Hz train of APs in a presynaptic Golgi cell (top) evoked depressing IPSCs in the postsynaptic granule cell (bottom; individual traces in gray and average in black). **F**, Extracellular stimulation of inhibitory inputs to granule cell isolated in the presence of NBQX and R-CPP evoked an IPSC that was almost completely blocked by a 300 nM concentration of the mGluR2/3 agonist LY 354740, suggesting that all inhibitory inputs to granule cells express mGluR2/3 receptors. Arrow indicates the timing of stimulation. Stimulus artifact has been removed for clarity. Note that IPSC is inward due to the use of a high- Cl^- intracellular solution (see Materials and Methods).

soned that if an mGluR2/3 receptor agonist reduced the evoked IPSC amplitude, this would strongly suggest that Golgi cells are the primary source of inhibition to cochlear nucleus granule cells. Bath application of 300 nM to 1 μM concentrations of the mGluR2/3 agonist LY354740 blocked $92 \pm 4\%$ of the IPSC ($n = 5$; Fig. 3F). These results suggest that all inhibitory inputs to granule cells are mGluR2-expressing Golgi cells. This conclusion allowed for estimation of the average number of Golgi cells synapsing onto a granule cell by dividing the inhibitory synaptic conductance using strong extracellular fiber stimulation (5.04 ± 2.37 nS, $n = 13$) by the unitary inhibitory synaptic conductance obtained in paired recordings (1.14 ± 0.18 nS, $n = 35$), which indicates that at least 5 Golgi cells contact each granule cell.

Reciprocally connected granule-Golgi cell pairs

Of 98 dual recordings between granule and Golgi cells in which we were able to test for both inhibitory and excitatory connections, 13 pairs were connected reciprocally (13% of dual recordings). The percentage of reciprocally connected granule-Golgi cell pairs we observed is equal to the product of the excitatory and inhibitory connection probabilities ($0.33 \times 0.38 = 0.13$), suggesting that the reciprocal connections occurred at the frequency expected given the excitatory and inhibitory connection proba-

bilities. An example reciprocal pair is shown in Figure 4. Spiking in the granule cell evoked EPSPs in the Golgi cell (onset of EPSPs denoted by asterisk in Fig. 4A) and a train of Golgi cell APs triggered by current injection inhibited firing of the granule cell (duration of Golgi cell spiking shown by gray bar in Fig. 4A). The granule cell spike-triggered average of Golgi cell membrane voltage for the same pair as in Figure 4A is shown in Figure 4B, confirming that granule cell spikes evoked short-latency EPSPs in the Golgi cell.

Single granule cells trigger Golgi cell spiking and IPSCs in granule cells

Can single granule cells provide suprathreshold excitation to Golgi cells? To answer this question, a 100 Hz train of 10 APs was evoked in granule cells in connected granule-to-Golgi cell pairs (Fig. 5A). The AP train evoked Golgi cell spikes in 7 of 17 pairs (41% of pairs), with Golgi cells firing an average of 0.8 ± 0.2 spikes per granule cell train (range, 0.1–2.0 postsynaptic APs per presynaptic train). Golgi cell spikes typically occurred after the granule cell had fired several times (average latency from first granule cell spike to first Golgi cell spike: 73.6 ± 1.9 ms; Fig. 5B). Although we cannot rule out the contribution of temporal summation in bringing the Golgi cell to threshold, the relatively low initial efficacy and the pronounced synaptic facilitation of the granule-to-Golgi cell synapse may explain the relatively long latency between the start of the granule cell AP train and the first spike in the Golgi cell.

The results shown in Figure 5, A and B, led to the prediction that a burst of APs in a single granule cell will after some delay evoke IPSCs onto the various granule cells innervated by that Golgi cell. We first tested this prediction by evoking spiking in one granule cell in current clamp while simultaneously recording from another granule cell in voltage clamp (Fig. 5C). In 6 of 69 dual recordings, a 100 or 200 Hz train of 10 APs in one granule cell resulted in IPSCs in the simultaneously recorded granule cell (6 of 132 directions tested for probability of 5%). Each granule cell train evoked 0.9 ± 0.1 IPSCs in the simultaneously recorded granule cell (range, 1.4–0.5 IPSCs per granule cell train). The IPSCs were blocked by NBQX (Fig. 5C, red line; $n = 2$) and by SR 95531 and strychnine ($n = 1$), as expected for disynaptic IPSCs evoked by glutamate release from granule cells onto Golgi cells and subsequent release of GABA and/or glycine. The IPSC occurred 54.2 ± 3.1 ms after the peak of the first granule cell spike for the 100 Hz train ($n = 4$) and 32.6 ± 2.1 ms for the 200 Hz train

($n = 2$; Fig. 5D). The apparently earlier onset of Golgi cell spiking in the experiments shown in Fig. 5, C and D, compared with those in Fig. 5, A and B, as inferred from the timing of the IPSC onto the granule cell, may have resulted from the hyperpolarizing current that was typically injected into Golgi cells to prevent spontaneous spiking in the paired granule-to-Golgi cell recordings (see Materials and Methods).

It was difficult to determine whether the spiking granule cell also evoked inhibition onto itself (i.e., feedback inhibition) in the experiments in Figure 5, C and D, possibly because the large spike-mediated conductances obscured IPSPs. However, because some granule cells are reciprocally connected to Golgi cells (Fig. 4), we reasoned that evoking escaping spikes in voltage-clamped granule cells (Barbour, 1993) might evoke IPSCs that could easily be distinguished from action currents by their relatively slow decay (Table 1). Escaping spikes were evoked in granule cells patched with a KCl-based intracellular solution by 1 ms depolarization to 0 mV from a holding potential of -60 mV. A 10 AP, 100 Hz train of escaping spikes evoked IPSCs in 5 of 85 recordings from single granule cells (6% of cells). The IPSC was blocked by NBQX (Fig. 5E; $n = 2$) and by SR 95531 and strychnine ($n = 2$), confirming the disynaptic nature of the IPSC. Each granule cell train evoked 1.2 ± 0.3 IPSCs (range, 0.7–2.0), and occurred 64.1 ± 3.6 ms after the peak of the first granule cell action current (Fig. 5F). Together, these results indicate that single granule cells can excite Golgi cells and thereby evoke IPSCs onto themselves and other granule cells. Because single Golgi cell spikes can inhibit granule cell firing (Fig. 3C,D), our results show that activity in a single granule cell can lead to inhibition in other granule cells.

Computational modeling of granule-cell-evoked inhibition

What effect does a high-frequency burst of spikes in a small number of granule cells have on the activity of a larger population of granule cells? A single Golgi cell projects to a large number of granule cells (Eccles et al., 1967), so even a single Golgi cell spike evoked by a single bursting granule cell could affect the activity of many granule cells. We turned to a simplified computational model of the cerebellar granular layer to answer this question (Fig. 6A; see Material and Methods). We hypothesized that a burst of spikes in one granule cell synapsing onto a Golgi cell will temporarily silence the spontaneous firing of the larger population of granule cells receiving an inhibitory input from the Golgi cell. The nonbursting granule cells will be referred to as “background” granule cells.

In the model, 490 background granule cells received input from 4 mossy fibers firing at an average rate of 5 Hz each, similar to the observed spontaneous firing rates from *in vivo* whole-cell recordings of mossy fibers (Rancz et al., 2007). The low-frequency mossy fiber input evoked low-frequency background granule cell spiking (1.0 ± 0.1 Hz) caused mainly by coincidence of two or more EPSPs, similar to rates observed *in vivo* (Chadderton et al., 2004; Loewenstein et al., 2005; Ruigrok et al., 2011; Duguid et al., 2012). One granule cell received a burst of mossy fiber input that evoked a train of 10.2 ± 0.1 spikes at 109.1 ± 0.7 Hz, similar to bursts evoked in granule cells by sensory stimula-

Figure 4. Reciprocally coupled granule-Golgi cell pairs. **A**, Granule cell was depolarized with current injection to evoke spiking (top). Granule cell spikes led to EPSPs in the Golgi cell (bottom, asterisk indicates the onset of EPSPs). After ~ 300 ms of granule cell spiking, 100 Hz Golgi cell spiking was evoked by suprathreshold current injection (gray bar indicates duration of Golgi cell spiking), which evoked IPSPs in the granule cell and paused spiking. Spikes in the Golgi cell have been truncated to allow higher magnification of EPSPs. **B**, Granule cell spike-triggered average confirms that granule cell spikes led to short-latency EPSPs in Golgi cell. Same pair of cells as in **A**.

tion *in vivo* (Chadderton et al., 2004; Jörntell and Ekerot, 2006; Bengtsson and Jörntell, 2009) and to our paired recordings in Figure 5. The burst of spikes in the single granule cell evoked a single Golgi cell spike, as in our experimental data. The IPSP evoked by the Golgi cell spike led to an inhibition in the firing rate of the background granule cells that was statistically significant for 30 ms (Fig. 6B,C).

Because granule cells may be either reciprocally or nonreciprocally connected to the Golgi cell (Fig. 4), the firing rates of bursting granule cells with and without reciprocal inhibition were compared. The impact of the IPSP on bursting granule cells was relatively weak, reducing the average number of APs fired by bursting granule cells by 0.6 ± 0.1 spikes and the average frequency of spiking by only 6.6 ± 0.7 Hz. Therefore, the main effect of the IPSP was to inhibit the firing of the background granule cells. Because a single granule cell will not synapse onto all of the parallel fiber target cells (Barbour, 1993; Roberts and Trussell, 2010), the Golgi cell may act to “inform” several target cells that a single granule cell has fired a burst by inhibiting the activity of the background granule cells (see Discussion).

In the cerebellum, a single mossy fiber terminal synapses onto 10–100 granule cells (Jakab and Hámos, 1988; Billings et al., 2014; Ritzau-Jost et al., 2014). Assuming that one-third of the granule cells receiving input from a particular mossy terminal synapse onto a given Golgi cell, this suggests that bursts of activity at a single terminal may result in an increase in the activity of several granule cells. Therefore, the number of bursting granule cells was progressively increased from 1 to 10 granule cells, which resulted in an increasingly prolonged duration of inhibition of the background population of granule cells (Fig. 6D, black line). However, the effect of changing the number of bursting granule cells on the duration of inhibition appeared to saturate with four or more bursting granule cells.

For the simulations described so far, the Golgi-to-granule cell synaptic latency and synaptic strength was the same for all of the granule cells in the simulation. To determine how the duration of inhibition is affected by changing the number of bursting granule cells under more realistic conditions, simulations were repeated with randomized synaptic latencies and strengths drawn from distributions based upon the experimental data from Golgi-to-

Figure 5. Single granule cells can evoke spiking in Golgi cells and IPSCs onto granule cells. **A**, A 100 Hz, 10 AP train of granule cell spikes (top) evoked spikes in the Golgi cell with variable latency and that typically occurred only after multiple granule cell APs. **B**, Summary graph of distribution of timing of Golgi cell APs for all pairs in which granule cell firing evoked Golgi spiking ($n = 7$). The timing of Golgi cell spikes was binned into 2 ms bins and 0 ms was set as the peak of the first granule cell AP in the train. Vertical black marks indicate the timing of granule cell APs. **C**, A 100 Hz, 10 AP train of APs in one granule cell (top) evoked IPSCs in a second granule cell (bottom). Gray traces show overlaid single trials and the thick black line is the average of the trials. NBQX blocked the IPSCs (as shown by thick red line, which is the average of several trials in the presence of NBQX), confirming that the IPSCs were evoked by glutamate release from the granule cell train. **D**, Summary graph as in **B** for distribution of timing of IPSCs for 2 pairs in which granule cell fired at 200 Hz (blue bars) and 4 pairs in which granule cell fired at 100 Hz (red bars). Blue vertical bars indicate the timing of granule cell APs for the 200 Hz train and black bars indicate the timing for the 100 Hz train. **E**, A 100 Hz, 10 action current train evoked by 1 ms depolarization of granule cell from -60 to 0 mV triggers IPSCs after the third or later action current in the train. Gray traces show overlaid trials and black line is the average of trials. NBQX blocked the IPSCs (red line). Granule cell was patched with a KCl-based intracellular solution, leading to inward-directed IPSCs (see Materials and Methods). Action currents have been partially deleted for clarity. **F**, Summary graph as in **D** for IPSCs evoked in single granule cells by escaping spikes ($n = 5$).

granule cell paired recordings (see Materials and Methods). Even under randomized conditions, bursting in granule cells was still able to significantly inhibit background granule cell firing, with the duration of inhibition increasing with the number of bursting granule cells (Fig. 6D, red line). Interestingly, under these more

realistic conditions, there was a greater range over which the duration of inhibition increased with the number of bursting granule cells. Therefore, bursts of spikes in a small number of granule cells can inhibit other granule cells firing at low rates in response to mossy fiber input and the time during which the nonbursting granule cells are inhibited increases with the number of bursting granule cells.

Discussion

We have shown that a burst of spikes in single granule cells can evoke Golgi cell spiking and recruit inhibition onto granule cells of the cochlear nucleus. Using paired patch-clamp recordings, we first characterized the granule-Golgi cell network, which has been extensively studied in cerebellum but has received little attention in the cerebellum-like cochlear nucleus and electrosensory lobe of weakly electric mormyrid fish. Single Golgi cells released GABA and/or glycine onto granule cells and mediated potent inhibition of granule cell firing. Granule cells made excitatory synapses onto Golgi cells that released glutamate onto postsynaptic Ca^{2+} -permeable AMPARs and underwent short-term synaptic facilitation.

Trains of APs at 100 Hz in a single granule cell were sufficient to evoke spiking in $\sim 40\%$ of connected granule-to-Golgi cell pairs. Firing of one granule cell evoked IPSCs in another granule cell in 5% of granule-granule cell dual recordings and in the same granule cell in 6% of single granule cell recordings. Last, simulations using experimentally constrained parameters confirmed that a train of APs in a single granule cell could inhibit the firing of other granule cells for tens of milliseconds. Furthermore, as the number of bursting granule cells was increased, the duration of inhibition of the nonbursting granule cells lengthened. Therefore, bursting in a small number of granule cells may inhibit activity in the larger population of granule cells innervated by a Golgi cell. Our results challenge the view that granule cell synapses onto Golgi cells are too weak to excite Golgi cells (Dieudonné, 1998; Xu and Edgley, 2008; Prsa et al., 2009), at least in cochlear nucleus.

A single granule cell would be expected to influence the firing of a limited subset of all other granule cells; in this way, feed-

back inhibition can control transmission of signals from mossy fibers but still maintain independence among different groupings of cells. Although it is difficult to extrapolate findings from dual recordings to the circuit level (Rieubland et al., 2014), our esti-

mate of 5–6% granule cells sensing feed-back from one granule cell is what would be expected based on connection probabilities and synaptic strengths in the circuit. If it is assumed that each granule cell only provides excitatory input to one Golgi cell (as might be expected from the low degree of shared parallel fiber input in cochlear nucleus; Roberts and Trussell, 2010), then the probability that a granule cell synapses onto a particular Golgi cell that then synapses onto the other granule cell is 13% (product of granule-to-Golgi cell and Golgi-to-granule cell connection probabilities: $0.33 \times 0.38 = 0.13$). Moreover, if 41% of these granule-to-Golgi synapses are strong enough to evoke Golgi cell spiking (as observed in paired granule-to-Golgi cell recordings), then the probability that spiking in one granule cell evokes IPSCs in another is only 5% ($0.13 \times 0.41 = 0.05$), as was observed. However the effects of tissue slicing may reduce the magnitude of Golgi cell projections so this value must be a lower estimate. Another contributing factor is that, with the convergence of even two granule cells onto a Golgi cell, the spike threshold will be more reliably reached and inhibition more pronounced. Optical methods for circuit analysis applied to thicker tissue sections may provide more accurate evaluation of the impact of single or multiple granule cells.

Inhibition of granule cells in the cerebellum and cerebellum-like systems

Classically, Golgi cells have been thought to have a gain control function in the cerebellum, whereby increased granule cell or mossy fiber activation excites Golgi cells and inhibits granule cell spiking (Marr, 1969; Albus, 1971; Billings et al., 2014). Similarly, cerebellar granule cells are subject to tonic inhibition through GABA_ARs containing both the $\alpha 6$ and the δ subunit (Brickley et al., 1996; Hamann et al., 2002; Rossi et al., 2003), which controls the gain of granule cell spiking in response to mossy input (Mitchell and Silver, 2003; Duguid et al., 2012). Despite the considerable developmental, genetic, morphological, and physiological similarity between granule cells in the cerebellum and in cerebellum-like systems (Funfschilling and Reichardt, 2002; Bell et al., 2008), granule cells in the cochlear nucleus and mormyrid electrosensory lobe appear to lack tonic inhibition (Zhang et al., 2007; Balakrishnan and Trussell, 2008). Therefore, although tonic inhibition seems to be important enough in the cerebellum that knock-out of the $\alpha 6$ and the δ subunit is compensated fully by upregulation of a K⁺ leak conductance (Brickley et al., 2001), tonic inhibition onto granule cells is not a general operating principle of cerebellum-like systems. However, Golgi cell firing induces inhibitory postsynaptic events in granule cells in the cerebellum and in cerebellum-like systems (Rossi and Hamann, 1998; Zhang et al., 2007; Balakrishnan et al., 2009), suggesting that Golgi-cell-mediated fast synaptic inhibition is a conserved network motif in these systems. The sources of excitatory input driving Golgi cell spiking *in vivo* are

Figure 6. Computational modeling predicts that bursts of spiking in a small number of granule cells can evoke inhibition of granule cells firing in response to spontaneous mossy fiber EPSPs. **A**, Schematic of the model. A total of 490 granule cells (Gr, red), called "background" granule cells, received excitatory input from 4 mossy fibers (blue) firing at 5 Hz, generating low-frequency granule cell firing (see text). The background granule cells all received inhibitory input from a single Golgi cell (Go, green), but for simplicity, the background cells did not synapse onto the Golgi cell. One to 10 granule cells received a burst of mossy fiber input that evoked a burst of APs. The bursting granule cells provided excitatory input to the Golgi cell and approximately half of the bursting granule cells also received feedback inhibition from the Golgi cell (see Materials and Methods). **B**, Raster plot from 5 runs of the simulation in which the Golgi cell fired an AP at a time of 0 ms (as indicated by arrow) in response to a burst of 10 APs at ~ 100 Hz in a single granule cell. Each red mark indicates the timing of an AP in a background granule cell. Bursting granule cell APs are not shown. **C**, APs in the background granule cells were binned into 1 ms bins for 100 runs of the simulation. The Golgi cell AP occurred at 0 ms. Red dotted line indicates the average number of APs per bin for the 50 ms period immediately preceding the Golgi cell spike. The black bar indicates the duration of inhibition, the time over which the number of APs per bin was significantly different from the bin count for the control period (t test, $p < 0.05$). **D**, The number of bursting granule cells was varied from 1 to 10 under 2 different network conditions: in the first condition, the synaptic latency and synaptic strength of the inhibitory Golgi-to-granule cell synapse was uniform (black circles); in the second condition, the parameters were randomized among the different background granule cells (red triangles). The duration of inhibition was measured as in **C**.

unknown, but Golgi cells receive excitatory synaptic input from granule cells, mossy fibers, and possibly climbing fibers in the cerebellum (Eccles et al., 1967; but see Galliano et al., 2013a) and auditory nerve fibers in cochlear nucleus (Mugnaini et al., 1980; Ferragamo et al., 1998).

Possibly because granule cell synapses onto Golgi cells have previously been considered less important in exciting Golgi cells (Dieudonné, 1998; Xu and Edgley, 2008; Prsa et al., 2009; but see Cesana et al., 2013), most studies have focused on firing of Golgi cells evoked by mossy fiber stimulation, which leads to feed-forward inhibition of granule cells (Mapelli and D'Angelo, 2007; Kanichay and Silver, 2008). Mossy fiber activation has been hypothesized to lead to a limited time window during which the granule cell can spike in response to mossy fiber EPSPs before it is inhibited by feed-forward inhibition (D'Angelo and De Zeeuw, 2009). However, because suprathreshold excitation of Golgi cells requires the activation of multiple mossy fibers (Kanichay and Silver, 2008; Vervaeke et al., 2012), whereas even a single mossy fiber can drive granule cell firing (Rancz et al., 2007; Arenz et al., 2009; Rothman et al., 2009), feed-forward inhibition is not likely to occur under conditions of sparse mossy fiber activation. In contrast, high-frequency firing in only a single mossy fiber could lead to a burst of spikes in its target granule cells, thereby generating feedback inhibition in other granule cells. Therefore, whereas feed-forward inhibition may occur under conditions of

abundant mossy fiber activation, bursts in a small number of granule cells may evoke feedback inhibition under conditions of sparse mossy fiber activation. Although we have shown that a train of high-frequency spikes in a single granule cell evokes inhibition onto granule cells in cochlear nucleus, such an experiment has not been performed in the cerebellum, so whether this phenomenon occurs in the cerebellum awaits experimental verification.

Possible circuit functions of bursts in single granule cells

Cerebellar granule cells appear to encode sensory stimuli by a burst of spikes (Chadderton et al., 2004; Jörntel and Ekerot, 2006; Arenz et al., 2009). However, mossy fibers in the cerebellum and electrosensory lobe are also spontaneously active (van Kan et al., 1993; Sawtell, 2010; Kennedy et al., 2014), which leads to low-frequency granule cell firing (Chadderton et al., 2004; Ruigrok et al., 2011; Duguid et al., 2012). Furthermore, cochlear nucleus, electrosensory lobe, and the vestibulocerebellum contain unipolar brush cells, local excitatory interneurons that provide input to granule cells and are spontaneously active (Russo et al., 2007; Ruigrok et al., 2011; Kennedy et al., 2014). The resulting low-frequency firing of granule cells appears to be quite sensitive to inhibition from Golgi cells (Fig. 6). Therefore, parallel fiber target neurons that do not receive input from the bursting granule cell(s) may receive reduced excitation from their source granule cells due to the inhibition triggered by the bursting granule cell(s). Due to the high rate of divergence of Golgi cell axons onto granule cells, Golgi cells may act to inform parallel fiber target neurons of bursts in one or more granule cells by decreasing the activity of the “background” granule cells, thus broadcasting the activity of a small number of bursting granule cells to a large number of parallel fiber target neurons. This function of Golgi cells may be particularly relevant in the cochlear nucleus, where there is little shared parallel fiber input among target neurons (Roberts and Trussell, 2010), and in the cerebellum, where the majority of granule cell synapses onto Purkinje cells are silent (Isope and Barbour, 2002; Brunel et al., 2004). The major effect of the decrease in background parallel fiber input evoked by a burst in a small number of granule cells may be a decrease in the activity of molecular layer interneurons because parallel fibers are particularly effective at exciting these cells (Barbour, 1993; Carter and Regehr, 2002). Therefore, a burst of spikes in even a single granule cell may have a circuit-wide effect.

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