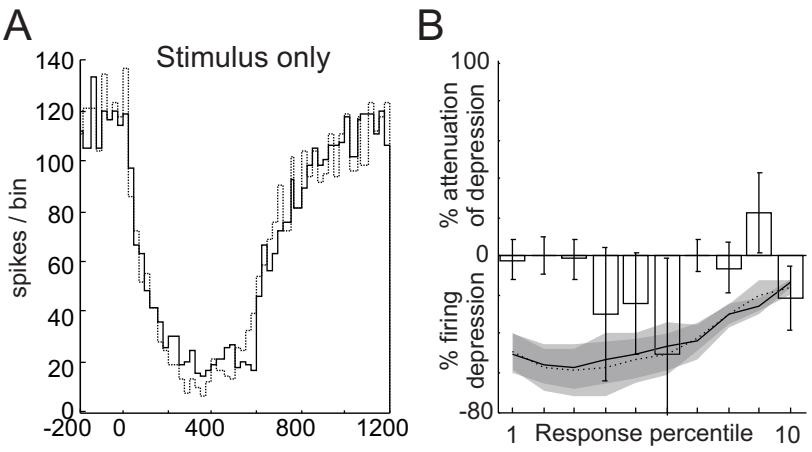
SUPPLEMENTARY 1

Figure S1 – Stability of Golgi cell responses over experimental time-course. The PSTHs shown in **A** depict the long-lasting depression responses of an example Golgi cell following stimulation of ipsilateral hindlimb afferents: control = solid line, test = broken line. In this example, the experiment was run over the same time-course as if drugs were applied, except that no drugs were used, instead the aim of the experiment was to test the stability of the Golgi cell long-lasting depression response. Grouped data for 8 Golgi cells tested in the same way is shown in **B** – for this analysis the inhibitory response of each cell was divided into 10 deciles (onset and offset assessed using turning points in the CUSUM trend-line – see Experimental Procedures) and firing rate ratios relative to pre-stimulus firing in each were calculated. The mean amplitude of depression of spontaneous firing for both control bracket (solid line) and drug (broken line) following the stimulus is shown in the lower plots of **B**, the grey shaded area around these lines represents 95% confidence limits for the mean, error bars represent 2 standard errors from the mean.

Holtzman et al Figure S1



SUPPLEMENTARY S2

Identifying granule cells *in vivo* is difficult so our classification is based on a number of lines of evidence, namely location in the granular layer alongside identified Golgi cells, characteristic irregular spontaneous firing pattern or indeed near lack of spontaneous activity, and their ability to fire at high frequency (Chadderton et al., 2004; Jorntell and Ekerot, 2006; Arenz et al., 2008; Barmack and Yakhnitsa, 2008) combined with juxtacellular labelling and antidromic collision. Granule cells are very small (~ 5 μm soma) and difficult to record from in vivo. The signals we attribute to putative granule cells could be recorded very focally and electrode movements in excess of 5 μm usually resulted in loss of signal, in close agreement with earlier studies (Shambes *et al.*, 1978; Huang *et al.*, 1993), whilst the signals from Golgi cells were much more robust (Holtzman et al., 2006). Figure S2A&B show interspike interval histograms (ISIH) for a Golgi cell granule cell pair recorded simultaneously on the same electrode (see Figure 4A); inset in panel B is the first 10 ms of the ISIH of the putative granule cell revealing the highest frequency firing.

In the absence of specific stimulation, half of our sample of granule cells were near silent (n = 22/45). The majority of those active units (n = 19/22) had irregular activity which included brief but vigorous high frequency firing (c.f. Figure 4A&B; range 140 - 833 Hz maximum instantaneous frequency, mean ± S.E.M: 663 ± 58 Hz), although 3 units did not fire at such high frequencies. Mean firing rates across the spontaneously active population were generally low (mean ± S.E.M: 3.9 ± 0.7 Hz, range 0.44 – 13.8 Hz) in agreement with other studies (0.3-0.7 Hz – rat; Chadderton et al., 2004; 3 – 19 Hz decerebrate cat; Jorntell and Ekerot, 2006). In one case were we able to juxtacellularly label an individual granule cell with neurobiotin. This neuron is illustrated in Figure S2C-E showing the ISIH and spike-shape, successful juxtacellular spike entrainment and recovered histology for the juxtacellularly labelled granule cell. We were also able to identify granule cells by antidromic activation following parallel fibre stimulation (n=3). The traces shown in Figure IDF show the responses of one such cell to a train of 3 brief stimulation pulses; note the evoked spike following each stimulus at invariant latency of 1.6 ms. When stimuli were triggered 1 ms after spontaneous spikes, no evoked spikes were seen (expected latency indicated by *) indicating collision of the antidromic spike with the spontaneously occurring orthrodromic spike. Units with 'dual-component' action potentials, a signature of mossy fibre terminals (Garwicz et al., 1998; Jorntell and Ekerot, 2006), were excluded from the main study. The PSTHs shown in Figure S2G&H show a paired recording of a granule cell (upper panels) and unit with a dual component action potential. Following stimulation of hindlimb inputs, the granule cell shows a prolonged excitation (see Figure 5) in tandem with a broadly similar increase in activity in the mossy fibre unit. When the same cell pair were tested with parallel fibre stimulation (5 pulses, 100 Hz), the granule cell showed a delayed onset, prolonged increase in firing likely to arise from the inhibitory effect the same stimuli have on Golgi cells (c.f. Figure 2). In contrast, as expected the mossy fibre showed no detectable change in activity following parallel fibre stimulation. Overall, as we were not able to juxtacellularly label or antidromically identify all of our cells, for the remainder of the study, those units located in the granular layer with small tuning distances, non-dual component action potentials, with a near absence of spontaneous activity or erratic often high frequency firing patterns are classified as putative granule cells.

Figure S2 – Identification of granule cells. A & **B** plot interspike interval histograms (ISIH) for each cell of the pair shown in Figure 4A. Overlaid spike waveforms are shown inset, and in addition the first 10 ms of the granule cell ISIH is shown on an expanded timescale, highlighting the highest instantaneous frequency activity of this cell. **C** plots and ISIH and inset spike waveform for a different granule cell, the spontaneous activity of which was devoid of high frequency activity (note the different time-scales c.f. **A** & **B**). This same cell underwent juxtacellular labelling, spike entrainment shown in **D** and was recovered histologically, revealing a labelled affair granule cell with three claw-like dendrites shown in **E**. Scale bar 5 μm, ISIH bin-size 5 ms, 1 ms and 0.1 ms. **F** plots raw data from a different granule showing antidromically evoked spikes (note fixed latency ~1.6 ms) following parallel fibre stimulation (stimulus artefacts obscured by grey boxes, 3 pulses, 3 ms pulse interval – top panel). When parallel fibre stimuli were delivered 1 ms after orthodromic (i.e. spontanesous) spikes, no antidromic spikes were evoked, indicated by * in the 3 traces shown in the bottom panel, confirming antidromic collision. **G** & **H** show PSTHs from a paired recording (same electrode) of a granule cell (upper panels) and mossy fibre unit following stimulation of ipsilateral hindlimb afferents (**G**) - both units show prolonged excitation. Following parallel fibre stimulation, only the granule cell responds with prolonged excitation, which is likely to be due to reciprocal decreases in local Golgi cell activity (see Figure 2). Insets show action potential waveforms for each unit.

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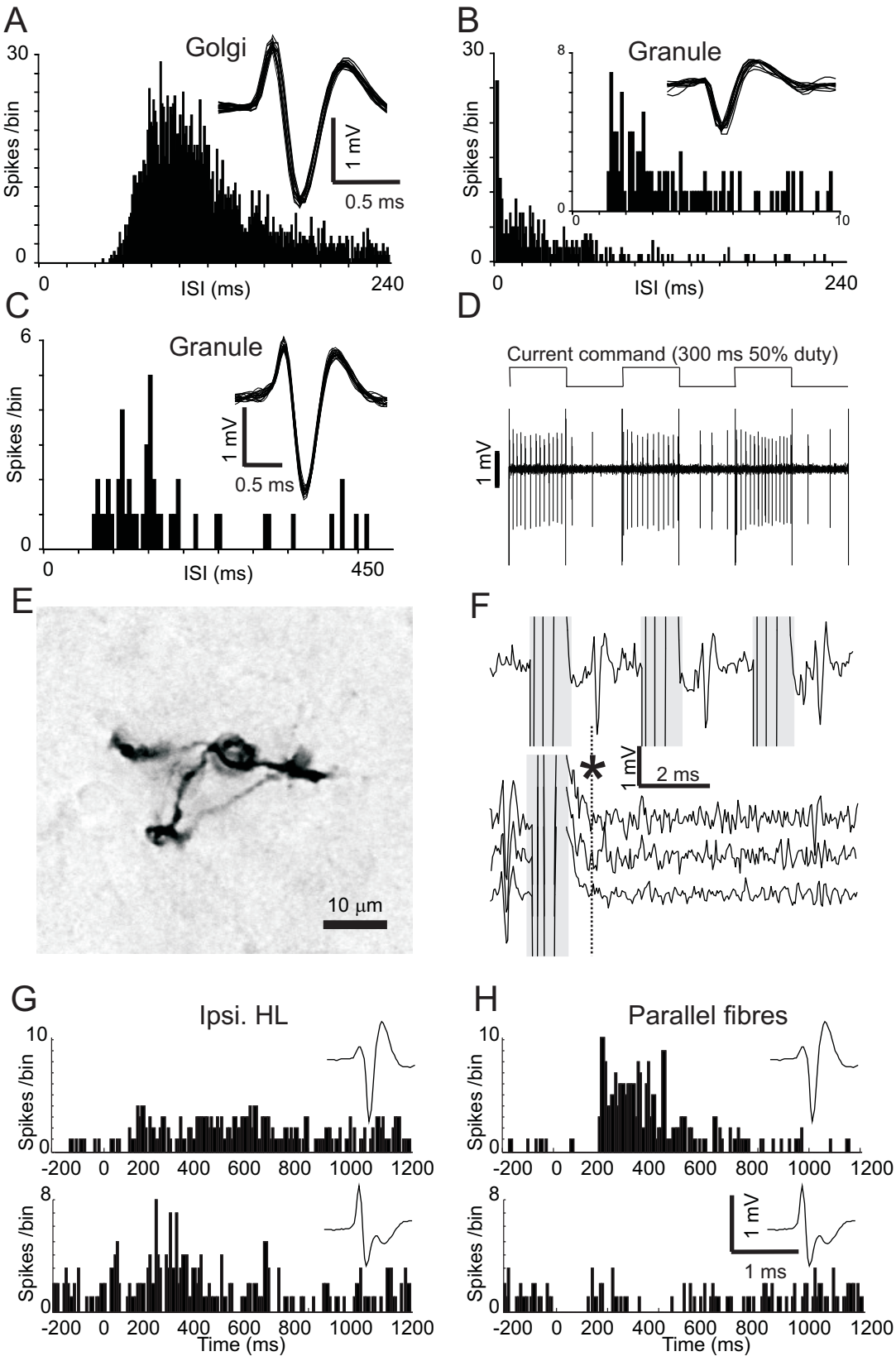
Holtzman et al Figure S2



SUPPLEMENTARY 3

Categorisation and analysis of granule cell responses

Granule cell responses fell into four categories consisting of excitation and depression, either of which could be early or late onset, with the general pattern that early responses were briefer-lived than late onset responses. Individual cells often showed complex mixtures of these various responses types, which are summarised in Supplementary Figure S3.

Considering the categories of response separately, we examine short latency excitations in detail in Supplementary Figure S4. Summary data illustrating the frequency of occurrence of long-lasting excitation (LLE) responses (irrespective of any depression responses) is drawn in Figure 6A. Receptive field analysis showed that long-lasting excitations in granule cells were evoked with equal likelihood from all inputs tested (n = 25 cells, Chi-squared test – see Figure 6A) suggesting that LLE responses are equally likely from any of these areas. Onset latencies and durations of the LLE responses are summarised in Figure 6B. Comparing the LLE onset latencies across each of the standard sensory inputs, no significant differences were apparent (Student's t-tests and Wilcoxon ranksum tests, as appropriate) although significant differences in LLE duration were found between the ipsilateral vibrissal skin and the contralateral hindlimb ($p < 0.014$, Student's t-test). Similarly, differences were also apparent between the duration of contralateral vibrissal SLEs and those from either of the hindlimbs ($p < 0.031$ & $p < 0.002$, ipsi- and contralateral hindlimb respectively, Student's t-test). These observations mirror those for long lasting Golgi cell responses, consistent with the general pattern that limb responses are more robust, i.e. 'longer and stronger', than vibrissal responses (see Holtzman et al., 2006, and Figure 8). Overall, the 'average' onset latency for LLE responses in the region of 110-150 ms, irrespective of the location of the sensory input, suggesting that LLE responses are a generalised pattern of granule cell response (see Figure S3C-G).

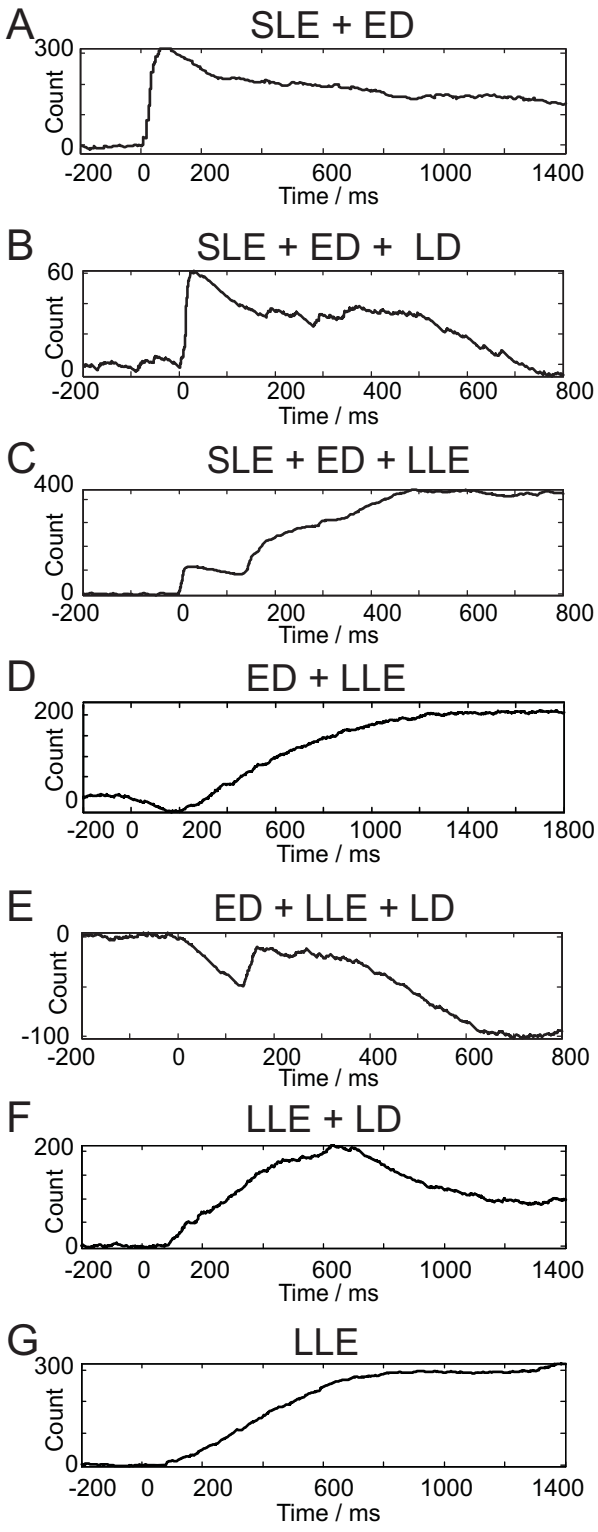
Granule cells also showed periods of early and late depressed activity which could occur independently from early excitations (see Figure S3D&E). Across our sample of 25 granule cells, early depressions were most likely following vibrissal stimulation, observed in 36 % (ipsilateral) and 20 % (contralateral) of neurones (Figure 6C). However, detection of depressions requires ongoing spontaneous activity (which was absent in many granule cells) so the frequency of these responses may be underestimated. Late depressions (Figure 5C & D, Figure S3B, E & F & Figure 8) were also observed in roughly 15% of our sample of granule cells (Figure 6E) from a variety of skin areas. Onsets and durations of early and late depressions are plotted in Figure 6D and 6F, respectively – the small number of observations in some groups precluded a detailed statistical analysis of these responses. Interestingly, individual cells could respond to different inputs with different mixtures of short and long excitation mixed with early and late depression, as shown in Figure S3, consistent with these responses being underpinned by independent excitatory and inhibitory processes in single granule cells.

Supplementary Figure S3 – Granule cell response categories. In each panel, CUSUM trendlines illustrate an example granule cell response. **A-C** show responses, each of which began with early excitations (SLE) and were accompanied by early depressions (ED) and either late depressions (LD, shown in B) or late excitations

(LLE, shown in C). The responses shown in **D** & **E** both began with early depressions (in the absence of any early excitations, c.f. **A**-**C**) and were accompanied by long-lasting excitations, which could also be followed by late depressions (LD, shown in **E**). F & E show responses which lacked any early components, either SLE or ED, and instead begun with long-lasting excitation, which may (**E**) or may not (**F**) be followed by late depression. Note the different time-scales in the CUSUM plots.

Holtzman T, Rajapaksa T, Mostofi A, Edgley SA (2006) Different responses of rat Cerebellar Golgi cell and Purkinje cells evoked by somatosensory afferent inputs. J Physiol 574:491-507.

Holtzman et al Figure S3



SUPPLEMENTARY S4

Short-latency, short-lasting excitations in Golgi cell and granule cells

Many early studies investigated responses evoked by the dense trigeminal afferent projection to lobule Crus II in the rat and some of these studies have specifically examined responses in the granular layer (Shambes et al., 1978; Bower and Woolston, 1983; Vos et al., 1999; Chadderton et al., 2004; Holtzman et al., 2006; Rancz et al., 2007). All of these studies studies reported short latency (~2-10 ms) activation in the granular layer following stimulation of the trigeminal afferents, predominantly ipsilateral. Our sample of 45 granule cells showed short latency excitations (SLEs) following activation of trigeminal inputs which consisted of brief but vigorous 'bursts' of action potentials, typically 2-4 spikes up to ~700 Hz instantaneous frequency. The PSTHs in Figure S4A show the responses of a typical Golgi cell (upper panel) and granule cell (lower panel) following stimulation of ipsilateral trigeminal inputs. The Golgi cell shows its typical 'triplet' SLE response of spikes within three different time windows (Vos et al., 1999; Holtzman et al., 2006) whereas the granule cell shows a less structured SLE response in a comparable latency range (c.f. Rancz et al., 2007). Receptive field analysis using neurones tested for responses from six routinely tested areas (bilateral trigeminal skin and all four limbs) included our original dataset of 51 Golgi cells (see Holtzman et al., 2006) and 25 granule cells from the current study, revealed that trigeminal territories most commonly evoked SLEs (see Figure S4B – note only one granule cell showed SLE responses from the limbs, precluding a detailed analysis. However, limb-evoked granule cell SLE responses were seen in other granule cells (although not included in this analysis as only partial mapping of receptive fields was achieved) and furthermore their presence is suggested by our observation that some Golgi cell SLE responses are THIP sensitive (see Figure 3A), thus mediated by underlying granule cell SLEs). SLE-only responses were more commonly evoked from the contralateral vibrissae compared to the ipsilateral, observed in 28 % and 12 % of neurones respectively (Figure S4B open bars; $p<0$ .01, Fisher-exact test). Mixed responses (SLE + LLE – see Figure RESPC; described later) were seen in 28 % and 12 % of the Golgi and granule cells respectively (ipsilateral vs. contralateral trigeminal skin - black bars in Figure S4B). Considered together, i.e. SLE-only and mixed responses, surprisingly SLEs were equally likely from the trigeminal territory of either side (40 % ipsilaterally and 40 % contralaterally, Figure S4B, open + black stacked bars), and when considered as a group, 24 % of cells had receptive fields that included SLEs from both sides of the face. An example SLE response evoked from stimulation of the *contralateral* trigeminal skin is shown Figure RESPC (upper panel, c.f. Figure S4A lower panel). Similar responses have been described in the same lobule in granule cells and some mossy fibres (Crus II - Chadderton *et al.*, 2004; Rancz *et al.*, 2007) using ipsilateral trigeminal stimulation, although contralateral trigeminal inputs were not tested in those studies. In comparison, Golgi cells are much less likely to show SLE responses from these same territories (grey bars Figure S4B) with 14% and 7% of responses from ipsi- and contralateral trigeminal inputs, respectively (indicated by *; Fisher-exact tests, $p<0.006$ ipsilateral, $p<0.001$ contralateral). In terms of onset latencies, trigeminal granule cell SLE response latencies were 7.6 ± 1.2 ms (ipsilateral, mean ± S.E.M) and 8.9 ± 1 ms (contralateral) which are significantly later than the earliest

spikes evoked in Golgi cells (3.4 ± 0.2 ms, ipsilateral; 4.3 ± 0.3 ms, contralateral; Students t-test, $p<0.01$ indicated by *) and significantly earlier than the 3rd spikes (18.7 ± 2.3 ms, ipsilateral; 15.6 ± 0.6, contralateral; Students t-test, $p<0.01$ indicated by *) of these responses. Thus the earliest spikes in the Golgi cell SLEs occur ~3-6 ms before the earliest granule cell SLE spikes, which in turn coincide with the 2nd spike of the Golgi cell SLE. Overall, this suggests that granule cells and Golgi cells may share a common source of trigeminal mossy fibre input or that granule cell SLEs potentially mediate secondary and tertiary spikes in Golgi cell triplets. Our observation that Golgi cell SLEs may precede similar responses in granule cells, as proposed by Eccles et al (1966) may be underpinned by differences in synaptic integration of these two cell types (for granule cell EPSP rise times, see Jorntell and Ekerot, 2006; Rancz et al., 2007).

Figure S4 – Short latency excitations in Golgi cells and granule cells. A: PSTHs (bin-size 0.5 ms) showing responses of an example Golgi cell (*upper* panel) and a granule cell to stimulation of ipsilateral trigeminal afferents. Each cell shows a short latency excitation (SLE), the Golgi cell consisting of a 'triplet' response with spikes at three distinct times in comparison to the less structured granule cell 'burst'. **B** plots the frequency of occurrence of these responses from each of six routinely tested skin areas –trigeminal evoked SLEs were equally likely from either side of the face in the majority of granule cells and significantly more likely in granule cells than Golgi cells. **C** plots onset latencies for the Golgi cell SLE responses (triplets are broken down by component) and short latency responses in granule cells. Granule cell SLE onsets from trigeminal inputs were not significantly different the onset of the second component of the Golgi cell triplets, although were significantly earlier than the 3rd Golgi cell spikes and significantly later than the 1st. Error bars represent 2 standard errors from the mean.

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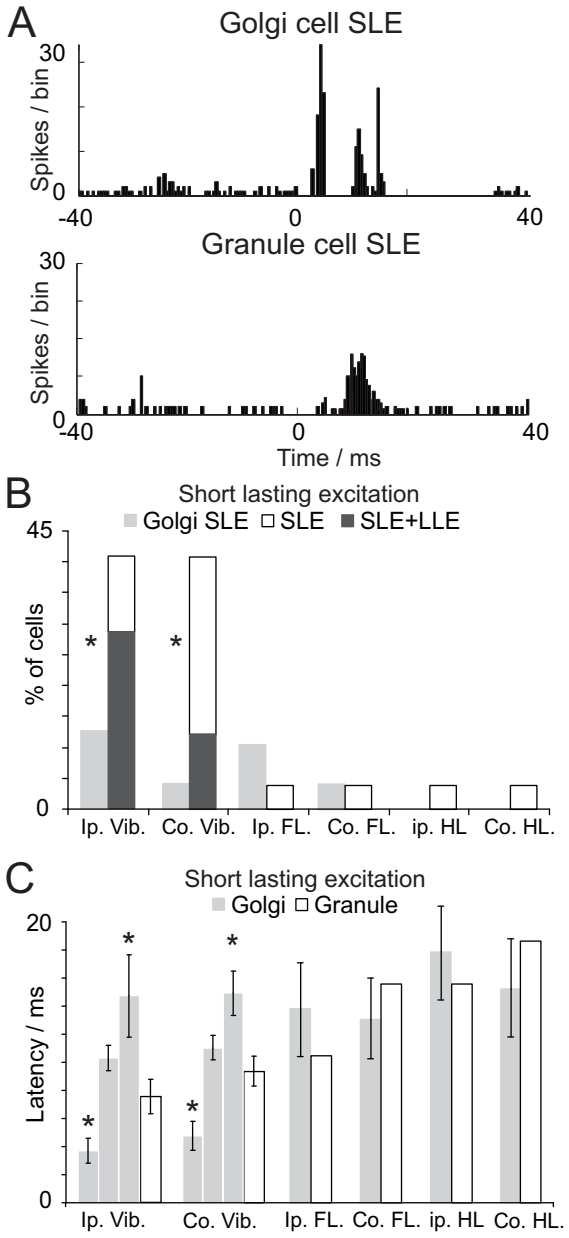

SUPPLEMENTARY 5

Widespread, convergent receptive fields in granule cells

Major inputs to Crus II are considered to arise from the facial dermatomes giving rise to a dense projection of trigeminal afferents (see Shambes et al., 1978 for details; Bower and Woolston, 1983). The majority of these afferents are reported to convey information from the ipsilateral facial structures and skin and so it was particularly surprising that many granule cells in Crus II were responsive to limb afferent inputs (see Figure 5A&B). Furthermore, the same neurones frequently responded to trigeminal inputs from both sides of the face, indicating a widespread and bilateral convergence of information onto single granule cells, in common with convergence reported for Golgi cells and Purkinje cells in the same cortical area (Holtzman et al., 2006a). As described above, individual granule cells could show a variety of 'mixed' responses (i.e. SLEs / LLEs mixed with early / late depressions) to different inputs (e.g. SLE from the face, LLE from the limbs and so on), indicating heterogeneity across the granule cell population. None-the-less, co-modulation in the population occurred over similar time-scales (see Figure 5C&D and Figure 6). Comparing the degrees of convergence observed across the sample of 25 granule cells tested for all standard inputs, 4 neurones responded to all six inputs, 2 neurones responded to five inputs, 5 neurones responded to 4 inputs, 4 neurones to 3 inputs and each of 5 neurones were responsive to 1 and 2 inputs, respectively. A minority of the sampled neurons had restricted receptive fields, responses were evoked only from trigeminal receptive fields (ipsi- and/or contralateral) in 4 neurones and 3 neurones were restricted to a limb receptive field, indicating that the remaining 72 % of the sample of granule cells received convergence of trigeminal and limb inputs. These observations run contrary to the accepted notion that the granular layer of Crus II is predominantly an ipsilateral trigeminal receiving area of the cerebellar cortex, and provide further evidence of the close relationship between the receptive fields of Golgi cells and granule cells. Routes for these inputs could include long range parallel fibres, and spinoreticular pathways and cerebro-pontocerebellar pathways that may have widespread convergent is (for discussion see Holtzman et al., 2006b).

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SUPPLEMENTARY 6

Figure S6 – Lugaro cell responses are appropriately timed to provide GABAergic / glycinergic inhibition of Golgi cells. The photomicrograph shown in **A** depicts a neurone filled with neurobiotin following juxtacellular recording / labelling. The somatic location in the granular layer close to the Purkinje cell layer (indicated by broken white line) and bifurcating (presumed) dendrites extending in the sagittal plane are consistent with this neurone belonging to the Lugaro cell class (see Figs. 2,3 10-12 of Laine and Axelrad, 1996; Laine and Axelrad, 2002). Abbreviations: ml = molecular layer, gl = granular layer, wm = white matter; scale bar 50 µm. **B** plots the interspike interval histogram taken from a period of resting firing of this neurone – inset 20 superimposed action potential waveforms. The PSTHs plotted in **C** show the responses of this neurone following stimulation of ipsilateral trigeminal, fore- and hindlimb inputs, respectively. In each example, a substantial increase in Lugaro cell activity with onset latency ~15 ms (trigeminal) or ~50 ms (limbs) lasting approximately 50-60 ms is seen. In addition, each limb response includes a long-latency prolonged increase in spiking, onset ~300ms lasting until ~600 ms – high bins indicated by *. Although subtle compared to the initial excitations, these prolonged responses represented significant increases above pre-stimulus levels (indicated by red lines; $p<0.005$ – see Experimental Procedures). Such activity is ideally suited to increase inhibition over Golgi cells via glycinergic / GABAergic co-transmission (Dugue et al., 2005). In this regard, it is also noteworthy this Lugaro cell shows convergence of widely separated receptive fields, similar to the receptive fields of nearby Golgi cells (see Figure 6 and Holtzman et al., 2006)

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