



PERIPHERAL STIMULI EXCITE CORONAL BEAMS OF GOLGI CELLS IN RAT CEREBELLAR CORTEX

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Abstract—Cerebellar granule cells constitute the largest neurone population of the brain. Their axons run as parallel fibres along the coronal axis, and the one-dimensional spread of excitation that is expected to result from this arrangement is a key assumption of theories of cerebellar function. In many studies using various techniques, however, it was not possible to evoke such a beam-like propagation of excitation with natural stimuli.

We recorded, in Crus I and II of anaesthetised rats, pairs of Golgi cells aligned along the parallel fibre axis and synchronising spontaneously. Each pair was subjected to two stimulation protocols: punctate and semi-continuous. Local punctate facial stimulation evoked distinct fast and late responses of variable strength and latency (fast: 4.0–10.2 ms; late: 13.6–22.7 ms). Semi-continuous stimulation with a brush increased the firing rate, and modified the precision and phase of synchronisation. Differences between a pair in response strength and phase to brush stimulation correlated strongly with the difference in latency to punctate stimulation.

These observations were reproduced in a model of the granular layer. The stimulus activated a central patch of mossy fibres, and Golgi cells received short- and long-range excitation from mossy and parallel fibres, respectively. The strength and latency of the punctate response of a model Golgi cell were found to vary with its position, reflecting a systematic change in the contribution of mossy and parallel fibres to its excitation with distance from the activated patch. During brush stimulation, model Golgi cells inside the patch fired more precisely synchronised, whereas the other Golgi cells responded with a lag proportional to their distance from the patch, thereby reproducing the experimentally observed changes in synchronisation.

Taken together with the previously reported large receptive fields of Golgi cells and with their spontaneous synchronisation, the variable, position-dependent latency of evoked Golgi cell responses indicates a beam-like spread of excitation along the parallel fibres in rat cerebellar cortex. © 2002 IBRO. Published by Elsevier Science Ltd. All rights reserved.

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The alignment of parallel fibres (PFs) along the length axis of a folium (the PF or coronal axis) and perpendicular to the plane of Purkinje cell (PC) dendrites is the basis for most theories of cerebellar functioning (Braitenberg and Atwood, 1958; Marr, 1969; Eccles, 1973; Braitenberg et al., 1997). However, most experimental studies failed to demonstrate the beam-like patterns of PC activity that were predicted to emerge from this anisotropy (Bower and Woolston, 1983; Cohen and Yarom, 1998; but see Garwicz and Andersson, 1992). These negative findings raised doubts about the effectiveness of PFs in activating PCs, and were explained by a predominant excitation of PCs through synapses on the ascending segment of the granule cell axon (Llinás, 1982; Bower and Woolston, 1983; Cohen and Yarom, 1998; Gundappa-Sulur et al., 1999).

As other mechanisms might hinder a transverse spread of PC activation, including for example failure of transmission along PFs (Swadlow et al., 1980; Debanne et al., 1997), it is important to establish the nature of PF activity in response to physiological stimuli. In the present study we used pairs of Golgi cells aligned along the PF axis as sensors of the PF activity, and applied electrophysiological and modelling techniques to investigate their response patterns to both punctate and semi-continuous stimuli.

In our previous studies, Golgi cells proved to be driven, at least in part, by PF excitation. First, a computer model of the granular layer (Maex and De Schutter, 1998) predicted that PFs would synchronise the spontaneous activity of coronally aligned Golgi cells, but not of sagittal pairs. This prediction was confirmed experimentally in the anaesthetised rat (Vos et al., 1999a; Maex et al., 2000). Subsequent observations of the responses to punctate tactile stimulation suggested that PFs also account for the large receptive fields (RFs) of Golgi cells (Vos et al., 1999b). In the latter study, a particular response pattern of Golgi cells, consisting of an early double peak, was assumed to be caused by monosynaptic mossy fibre (MF) excitation

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Abbreviations: CCH, cross-correlation histogram; CV, coefficient of variation; ISI, interspike interval; MF, mossy fibre; MI, modulation index; PF, parallel fibre; PC, Purkinje cell; PSTH, peri-stimulus time histogram; RF, receptive field.

whereas the other response components were presumably due to a mixture of MF and PF excitation (Vos et al., 1999b, 2000). One goal of the present study is to assess the respective contributions of these two afferent pathways in more detail.

A second prediction of the network model was that the degree of synchronisation would increase with the level of granular layer activity (Maex and De Schutter, 1998). While we found indirect evidence for this in previous experimental studies – the precision of synchrony was positively correlated to the mean Golgi cell firing rate (Vos et al., 1999a) – a more direct demonstration requires experimentally activating the circuit. Punctate stimuli were inappropriate because strong phase locking of the response to the stimulus conceals the genuine contribution of granular layer dynamics to synchrony. A sweeping stimulation by a paint brush provided for a semi-continuous activation of MFs with the desired properties.

EXPERIMENTAL PROCEDURES

Surgical preparation

Anaesthesia, surgery, and recordings were performed as detailed before (Vos et al., 1999a,b). All efforts were made to minimise animal suffering and to limit the number of animals used. All animal procedures were approved by the Ethical Committee of the University of Antwerp, Belgium, in accordance with Federal Laws.

Eleven male Sprague–Dawley rats (300–400 g, Iffa Credo, Brussels, Belgium) were anaesthetised with a mixture of ketamine HCl (75 mg/kg; Ketalar, Parke-Davis, Warner Lambert Manufacturing, Dublin, Ireland) and xylazine HCl (3.9 mg/kg; Rompun, Bayer, Leverkusen, Germany) in normal saline (0.9% NaCl, Baxter, Lessine, Belgium). Supplementary i.m. injections (a third of the initial dose) were given when required. Throughout the experiment toe-pinch reflexes were assessed to control the level of anaesthesia. The rat's head was fixed in a Kopf stereotaxic frame (David Kopf Instruments, Bilaney Consultants, Düsseldorf, Germany) with blunt ear bars and a standard mouthpiece with incisor bar. A homeothermic blanket system (Harvard Apparatus, Holliston, MA, USA) was used to maintain core temperature ($\sim 37^{\circ}\text{C}$). A midline incision was made and dorsal cranial muscles were removed to expose the caudal part of the skull. The squamous part of the occipital bone was removed to expose Crus I and II of the left hemocerebellum. Before placement of the electrodes, the dura was reflected and removed. The cisterna magna was punctured and the cerebrospinal fluid drained to reduce cerebellar pulsation. The exposed cerebellar surface was covered with warm agar (2% in 0.1 M phosphate-buffered saline).

Recording procedures

Simultaneous unitary extracellular recordings were made with two or three sharp (1 μm tip) tungsten microelectrodes ($\sim 9.0\ \text{M}\Omega$, Frederick Haer and Co., Bowdoinham, ME, USA), lowered individually. Signals were amplified and filtered (gain, 5000–10000; band pass 0.4–5 kHz), digitised and discriminated using a multi-channel neuronal acquisition processor (Plexon, Austin, TX, USA). Isolated units were identified as Golgi cells using several quantitative criteria: low discharge rate at rest (interspike interval (ISI) $> 20\ \text{ms}$) with no bursting, large bipolar spikes of duration $> 0.8\ \text{ms}$, long tuning distances (50–150 μm), no complex spikes, and location in the granular layer (Vos et al., 1999a,b).

Peripheral stimuli

After several 600-s periods of rest, during which spontaneous Golgi cell discharges were recorded, facial RFs were explored using a cotton-tipped wooden rod. A mechanical stimulation device (1-mm-diameter cylindrical stainless steel probe with a flat surface, maximal excursion 2.5 mm) driven by a Grass 11S stimulator (Astro-Med) was then positioned at the centre (red dot in Fig. 2A) of the RF in order to deliver controlled innocuous tap stimuli (10 ms) to (primarily) facial dermatomes (Vos et al., 1999b). The punctate stimulus was applied at 1 Hz during 200 s.

Animals were then subjected to a second stimulation paradigm in which a larger area of the face (vibrissae and surrounding skin), around and including the point of punctate stimulation (black circle in Fig. 2A), was manually stimulated, using a hand-held brush (15 mm^2 area) (Panetsos et al., 1998). Stimulation was presented in blocks of 100 s ('ON period'), alternated with 100-s blocks without stimulation ('OFF period'). The cross-correlation histograms (CCHs) became periodic during sensory stimulation, with symmetrical side peaks at 250 ms, indicating a brushing frequency of about 4 Hz. This second paradigm was used to increase the firing rate of Golgi cells in a semi-continuous way. All stimuli were applied ipsilateral to the recording site.

Histological procedures

At the end of the experiment, electrolytic lesions (15 μA , 2 s, cathodal DC current) were made to mark the location of the electrode tips, and rats received a lethal dose of sodium pentobarbital (120 mg/kg i.p.; Nembutal, Sanofi, Libourne, France). The brain was removed and fixed in 4% formalin. The cerebellum was embedded in paraffin, and 5- μm -thick coronal sections were cut and stained with Cresyl Violet. For six out of 10 transverse electrode pairs, both lesions could be visualised histologically so that the distance between the units could be measured. The lesions for these pairs were located in the same coronal section, indicating that the two units were positioned along the same PF beam.

Data analysis

Collected spike trains were analysed off-line using NEX (Plexon) and MATLAB software (MathWorks, Natick, MA, USA). To characterise Golgi cell firing at rest, the average firing rate (spikes/s) and the coefficient of variation (CV) of the ISI (standard deviation ISI/mean ISI) were calculated. A smoothed CCH of the spontaneous activity of each pair of Golgi cells was computed from the recorded spike trains at rest, as described before (Vos et al., 1999a; Maex et al., 2000). The significance of the central peak of the CCH was assessed by calculating the standard Z score, with the critical value for significance set at $Z > 3$. The position of the peak on the CCH was given by the bin corresponding to the highest Z score. The precision of coherence was measured as the full width at half height of the central peak.

The latencies of the evoked responses to mechanical stimulation were measured on fine resolution peristimulus time histograms (PSTHs; 0.25 ms bin width) constructed after 200 stimulus presentations. Individual response components were isolated as distinct peaks on the PSTH using an algorithm described previously (Vos et al., 1999b). The mean and standard deviation of the isolated peak determined the latency and accuracy of the response.

To quantify the spike discharge modulation during brush stimulation, a modulation index (MI) was computed for each Golgi cell: $\text{MI} = (f_{\text{ON}} - f_{\text{OFF}}) / (f_{\text{ON}} + f_{\text{OFF}})$, with f_{ON} and f_{OFF} the mean firing rate during the stimulation and rest period, respectively. Changes in synchrony were monitored by subjecting all pairs to cross-correlation analysis during the 'OFF' and 'ON' periods of brush stimulation separately.

Correlations were tested with the z-test's correlation coeffi-

cient (R). A statistically significant relationship required a P value < 0.05 .

The model granular layer

We simulated a slightly modified version of the feedforward inhibition model described in Maex and De Schutter (1998). This granular layer network consists of MFs firing randomly at a given rate, and of single-compartmental, active Golgi cells and granule cells. Along a 14-mm-long one-dimensional array, 756 MFs, 42 Golgi cells and 3009 granule cells were evenly distributed. Each MF innervated the nearest six Golgi cells, resulting in each Golgi cell receiving monosynaptic excitation from 108 MFs. In addition, each Golgi cell received excitation from on average 240 granule cells through PF synapses (connection probability from granule cells to Golgi cells 0.2; PF length 5 mm, PF conduction speed 0.3 m/s). This way Golgi cells exerted both feedforward (MF \rightarrow Golgi cell \rightarrow granule cell) and feedback inhibition (MF \rightarrow granule cell \rightarrow Golgi cell \rightarrow granule cell) on granule cells (Fig. 1A). In the present simulations, the cumulative strength of all MF synapses on a Golgi cell equaled half the strength of all its PF synapses. Each granule cell was excited by a unique set of four MFs selected from the nearest five MFs, and received inhibition from the nearest four Golgi cells. The PF and the ascending segment of a granule cell axon were not modelled explicitly; the delay of activation of a PF synapse on an efferent Golgi cell was set to the distance to the source granule cell divided by the PF conduction speed. As before, synaptic weights and resting potentials were randomised around the median.

In addition to the lower PF conduction speed (0.3 versus 0.5 m/s) and the weaker Golgi cell inhibition of granule cells, which will be motivated in the Discussion, some parameters were changed for practical reasons only: the simulated array was longer than in Maex and De Schutter (1998) to avoid boundary errors during stimulation; the density of granule

cells was lower to speed up simulations; the MF refractory period was decreased from 5.0 to 0.5 ms so as to generate spike doublets during simulated punctate stimulation, and Golgi cells had less spontaneous activity to distinguish easier their stimulus-induced responses.

Model stimuli

Spontaneous activity in the model granular layer was measured during random MF activity. Throughout such a simulation, each MF fired Poisson-distributed spikes at a mean rate of 5 spikes/s.

Model stimuli were applied only to a central subset of MFs, as schematically depicted in Fig. 1B. During simulations of the punctate stimulation experiment, MFs fired randomly as well, but every 360 ms the firing rate of the central subset of MFs was temporarily increased during two 0.5-ms rectangular pulses with a 1-ms interval. This procedure generated a single or double spike in all stimulated MFs (see Fig. 5A). In order to allow for a comparison with the experimentally obtained responses, a 5-ms delay was introduced for the calculation of the PSTHs (Figs. 5B and 6). This delay accounted for the time elapsing in the experiments between the stimulus trigger and the arrival of a MF spike in the granular layer.

Brush stimulation was simulated as follows. From symmetrical side peaks on the experimentally obtained CCHs, it could be concluded that the hand-held paint brush was swept over the RF at a rate of about 4 Hz. We therefore modulated the firing rate of the central subset of MFs in the model by a sine wave with 256 ms period and with amplitude equal to the background spontaneous activity.

Simulations were performed with GENESIS (Bower and Beeman, 1998), using Crank–Nicolson integration with a step size of 20 μ s.

RESULTS

Experimental results

Multi-electrode recordings were obtained from 10 pairs of Golgi cells aligned along the same PF beam. The distance between the six reconstructed pairs ranged from 0.3 to 2.0 mm. Golgi cells showed low activity at rest (mean 8.78 spikes/s) with a fairly irregular firing pattern (mean CV of ISI 0.38). As in our previous study (Vos et al., 1999b), Golgi cells had large RFs, covering the entire territory of the maxillary branch of the trigeminal nerve (rhinarium, all rows of vibrissal pad and upper lip; Waite and Tracey, 1995) but exhibiting local variations in response strength and timing. As a result, the RFs of cells of a pair were often overlapping: stimulation at a particular facial location evoked strong responses in both cells (Fig. 2A).

Responses to punctate stimuli. The majority of Golgi cells (15/17) responded to the punctate stimulus (red dot, Fig. 2A) with both an early (4.0–10.2 ms) and a late (13.6–22.7 ms) excitatory component. Following the classification we introduced previously (Vos et al., 1999b, 2000), four different PSTH profiles were observed. In 12 Golgi cells, both the early and the late component consisted of a single peak. Such a ‘single/single’ response is exhibited by units Me0ca1 and 2 in Fig. 2B. Responses consisting of an early double peak and a late single peak (‘double/single’) were observed in three units. This type of PSTH was evoked in unit Ap9cb1 of Fig. 2B. Two

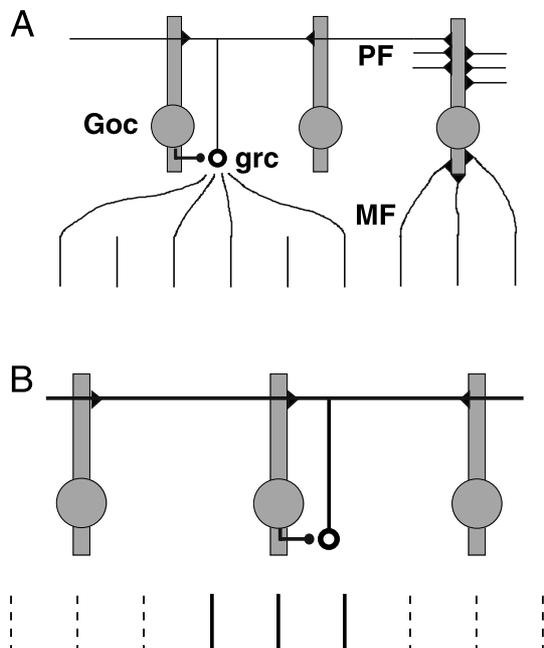


Fig. 1. The model granular layer. (A) The model granular layer is a one-dimensional array of MFs, granule cells and Golgi cells. Granule cells receive excitation from MFs and inhibition from Golgi cells. Golgi cells receive excitation monosynaptically through MFs, and disynaptically through the transverse parts of granule cell axons or PFs. The distance between neighbouring Golgi cells is 300 μ m. (B) The model stimulus is applied to a central subset of MFs (bold lines); the other MFs have a constant firing rate (broken lines).

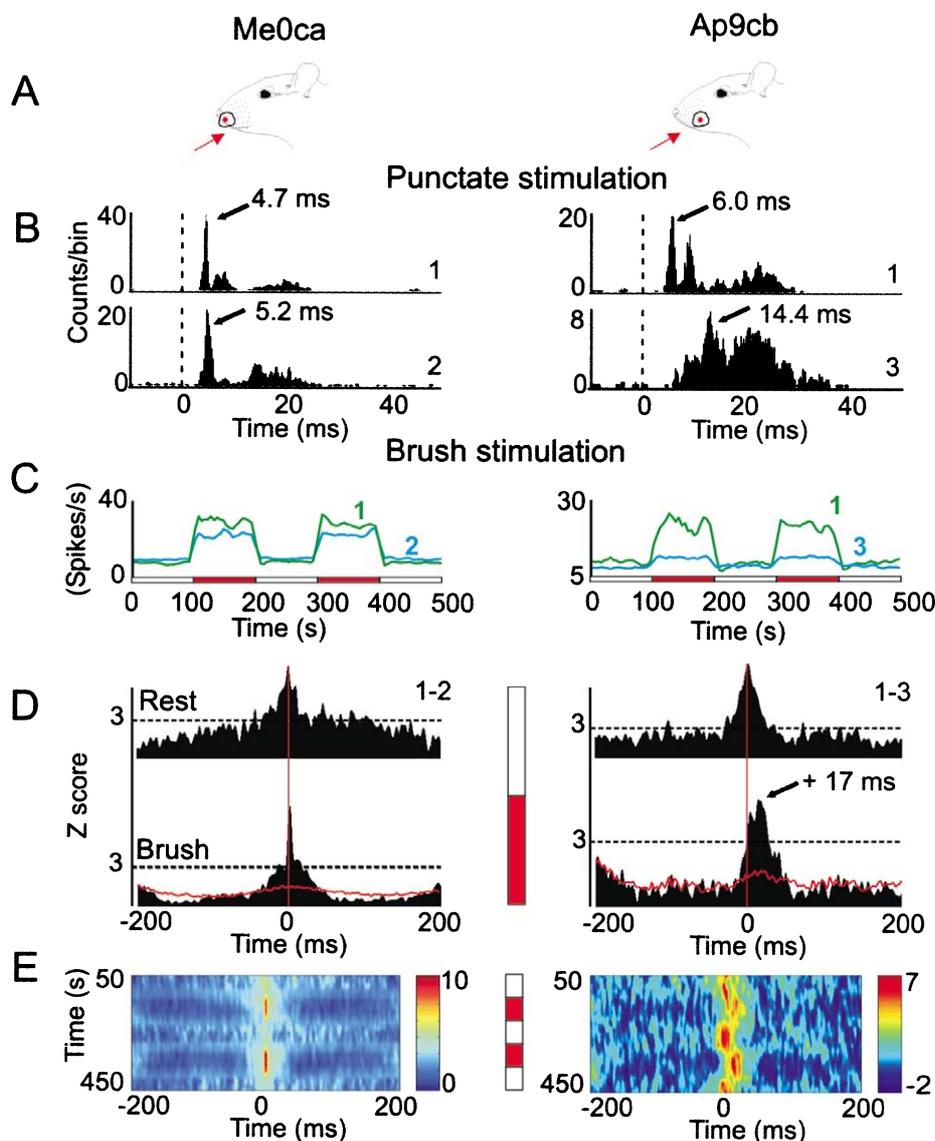


Fig. 2. Responses of two pairs of rat Golgi cells to punctate and brush stimulation. (A) Location of punctate (red dot, vibrissa D7 for pair Me0ca and D2 for pair Ap9cb) and brush stimulation (open circle). (B) Responses to punctate stimulation: PSTHs counting the number of spikes over 200 trials (bin width 0.25 ms). Response latency was measured at the PSTH peak marked by an arrow (see Experimental procedures). (C–E) Responses to brush stimulation; stimulus-ON periods indicated by red bars. (C) Time course of the firing rate, averaged over 5-s non-overlapping sliding windows. Mean firing rate during stimulus-OFF/ON periods: 6.6/30.5 (Me0ca1), 8.8/21.2 (Me0ca2), 7.6/16.1 (Ap9cb1) and 6.0/7.6 spikes/s (Ap9cb3). (D) CCHs (bin width 1 ms; smoothed with a three-point averaging filter) during OFF ('rest', top panels) and ON periods ('brush', lower panels, shuffled CCH in red) expressed as Z score. Dashed lines indicate the significance level, $Z = 3$. (E) Time course of the CCH using 50-s sliding windows incremented every 10 s. Colour legends indicate Z scores.

units produced only a single response component (either early: 'single/–' or late: '–/single').

Responses to brush stimuli. The second stimulation paradigm consisted of a peripheral stimulation of a larger facial area using a manual brush. The stimulus was carefully centred at the locus of punctate stimulation (black circle, Fig. 2A). Using this protocol we were able to modulate the firing rate of the units in a significant manner: the mean Golgi cell activity increased from 8.3 spikes/s during rest (OFF period) to 15.6 spikes/s during stimulation (ON period; mean MI: 0.33). However, a large disparity was observed between units in the

modulation of their discharge rate during brush stimulation (MI 0.04–0.74). This difference in modulation is exemplified by pair Ap9cb in Fig. 2C. In response to the common stimulus, unit 1 greatly increased its firing rate (MI = 0.36), while unit 3 was only weakly modulated (MI = 0.11).

Responses to punctate and brush stimuli compared. The responses of individual Golgi cells to the two stimulation protocols were compared. The amplitude of the response to punctate stimulation was not correlated to the degree of firing rate modulation during brush stimulation ($R = 0.055$; $P = 0.826$). However, a significant

positive correlation was found between the *latency* of the punctate response and the MI. Again looking at pair Ap9cb in Fig. 2B,C, unit 1 had a short-latency double-peaked response and was strongly modulated, whereas unit 3 responded quite late and showed very little firing rate modulation. Figure 3A shows the highly significant correlation between the difference in MI for Golgi cells of the same pair and their difference in latency to punctate stimulation ($R=0.897$; $P=0.0001$).

Synchrony. In accordance with previous studies (Maex and De Schutter, 1998; Vos et al., 1999a), the spontaneous activity of Golgi cells aligned along the coronal axis of a folium (the PF axis) was synchronised. All such simultaneously recorded Golgi cells showed high levels of synchronisation at rest (mean height of the central peak on the CCH, as Z score: 6.65; range 4.45–8.43). Control experiments ($n=5$) confirmed the lack of synchrony at rest in sagittally oriented pairs of Golgi cells (Z score < 3 in all cases).

Brush stimulation led to an increased synchronisation of pairs of Golgi cells, but sometimes additional effects were observed. When one of the units did not strongly increase its firing rate during brush stimulation (unit Ap9cb3 in Fig. 2C), a lag of the central peak appeared on the CCH (Fig. 2D). In that case, the strongly modulated unit led the weaker one, by up to 17 ms. No lag was observed for pairs of which both units modulated strongly their firing rate (e.g. pair Me0ca in Fig. 2D). Indeed, the magnitude of the lag was positively correlated with the *difference* in MI between the two units ($R=0.779$; $P=0.0059$; Fig. 3B). The raw difference in firing rate between the units could not account for this lag ($R=0.055$; $P=0.826$). We also found a positive correlation between the latency difference of a pair to punctate stimulation and the lag of the central peak during brush stimulation ($R=0.837$; $P=0.0013$; Fig. 3C). In addition, the distance between units was positively correlated to the magnitude of the lag for the six pairs successfully reconstructed ($r=0.786$; $P=0.066$, near-zero intercept). Of these, the pair with the largest spacing (2 mm) had a correlation lag of 15 ms.

Finally, the positive correlation between the mean firing rate of a pair and its coherence, predicted by our network model (Maex and De Schutter, 1998), was confirmed for the four pairs that did not present a phase lag during brush stimulation (open circle data points in Fig. 3A–C). The increased discharge during stimulation raised the height of the central peak on the CCH (mean Z score, OFF: 5.74, ON: 10.35), and increased the precision of the coherence (mean peak width, OFF: 22.0 ms, ON: 11.75 ms). An example of this behaviour is shown by pair Me0ca in Fig. 2C, D: the firing rate of both units and their synchrony increased markedly during brush stimulation compared to rest.

Although, in control experiments, all five sagittal pairs of Golgi cells also had overlapping RFs and responded to the brush and punctate paradigms as did the coronal pairs (e.g. Fig. 4A, B), they never developed precise coherent firing during brush stimulation. As exemplified in Fig. 4C, brush stimulation resulted in a very wide

central peak (mean width 28 ms) accompanied with side peaks, reflecting a strong firing rate co-modulation of the two Golgi cells. This observation excluded stimulus-locked spikes as the source of the increased Golgi cell synchronisation observed in the coronal pairs (Fig. 2D).

Simulation results

We used computer simulations to explain the observed difference in response timing between two simultaneously recorded Golgi cells during peripheral stimulation. Equivalently, the model explains the difference in response timing of a single Golgi cell to stimuli presented at different locations in its RF (Vos et al., 1999b). In the simulated model, the Golgi cells received a monosynaptic

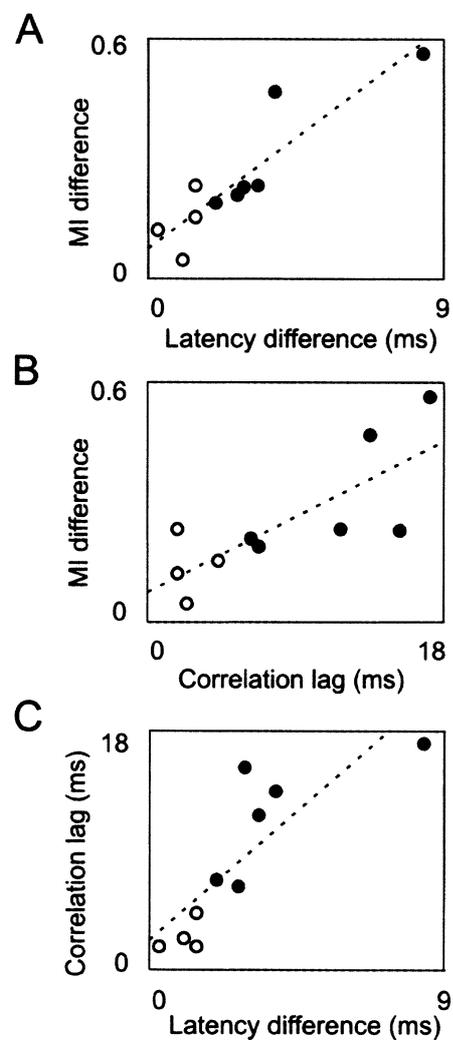


Fig. 3. Scatterplots of the relationships between the punctate and brush responses of all transverse rat Golgi cell pairs. Pairs which presented a phase lag < 3 ms during brush stimulation are represented as open circles. The measured response variables for each pair are: the difference in punctate response latency between the two cells (horizontal axes in A and C), the difference in brush response MI between the two cells (vertical axes in A and B), and the lag of the central peak on the CCH during brush stimulation (horizontal axis in B, vertical axis in C). Significant positive correlations were found for the three relations (see Results).

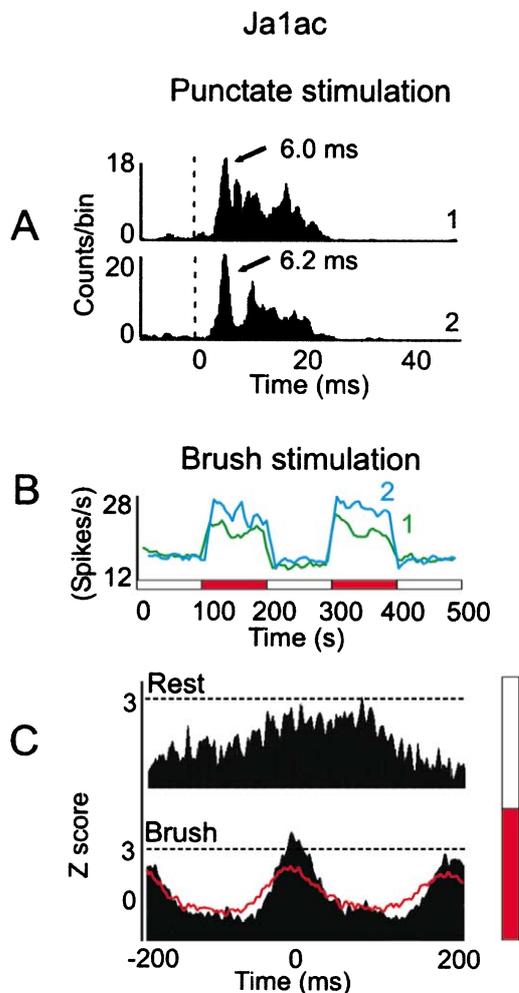


Fig. 4. Responses of a sagittal pair of rat Golgi cells to punctate and brush stimulation. (A) Responses to punctate stimulation. PSTHs counting the numbers of spikes over 200 trials. (B, C) Responses to brush stimulation; stimulus-ON periods indicated by red bars. (B) Time course of the firing rate (averaged over 10-s non-overlapping sliding windows) of the recorded units. Average firing rates during stimulus-OFF and ON periods were: 13.4/20.6 spikes/s (Ja1ac1, green curve), 13.5/25.2 spikes/s (Ja1ac2, blue curve). (C) CCHs (1-ms bin) during OFF ('rest', upper panels) and ON periods ('brush', lower panels, shuffled CCH shown as red dashed line) expressed as Z score. The black dashed lines indicate the significance level, $Z=3$. Notice similarity of shuffled and raw CCHs (compare to Fig. 2D).

excitation from MFs innervating their RF centre and a disynaptic excitation over PFs from an area, overlying and surrounding the centre (Fig. 1A). Peripheral (brush or punctate) stimuli activated only a local patch of MFs (Fig. 1B).

During simulations of spontaneous granular layer activity, Golgi cells and granule cells fired at 5.9 and 1.1 spikes/s, respectively. Model Golgi cells fired more regularly than those recorded from (mean CV of ISI 0.194), presumably because they were activated by MFs firing at a constant rate. The spontaneous firing rate of *in vivo* granule cells has not been reported.

Responses to punctate stimuli. Punctate stimulation was simulated by generating doublet spikes in a central

patch of MFs (Fig. 5A). As shown by the PSTHs of Fig. 5B (left column), the response of a model Golgi cell varied in a systematic way with position along the array. As the network is symmetric, only the upper half of the Golgi cell array is shown. Golgi cells in the centre of the patch (Golgi cells 22–25 in Fig. 5B, left column) fired first an early doublet spike, whereas those at the patch border (Golgi cells 27–28) fired only a single early spike because fewer of their afferent MFs originated from the stimulated patch. This early response was followed by a slower response in all Golgi cells positioned within one half a PF length from the activated patch (Golgi cells 22–33). Golgi cells not monosynaptically excited by the stimulus showed only this late response component (Golgi cells 29–33).

Following the response classification of Vos et al. (1999b), the Golgi cells along the array showed a transition from 'double/single' responses at the centre of the patch, to 'single/single' responses at the patch border, to a '-/single' response outside the activated patch. Responses with only an early single component ('single/-') were easily generated in other simulations in which a weaker MF stimulus was applied (not shown).

The strength of the late response component depended on the number of active PFs at each location, which decreases almost linearly with distance from the locus of MF stimulation. The latency of the late response increased with distance (Fig. 5B, left column), as it is primarily determined by the PF conduction delay.

In order to distinguish the contributions of the monosynaptic MF and disynaptic PF pathways to Golgi cell excitation, the PSTHs of the same Golgi cells were calculated in a model granular layer lacking granule cells and PFs (Fig. 5B, right column). From this comparison, it appeared that those response components with latencies ranging from as small as 7 ms (the second spike of an early doublet) to as large as 20 ms (a late single peak) depended, partially or completely, on PF excitation.

Finally, after their discharge in response to the punctate stimulus, model Golgi cells were silent for a variable period of time (between 50 and 200 ms; Fig. 6, upper curve) proportional in duration to the strength of their initial response (see Vos et al. (1999b) for the same, experimental observation). This silent period was not due to a suppressed input from the granule cell population, which remained silent for only 25 ms (Fig. 6 lower curve), but appeared to be caused by an intrinsic after-hyperpolarisation effectuated by the K_{Ca} current.

Responses to semi-continuous stimuli. Next, we modulated sinusoidally in time the firing rate of the central patch of MFs. This sinusoidal stimulus did not contain the noise inherent to hand-held stimulation, but it allowed us to separate mathematically the synchrony generated by firing rate modulation from synchrony generated in the granular layer circuit itself (Maex et al., 2000). The different effects of monosynaptic MF excitation and disynaptic PF excitation on response strength and timing were now apparent in the response amplitude and phase.

Model Golgi cells monosynaptically stimulated had

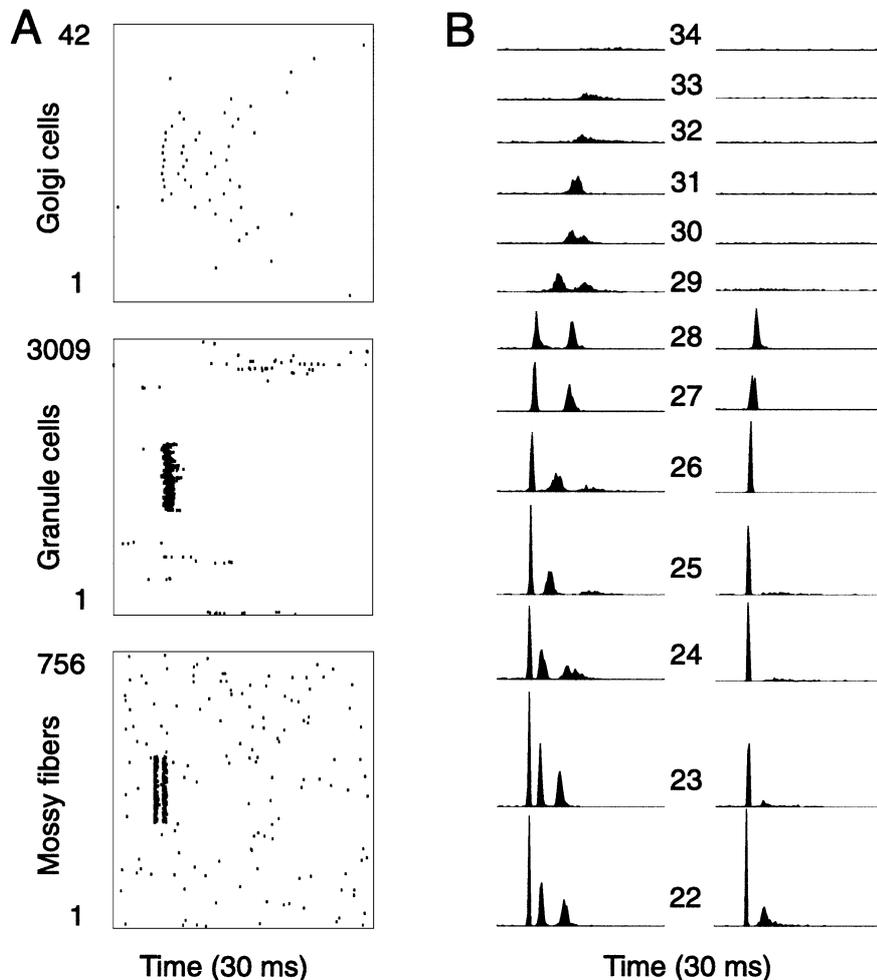


Fig. 5. Model responses to a punctate stimulus. (A) Thirty-millisecond time window of a single trial, with each dot representing a spike fired in the population of 756 MFs (lower panel), 3009 granule cells (middle panel) and 42 Golgi cells (upper panel; vertical axes indicate neurone numbers). The stimulus evoked a doublet spike in the central patch of 180 MFs. (B) Left: PSTHs (bin width 0.25 ms) of individual model Golgi cells from 305 repetitions of the trial in A. Golgi cell positions ranged from the patch centre (Golgi cell 22) to completely outside the patch (30–34). Right: PSTHs of the same Golgi cells in a network without granule cells, and hence without PF synapses on Golgi cells.

the largest response amplitudes. CCHs between a Golgi cell inside and one outside the MF patch exhibited a positively lagged central peak (Fig. 7B), similar to the lagged CCH peaks observed experimentally (pair Ap9ab in Fig. 2D). This lag was proportional to the distance of the second Golgi cell from the patch. Like in the experiments, the central peak lag also correlated with the difference in latency between the pair's responses to punctate stimulation (compare Figs. 5 and 7) and with the difference in MI during sinusoidal stimulation. In Fig. 7 the differences in MI between reference and target sets of Golgi cells measured 0, 0.07, 0.18 and 0.24, respectively. The corresponding CCHs peaked at 0, 3, 6 and 11 ms.

In addition, during stimulation, the synchrony was more precise (narrower central peak) than during spontaneous activity (Fig. 7A, B). This finding should be ascribed to the increased Golgi cell firing rate during the positive turns of the input modulation (see Maex and De Schutter (1998)).

We considered the possibility that the lag of the central CCH peak was caused by a stimulus-induced imbalance in firing rate between the component model Golgi cells. Indeed, a neurone firing at a higher rate tends to fire earlier than, and hence to lead, a less active neurone, the latter requiring a longer synaptic integration time to reach the firing threshold (Maex et al., 2000). However, in control simulations where the PF conduction delays were set to zero, the maximal peak lag was only 2 ms. This confirmed that the slowly conducting PFs generated the observed lag of the central peak.

DISCUSSION

In anaesthetised rats, pairs of Golgi cells simultaneously recorded from along the PF axis responded with a different strength and timing to a focal tactile stimulus. All response components to different stimulation paradigms could be reproduced with a detailed com-

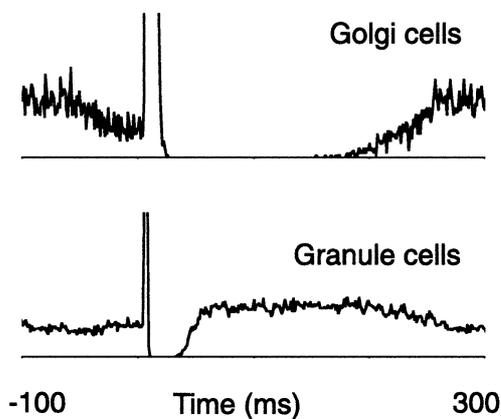


Fig. 6. Model Golgi cell discharges are followed by a long silent period, like in the experiment. The responses to the punctate stimulus of Fig. 5 are shown in a 400-ms window and averaged over the central 10 Golgi cells (upper PSTH, truncated at 20 spikes/s) and 600 granule cells (lower PSTH, truncated at 5 spikes/s).

puter model of the granular layer, in which the stimulus activated a patch of MFs, which monosynaptically excited granule cells and Golgi cells. The granule cell activity propagated along the PFs, giving rise to a beam-like spread of disynaptic Golgi cell activation. Differences in response strength and timing between simultaneously recorded Golgi cells were explained by putative differences in their distance from the centre of MF activation. This, together with the large RFs of Golgi cells, leads us to conclude that peripheral stimuli evoke a beam-like spread of activity along the PF axis.

Restrictions and constraints of the model

Several parameters of the model, especially those describing the feedforward inhibitory pathway from MFs over Golgi cells to granule cells, are incompletely constrained by available experimental data. The MF synapses on model Golgi cells needed to be strong enough in order to generate an early spike doublet in Golgi cells (Fig. 5), but when taken too strong they would prevent the spontaneous synchrony observed between Golgi cells during random MF activity (Fig. 7A) (Maex and De Schutter, 1998). In addition, the inhibition exerted by Golgi cells on granule cells needed to be taken half the strength of that used before (Maex and De Schutter, 1998). Indeed, because the model Golgi cells fired early doublet spikes to the punctate stimuli, the temporal summation of postsynaptic inhibitory currents in granule cells would otherwise have suppressed completely the PF component of the Golgi cell responses.

Quantitatively, the results depended on the PF conduction speed, which was set to 0.3 m/s. Reported values vary from 0.2 m/s at 28°C (Cohen and Yarom, 1998) and at 32°C *in vitro* (Vranesic et al., 1994), to 0.3–0.4 m/s *in vivo* (Eccles et al., 1966; Garwicz and Andersson, 1992), and even 0.3–0.7 m/s in experiments where the temperature of the agar over the exposed cerebellar surface was maintained at 35–37°C (Merrill et al., 1978). These values can be partly reconciled by taking into account the temperature dependence of the speed of spike propaga-

tion. The correction factor for conversion from 27 to 37°C is about 1.6 for unmyelinated axons (Hopkins, 1975; Hille, 1977).

It is remarkable that even with these parameter modifications, which reduced the strength and speed of the Golgi cell–granule cell feedback loop, the model granular layer exhibited synchronous oscillations when the MF firing rate slightly increased (e.g. during the peaks of the sinusoidal modulation, Fig. 7B).

However, none of the conclusions of the present study depended on the applied spatiotemporal pattern of MF stimulation. Stimulation of multiple, smaller MF patches yielded qualitatively the same results as those obtained with stimulation of a single patch.

Sources and timing of synaptic inputs to Golgi cells

The present study confirms our previous speculations on the origin of different types of Golgi cell responses (Vos et al., 1999b, 2000) and extends it to semi-continuous MF stimulation. The present model network of MFs, granule cells and Golgi cells explains the experimentally observed Golgi cell responses during a time window up to 20 ms after the presentation of a periph-

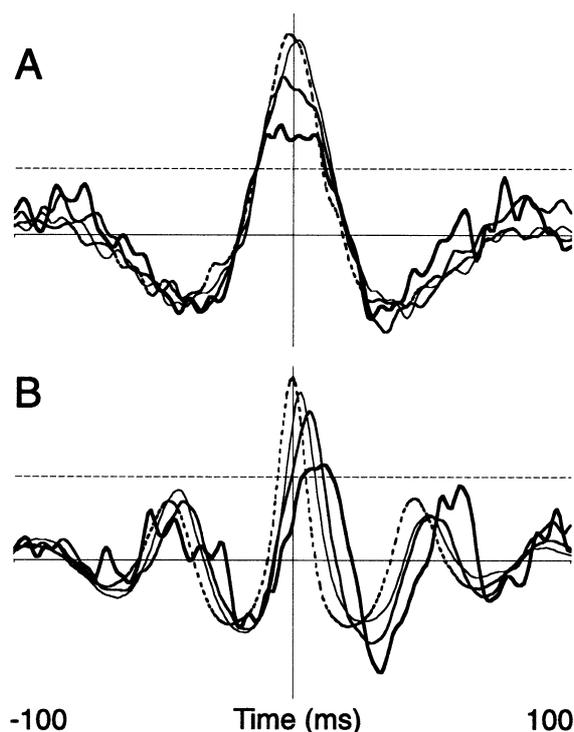


Fig. 7. Model Golgi cell responses to a sinusoidal stimulus. (A) CCHs during simulated spontaneous activity. (B) CCHs during sinusoidal firing rate modulation of the central patch of MFs. The stimulus-locked firing rate modulation of ~ 4 Hz was removed from the CCHs in B by zeroing its amplitude after fast Fourier transformation (Press et al., 1992). For robustness, the CCHs are mean CCHs calculated between two sets of three Golgi cells. The fixed reference set was inside the patch (Golgi cells 22–24; see Fig. 5). Target sets were either inside the patch (Golgi cells 19–21; broken line) or at increasing distances from the patch centre (Golgi cells 25–27, Golgi cells 28–30 and Golgi cells 31–33, represented by solid curves of increasing thickness). Dotted horizontal lines in A and B indicate the significance level, $Z=3$.

eral stimulus. The precisely timed response components were generated in the model with trigeminal MF and PF excitation alone, i.e. without a need for other, non-modelled synaptic inputs to Golgi cells originating from Lugaro cells (Dieudonné and Dumoulin, 2000), stellate cells, climbing fibres, the pontine nuclei (Leergaard et al., 2000) or the lateral reticular nucleus.

An important finding of the modelling study is that PF excitation contributed to the Golgi cell discharge as fast as 7 ms after stimulation. The second spike of an early doublet response disappeared almost completely when the PF input was cut in the model (Fig. 5B, right column), but also when a weaker MF input was applied (not shown). This indicates that this response component was the combined effect of MF and PF excitation.

The model late response component (13–26 ms) could be completely attributed to PF excitation. Conduction delays of more than 8 ms over a PF branch, combined with delays generated in granule cells, resulted in a disynaptic excitation of Golgi cells with latencies of up to 20 ms. The PF conduction delays were also responsible for the lag of the central peak on the CCH observed during brush stimulation.

It is probable, however, that the actual late response component, which was usually stronger in the experiments than in the model, is the combined effect of a disynaptic excitation through PFs and a multi-synaptic excitation involving the cortico-pontocerebellar pathway (the latter not being included in the model). Indeed, the late response component has been observed to decline during inactivation of the somatosensory cortex (Morissette and Bower, 1996). It must also be noted that the stimulus probe, which was in contact with the rat's face during 10 ms in the punctate experiments, may have evoked a response when retracted (OFF response; Kyriazi et al., 1994). The late response component, however, was also apparent when the contact duration was prolonged to 50–100 ms. At these stimulus durations, an OFF-response was clearly separable from the late ON response (data not shown).

Our minimal network model generated loose synchrony and response delays along the coronal axis, through the single mechanism of the PFs' slow spike propagation. Although other pathways and mechanisms cannot be excluded to have contributed to our experimental observations, it is unclear how they would generate delays with a spatially anisotropic pattern. For example, the inhibitory Lugaro cells were proposed to synchronise Golgi cells (Dieudonné and Dumoulin, 2000). It would indeed be difficult to distinguish, from the CCH, common inhibition from common excitation as the cause of synchrony (Maex et al., 2000). However, Lugaro cell axons are partly myelinated and thicker, and therefore probably faster than PFs, and develop also plexi along the sagittal axis (Dieudonné and Dumoulin, 2000). Similar arguments hold against a common Golgi cell excitation by mossy fibres.

Synaptic integration by Golgi cells

In the present single-compartmental model Golgi cells,

the conductances of MF and PF synaptic channels summed in a linear way. Hence, the stimulus-evoked responses of rat Golgi cells were well explained by a linear integration of MF and PF input. Nevertheless, intrinsic Golgi cell properties shaped the discharge to punctate stimuli in a nonlinear way, i.e. by saturating the strong, early monosynaptic MF response and thresholding the weaker disynaptic PF response (Fig. 5B). The excitatory discharge was followed by a long silent period (Fig. 2B; Vos et al., 1999b). The model suggests that the silent period is due to the activation of a K_{Ca} current, and not to a depressed granule cell input (Fig. 6). During semi-continuous brush stimulation on the other hand, the mean firing rate of Golgi cells increased, and linearised their responses. The above nonlinearities explain the absence of a significant correlation between the amplitude of the punctate response and the MI of the brush response (see Results). These variable response modes of Golgi cells may have important implications for their function (De Schutter et al., 2000).

Activation of parallel fibres

The present model of Golgi cell excitation combines a classic view of the PFs, i.e. waves of activity spreading along a PF beam (Braitenberg and Atwood, 1958; Eccles, 1973) with more recent insights into the anatomical organisation of MF inputs to the cerebellar hemispheres (Bower et al., 1981). An important conclusion of our study is that stimulation of a patch of MFs activates a beam of PFs. But whereas this PF beam causes Golgi cells to fire, the excitatory RF of PCs does not differ from that of the immediately subjacent granular layer (Bower and Woolston, 1983; Cohen and Yarom, 1998). Synapses made by the ascending segment of the granule cell axon may explain larger responses in PCs overlying the activated MF patch (Gundappa-Sulur et al., 1999), but it seems unlikely that this is sufficient to explain the absence of PC responses along the beam.

The smaller RFs of PCs, and the failure of pairs of on-beam PCs to fire in synchrony, indicate therefore that PFs exert different excitatory, or disynaptic inhibitory, effects on PCs and Golgi cells. First, it has been established that although PF synapses are more numerous on PCs, individual synapses are weaker on them. The mean number of PF synapses on a rat PC, estimated to be over 150 000 (Harvey and Napper, 1988), admittedly must be an order of magnitude larger than the number of PF synapses on Golgi cells. Indeed, PFs make 90% of their synapses on PC spines (Pichitpornchai et al., 1994), leaving no more than 10% for the equally numerous Golgi cells and the ten times richer stellate and basket cells (Korbo et al., 1993). However, *in vitro* stimulation of individual granule cells evoked stronger postsynaptic currents in Golgi cells (mean amplitude 38 pA, Dieudonné, 1998) than in PCs (mean 14 pA, Barbour, 1993), and from the low ratio of electrophysiologically connected granule cell–PC pairs it was concluded that most PF synapses on adult rat PCs are silent (Isope and Barbour, 2001). Other PF synapses on PCs could have been weakened by long-term depression (Linden

and Connor, 1995). Furthermore, even if the total excitatory current evoked by all active PFs were as large in PCs as in Golgi cells, it is known from computer simulations that the large number of weak synapses would be less effective to fire, and synchronise, the PCs than would be the activation of a smaller number of stronger synapses, as found on Golgi cells (Maex et al., 2000; Solinas et al., 2001).

Second, whereas both PCs and Golgi cells receive inhibitory synapses from stellate cells on their dendritic tree, basket cells form typical plexi on the soma and the axon initial segment of PCs (Palay and Chan-Palay, 1974). The PF response of PCs could therefore be more suppressed by on-beam inhibition (Cohen and Yarom, 2000). In accordance with this, PCs can be inhibited by peripheral stimuli from an area that is larger than

their excitatory RF (Eccles et al., 1972; Bower and Woolston, 1983; Ekerot and Jörntell, 2001). Conversely, our modelling study suggests that the inhibitory inputs on Golgi cells (Dieudonné, 1995) have little effect on the response patterns we investigated experimentally. Therefore measuring Golgi cell activity may be a good way to infer PF activity.

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