Available online at www.sciencedirect.com
SCIENCE DIRECT
Neuroscience Letters 390 (2005) 156–161
Neuroscience Letters
www.elsevier.com/locate/neulet

Temporal characteristics of tactile stimuli influence the response profile of cerebellar Golgi cells

Koen Tahon, Antonia Volny-Luraghi, Erik De Schutter*

Laboratory of Theoretical Neurobiology, Department of Biomedical Sciences, University of Antwerp, B-2610 Antwerp, Belgium

Received 7 April 2005; received in revised form 1 August 2005; accepted 5 August 2005

Abstract

An increasing number of studies have investigated the effect of stimulation parameters on neuronal response properties. Here, we describe the effect of temporal characteristics of tactile stimuli, more specifically the stimulation frequency and duration, on the response profile of simultaneously recorded cerebellar and cerebral cortical units in ketamine–xylazine anaesthetized rats. Long stimulus durations (>50 ms) elicited ON and OFF excitatory components in response to the stimulus onset and offset respectively, in both the cortex and the cerebellum. Golgi cells responded on average 7.5 ms later to the stimulus withdrawal than to the stimulus onset. Furthermore, the corticopontine OFF responses in the cerebellum and OFF responses in the cortex showed congruent latency decreases and amplitude increases for longer stimulus durations (50–200 ms). Decreasing the stimulus frequency similarly affected the latency and amplitude of the responses for inter-stimuli intervals shorter than 200 ms. In view of these results, we speculate that the stimulus offset is regarded as a novel input, because both paradigms resulted in similar response amplitude and latency modifications. Finally, the results suggest that a 100–200 ms time window can be of particular importance for cerebellar processing of information in the somatosensory system.

Keywords: SI cortex; Cerebellum; Golgi cell; Offset; Frequency; Stimulus duration

The somatosensory system of the rat comprises the parts of the nervous system necessary to carry and process information from receptors to cerebral cortex. Despite earlier studies [5,12,13,19–21] the role of the cerebellum in the somatosensory pathways remains to be defined. As the main inhibitory units of the cerebellar granular layer, Golgi cells play an important role in the preprocessing of afferent information [3,8,14,16]. A distinctive feature of these neurons is the combined trigeminocerebellar and corticopontine inputs they receive, which enable a direct comparison of the information processing in the two pathways [18,26,27].

The present work focused on the temporal aspect of tactile stimulation, specifically the stimulus duration and frequency of stimulation.

Anaesthesia, surgery, and recordings were performed as detailed before [26]. In brief, 25 male Sprague–Dawley rats (300–400 g) were anesthetized with a mixture of ketamine (75 mg/kg) and xylazine (3.9 mg/kg). A craniotomy was made to access the perioral representation of the cerebellum. The craniotomies were extended to include the contralateral SI cortex in five animals. Efforts were made to minimize 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.

Unitary extracellular recordings were made [26]. Golgi cells (Goc; $n = 31$) were identified using several quantitative criteria: low discharge rate at rest (theta frequency band; minimum interspike interval >20 ms) with distinctive rhythm and without bursting, large bipolar spikes of duration >0.8 ms, long tuning distances (50–150 μm), no complex spikes, and location in the granular layer [26]. Cerebral cortical units (CoU; $n = 8$) were recorded in deep layer V (1300 μm down).

A custom-built mechanical stimulation device was positioned at the centre of the receptive field in order to deliver controlled innocuous mechanical tap stimuli to the base of

* Corresponding author. Tel.: +32 3 8202616; fax.: +32 3 8202669.
E-mail address: erik@tnb.ua.ac.be (E. De Schutter).
URL: http://www.tnb.ua.ac.be.

0304-3940/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2005.08.018

the intact vibrissae (29% of recorded Gocs; 25% of recorded CoU) or perioral structures (71% of recorded Gocs; 75% of recorded CoU) [26]. The results described in this paper were independent of stimulus location (not shown).

We used two stimulation paradigms. Fifteen Golgi cells and eight SI cortical units were subjected to the first paradigm which consisted of a series of a minimum of 200 stimulus repetitions, with an interval between two successive stimulus onsets (interstimulus interval) fixed at 1000 ms. The stimulus duration, constant within a series, was increased in successive series from 10 to 700 ms. Paradigm 2, applied to a second group of Golgi cells ($n = 16$), consisted of 200 stimulus repetitions, with the stimulus duration fixed at 10 ms. The interval between successive stimulus onsets (interstimulus interval), constant within a series, varied from 100 ms (or 10 Hz) to 1000 ms (1 Hz) for successive series.

All parameters of the evoked responses to mechanical stimulation were measured by using fine resolution peristimulus time histograms (PSTHs; 1 ms bin width) constructed after 200 stimulus presentations.

Responses latencies to the stimulus onset (ON components) and offset (OFF components) for each unit were calculated as the time from the stimulus onset/offset at which the firing rate crossed the half-peak value (after subtracting background activity). The amplitudes were computed as the total number of spikes fired above background level within the component's time windows.

Linear regression analysis was used to fit lines to the response curves. r^2-Values were considered significant when ANOVA test for regression analysis yielded a P-value <0.05.

All recorded cerebellar units were identified as Golgi cells with spontaneous activity in the theta-frequency band, as detailed before [25]. Fig. 1 shows a typical response profile of a Golgi cell and SI cortical unit to an increase in stimulus duration. The top panel shows the response to a 1 Hz, 10 ms stimulation. Following the classification introduced in our previous work [26], the majority of the Golgi cells (17/31) responded to the punctate stimulus with both an early (8.21 ± 2.87 ms) and a late (17.37 ± 3.1 ms) excitatory component, mostly caused by input via the trigeminocerebellar and corticopontine projections, respectively [18,26]. The response of Golgi cells included a period of decreased activity, or "silent period" which started at 34 ± 3.5 ms (mean $\pm$ S.D.) and lasted for 200 ± 78 ms. In a large number of Golgi cells (20/31), the silent period was followed by a period of rebound activity starting at 203 ± 60 ms after the stimulus onset and ending at 382 ± 87 ms.

In response to increasing stimulus durations (first paradigm) an OFF component evoked by the stimulus offset appeared in all of the recorded Golgi cells; this OFF component was not present at 10 ms. In analogy with the early and late ON peaks, the OFF response was composed of early and/or late components.

The origin of the late OFF peak was investigated by simultaneously recording SI cerebral cortical units ($n = 8$, in five animals) and cerebellar units with overlapping receptive

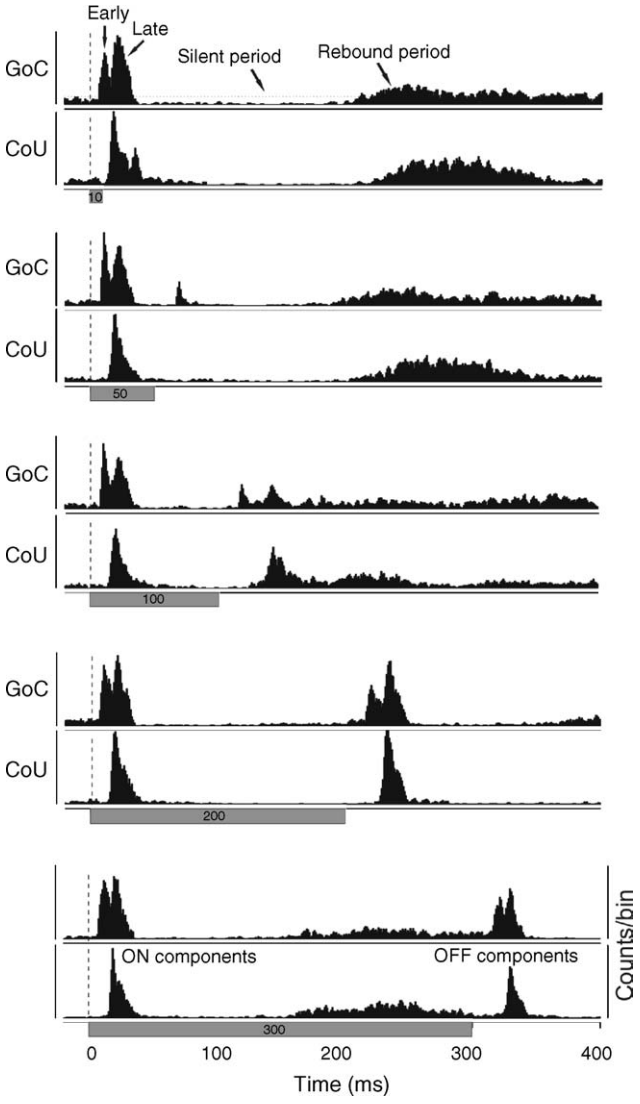

Fig. 1. Example of stimulus-evoked responses (RF: upper lip) in a simultaneously recorded cerebellar Golgi cell (GoC) and SI cortical unit (CoU) (PSTHs 1 ms bin). The Golgi cell exhibited both early and late excitatory components ("ON") in response to the stimulus onset (indicated by the vertical dotted line). The components were followed by a pronounced silent period and a rebound excitatory period. Lengthening the stimulus resulted in the appearance of "OFF" components evoked in response to the stimulus offset (stimulus duration indicated by grey bars). Note that the cerebellar late OFF component and the cerebral cortical OFF component appeared together.

fields (Fig. 1). The Golgi cell responded to the shortest stimulus (10 ms) with both early and late ON components (Fig. 1, top frame). Increasing the stimulus duration to 50 ms resulted in a small and single OFF component, evoked only in the Golgi cell (second frame). A further increase to 100 ms evoked a late OFF component in addition to the early one in the Golgi cell, and a single OFF component in the cerebral cortical unit. At a stimulus duration of 200 ms, ON and OFF responses reached the same amplitude level. Thus, the appearance of the late OFF cerebellar component coincided with the occurrence of the cerebral cortical OFF component. Furthermore, we observed that OFF components

were only evoked if the corresponding ON responses were also present. Taken together, these observations suggest that the early and late OFF components reflected the trigeminal and corticopontine inputs respectively, in analogy with the proposed origin for the early and late ON components.

While the ON responses remained constant for increasing stimulus duration, the OFF responses changed in both amplitude and latency (see infra). We compared these changes to the sensitivity of ON responses of Golgi cells to increasing stimulus frequency (second stimulation paradigm). We observed that the OFF response profile of Golgi cells to an increasing stimulus duration was similar to the ON response profile to a decreasing stimulation frequency (Fig. 2). This is easier to understand if one considers the second paradigm as a change in interstimulus interval. The ON response profile to a high frequency stimulus (Fig. 2B; 10 Hz) is comparable to the OFF response profile of a short stimulus duration (Fig. 2A, 100 ms, ON responses are omitted for clarity).

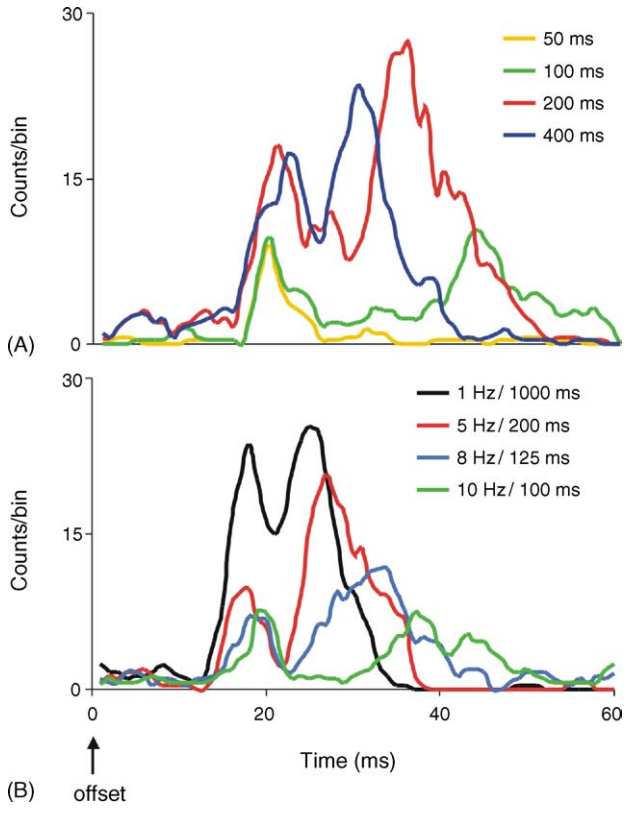

Fig. 2. Similarity between the effect of stimulus duration (A) and interstimulus interval (B) on different Golgi cells, exhibiting both an early and late component. The responses to the different stimulation conditions were superimposed on each other. (A) Same cell as GoC in Fig. 1. The latency of the late OFF component decreased with increasing stimulus duration, whereas the amplitude of both OFF components increased. The ON components were omitted for clarity. (B) Similar effects were seen when increasing the interval between two stimuli (equivalent to decreasing stimulation frequency). It resulted in an increase in amplitude of both components and a shift towards shorter latencies for the late component only.

The dependence of the response amplitudes for the cerebellar and cerebral cortical populations on stimulus duration is depicted in Fig. 3. Increasing the stimulus duration did not affect the amplitude of the ON components. For the three curves (Fig. 3A), regression lines fit to the responses had slopes that did not differ significantly from zero ($r^2 < 0.03$ and $P > 0.1$). The corresponding OFF responses showed a different profile (Fig. 3B). The responses to the stimulus offset gradually increased for increasing stimulus durations ($r^2 > 0.4$, $P < 0.001$, for the three curves, stimulus duration 30–200 ms), before reaching a plateau at ∼200 ms. Interestingly, the initial epoch of ∼200 ms of small OFF amplitudes mirrored the timing and duration of the silent period evoked by a single short stimulation in Golgi cells. It thus corresponded to a period during which the reduced excitability following the stimulus onset could influence the size of the OFF response. For stimulus durations of 200–400 ms, the amplitude of the OFF components slightly decreased, possibly corresponding to the period of rebound excitation observed after a single stimulus. Increasing interstimulus intervals resulted in a similar modification of the early and the late ON response components of a typical unit. The amplitude of both components showed an initial rapid increase ($r^2 > 0.2$, $P < 0.001$, intervals 100–300, Fig. 3D) before reaching a maximum for interstimulus intervals above 300 ms.

The latencies of cerebellar and cerebral cortical ON components were independent of the stimulus duration ($r^2 < 0.02$, $P > 0.1$, regression analysis, Fig. 3C), with mean latencies in agreement with previous observations [26]. The latency of the early OFF component, although significantly longer (mean latency: 16.48 ± 0.59 ms), showed the same level of stability. In contrast, the latencies of both the late cerebellar and the cerebral cortical OFF components clearly decreased for increasing stimulus duration ($r^2 > 0.4$; $P < 0.0001$, stimulus duration 50–250 ms, Fig. 3C) before stabilizing for durations >300 ms (mean latency: 25.69 ± 1.09 ms for cerebellar units, 19.98 ± 0.69 ms for cerebral cortical units, stimulus duration 300–700 ms). Again similar trends were observed when latency values for both early and late components were plotted against the interstimulus intervals (Fig. 3E). The latency of the early component (mean over all frequencies: 8.17 ± 0.69 ms) was unaffected by the change in the stimulus frequency). The latency of the late component, on the other hand, decreased steadily from 25 ms (24.83 ± 1.5 ms) before slowly stabilizing for long interstimulus intervals (mean latency: 17.03 ± 0.5 ms, intervals 500–1000 ms).

The response of a cell to the withdrawal of a stimulus, or stimulus offset, is frequently reported in the rat somatosensory pathway, including the trigeminal ganglion, the ventrobasal thalamus, and the barrel cortex [4,15,17,22]. Common to these studies is the long stimulus duration, over 200 ms, used. However, studies specifically investigating the effect of stimulus duration are sparse [15]. Here we examined whether this response to the stimulus offset, or OFF component could also be evoked in the cerebellum. This question was especially relevant as our previous work,

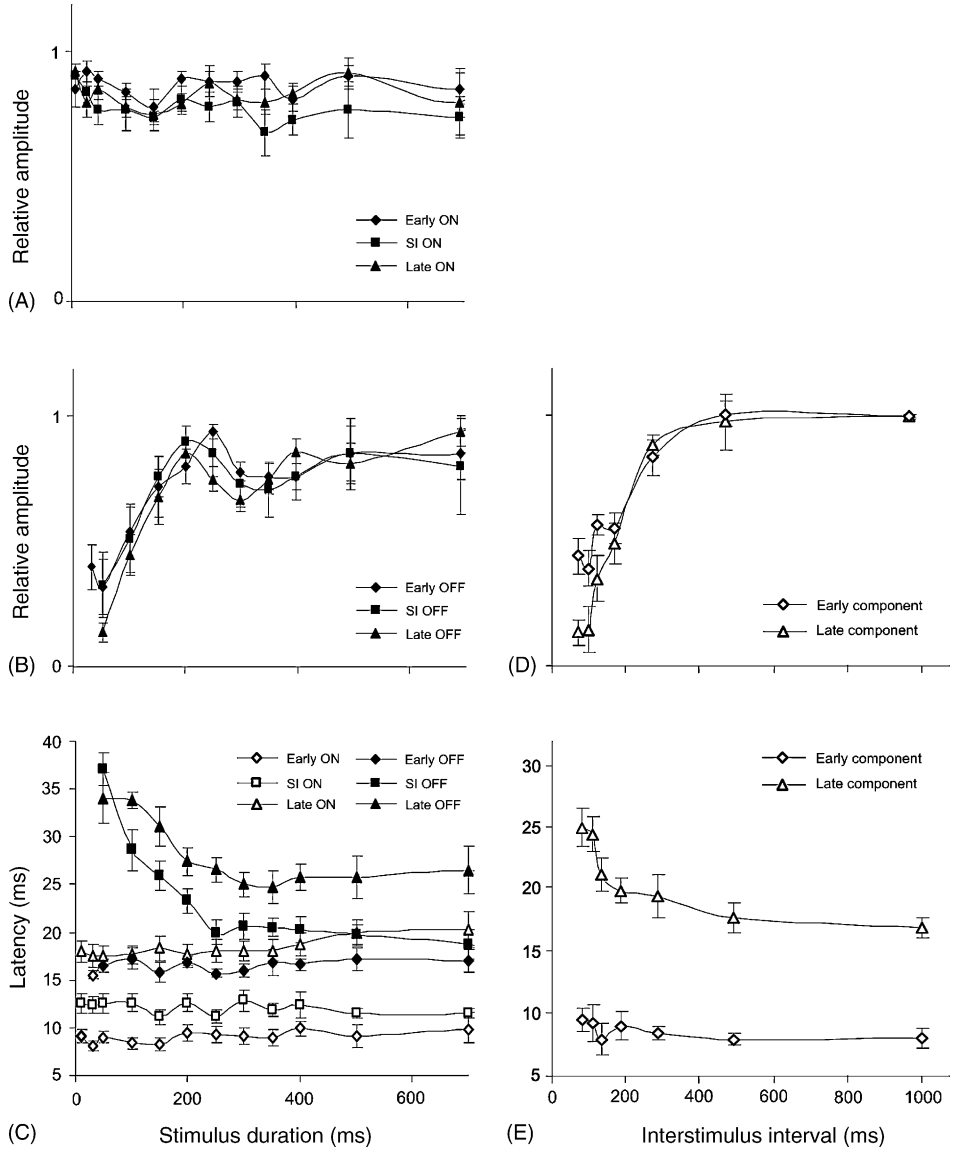

Fig. 3. Effects of temporal characteristics on the response profile of cerebellar Golgi cells and SI cortical units. (A) Increasing the stimulus duration (from 10 to 700 ms) has no effects on the ON response amplitudes. (B) OFF response amplitudes sharply increased before reaching a maximum for stimulus duration ~200 ms. (C) The latencies of the cerebellar late OFF and of the cerebral cortical OFF components decreased rapidly before slowly stabilizing. The other components were independent of the stimulus duration. (D) Effect of interstimulus interval (stimulation frequency) on the amplitude of the early and late cerebellar components. Both components increased, when the time between two stimuli was increased, before reaching a maximum. (E) Effect of interstimulus interval on the latencies of the early and late cerebellar components. While the latency of the early component was relatively stable, the latency of the late component slowly decreased with increasing interstimulus intervals.

performed with stimuli of 10 ms, did not reveal the OFF component. We showed that increasing the stimulus duration effectively modified the response profile of cerebral cortical units, in agreement with Kyriazi et al. [15], but also of cerebellar units. Stimuli that lasted longer than 30–50 ms evoked an OFF component, the amplitude of which sharply increased before stabilizing for longer stimulus durations. These results were observed not only for the cerebral cortical response but also for both the cerebellar trigeminal and cerebral cortical responses. While the early cerebellar OFF component was sometimes observed for a stimulus duration as short as 30 ms (above the 10 ms normally used in our studies), the late cerebellar OFF responses were never observed for stimulations less than 50 ms. Similar findings were reported in the cortex, but not in the trigeminal ganglion in which OFF responses were largely unaffected by stimulus duration [15]. In addition, we found that increasing the stimulus duration from 10 to 200 ms resulted in decreased OFF latencies of the cerebral cortical component and of the late cerebellar component (Fig. 3). The fact that the latencies of cerebellar components of presumably trigeminocerebellar and corticopontine origin were differentially affected indicates that the cerebral cortical response modifications may occur at central processing levels outside the cerebellum and

brainstem. It is interesting to note that cells responded on average 7.5 ms later to the stimulus withdrawal than to the stimulus onset. Kyriazi et al. [15] reported similar latency shifts of thalamic and cerebral cortical responses (7.4–8 ms), while the trigeminal ganglion OFF responses in Golgi cells took only an extra 5 ms compared to the ON responses.

These results indicate that stimulus characteristics, such as the duration, could have a critical effect on the cell response profile. The importance of another temporal parameter, the frequency, was recently highlighted in several studies investigating the frequency-dependent dynamics of the rat somatosensory system [1,2,23].

The cells were therefore subjected to a second protocol, which tested the effect of stimulation frequency on the cell response profile. The amplitude of the response increased with longer interstimulus intervals before reaching a "plateau" for intervals of more than 200 ms (i.e. for stimulation frequency below 5 Hz, Fig. 3). The latency of the cerebellar component of cerebral cortical origin decreased, while the latency of the early cerebellar component of trigeminal origin was unaffected. These observations were in agreement with recent findings of Ahissar and colleagues [1,2]. The authors reported that varying the stimulation frequencies affected the paralemniscal (POm thalamus, cerebral cortical layers I and Va and septa between barrels in layer IV) pathways of the rat. Whereas steady-state latencies of brainstem were almost constant for all the tested frequencies, those of the paralemniscal pathway increased with increasing frequencies. The authors offered an attractive explanation for the latency modifications in the thalamus and SI cortex by concluding that this pathway of the somatosensory system coded the input frequency as changes in latency, and was optimally tuned for temporal processing of vibrissal information around the whisker frequency range. The temporally encoded information could be translated to a rate code by thalamocortical loops.

The time-dependent effect of stimulus duration observed in the whisker/barrel system has been suggested to be a central phenomenon reflecting the waning of synaptically mediated inhibition, known to be evoked by the preceding stimulus onset [15]. The initial ~200 ms epoch during which changes in the OFF response were observed paralleled the silent period present in both cerebral cortical and Golgi cell units following a single stimulus (present study, [26]). Although the possible contribution of brain stem processing mechanisms can not be excluded, our observation that the Golgi cell OFF component of trigeminal origin also showed amplitude modification argues for processes operating at a cerebellar level.

Postexcitatory firing suppression has been characterized in multiple structures of the somatosensory system of several species, including the rat barrel cortex [11,22]. The epoch of reduced firing in our SI cerebral cortical recordings was significantly longer (up to 200 ms) and the rebound excitation stronger than reported in the above studies. However, previous control experiments performed in the lab under barbiturate anaesthesia and using the same stimulation paradigm as in the present study resulted in shorter postexcitatory firing suppression in the cortex. This observation indicates that the anaesthetic regime (ketamine–xylazine in this study) plays an important role in determining the firing profiles of the cerebral cortical units. By inhibiting NMDA receptors, ketamine could reduce granule cell excitation leading to diminished feedback excitation of Golgi cells by parallel fiber synapses. This would result in a modification of the silent period seen in the Golgi cells. This is, however, unlikely as the silent period also occurs under α-chloralose anaesthesia [26].

Another significant difference is that silent periods in cortex are observed in excitatory neurons while inhibitory neurons remain active. In the granular layer, the silent period is observed in the inhibitory neuron. The most likely cause of the silent period is a calcium activated afterhyperpolarisation intrinsic to Golgi cells [10]. This is supported by several properties of the silent period previously reported by us. We found a positive correlation between the initial firing rate of Golgi cells in response to stimulation and the duration of the following silent period [26]. At the same time the duration of silent periods simultaneously recorded in multiple Golgi cells can be very different [8], both suggesting an intrinsic process not linked to oscillatory processes. Moreover, in a recent modeling study the calcium activated afterhyperpolarisation was sufficient to cause the silent period in simulated Golgi cell responses [24]. All these findings suggest that intrinsic cell properties are the main cause for the long-lasting silencing of Golgi cells. Activation of postsynaptic metabotropic glutamate receptor subtype-2 by glutamate from granule cells [29] may further enhance this afterhyperpolarisation. Moreover, additional local circuit properties like the strong tonic component of granule cell inhibition [6,7,28], which might cause an interruption of parallel fiber excitation to the Golgi cell, or inhibition of the Golgi cell by molecular layer interneurons [9], or Purkinje cells collaterals could also contribute to the silent period. The extent of the tonic inhibition of granule cells [7] will determine to which degree the Golgi cell silent period corresponds to a disinhibition of granule cells [8].

Whatever its origin may be, interestingly the silent period seemed to affect the two stimulation protocols we used in a comparable manner. Increasing the stimulus duration or the interstimulus interval resulted in both a latency decrease of the corticopontine responses and an amplitude increase of both responses. However, the response profiles were slightly different for the latencies and the amplitudes, suggesting that amplitude and latency transformations might follow different processes.

In conclusion this study demonstrates that the silent period observed in Golgi cells has a strong effect on both offset responses for short stimulation durations and onset responses to high frequency stimuli. This suggests that the corresponding 200 ms time window may be important in cerebellar processing, at least for somatosensory stimuli.

Acknowledgements

A.V.L. is supported by the FWO (Flanders). The work was supported by the University of Antwerp, FWO, GSKE and the EC.

References

[1] E. Ahissar, R. Sosnik, K. Bagdasarian, S. Haidarliu, Temporal frequency of whisker movement. II. Laminar organization of cortical representations, J. Neurophysiol. 86 (2001) 354–367.
[2] E. Ahissar, R. Sosnik, S. Haidarliu, Transformation from temporal to rate coding in a somatosensory thalamocortical pathway, Nature 406 (2000) 302–306.
[3] J.S. Albus, A theory of cerebellar function, Math. Biosci. 10 (1971) 25–61.
[4] K.D. Alloway, M.J. Johnson, M.B. Wallace, Thalamocortical interactions in the somatosensory system: interpretations of latency and cross-correlation analyses, J. Neurophysiol. 70 (1993) 892–908.
[5] J.M. Bower, Is the cerebellum sensory for motor's sake, or motor for sensory's sake: the view from the whiskers of a rat? Prog. Brain Res. 114 (1997) 463–496.
[6] S.G. Brickley, S.G. Cull-Candy, M. Farrant, Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of GABAA receptors, J. Physiol. 497 (Pt. 3) (1996) 753–759.
[7] E. de Schutter, Cerebellar cortex: computation by extrasynaptic inhibition? Curr. Biol. 12 (2002) R363–R365.
[8] E. de Schutter, B. Vos, R. Maex, The function of cerebellar Golgi cells revisited, Prog. Brain Res. 124 (2000) 81–93.
[9] S. Dieudonné, Glycinergic synaptic currents in Golgi cells of the rat cerebellum, Proc. Natl. Acad. Sci. USA 92 (1995) 1441–1445.
[10] S. Dieudonné, Submillisecond kinetics and low efficacy of parallel fibre-Golgi cell synaptic currents in the rat cerebellum, J. Physiol. 510 (1998) 845–866.
[11] E.E. Fanselow, M.A. Nicolelis, Behavioral modulation of tactile responses in the rat somatosensory system, J. Neurosci. 19 (1999) 7603–7616.
[12] J.H. Gao, L.M. Parsons, J.M. Bower, J. Xiong, J. Li, P.T. Fox, Cerebellum implicated in sensory acquisition and discrimination rather than motor control, Science 272 (1996) 545–547.
[13] M.J. Hartmann, J.M. Bower, Oscillatory activity in the cerebellar hemispheres of unrestrained rats, J. Neurophysiol. 80 (1998) 1598–1604.
[14] M. Ito (Ed.), The Cerebellum and Neural Control, Raven Press, New York, 1984, p. 580.
[15] H.T. Kyriazi, G.E. Carvell, D.J. Simons, OFF response transformations in the whisker/barrel system, J. Neurophysiol. 72 (1994) 392–401.
[16] D. Marr, A theory of cerebellar cortex, J. Physiol. 202 (1969) 437–470.
[17] B.S. Minnery, D.J. Simons, Response properties of whisker-associated trigeminothalamic neurons in rat nucleus principalis, J. Neurophysiol. 89 (2003) 40–56.
[18] J. Morissette, J.M. Bower, Contribution of somatosensory cortex to responses in the rat cerebellar granule cell layer following peripheral tactile stimulation, Exp. Brain Res. 109 (1996) 240–250.
[19] S.M. O'Connor, R.W. Berg, D. Kleinfeld, Coherent electrical activity between vibrissa sensory areas of cerebellum and neocortex is enhanced during free whisking, J. Neurophysiol. 87 (2002) 2137–2148.
[20] K. Semba, B.R. Komisaruk, Neural substrates of two different rhythmical vibrissal movements in the rat, Neuroscience 12 (1984) 761–774.
[21] G.M. Shambes, J.M. Gibson, W. Welker, Fractured somatotopy in granule cell tactile areas of rat cerebellar hemispheres revealed by micromapping, Brain Behav. Evol. 15 (1978) 94–140.
[22] D.J. Simons, G.E. Carvell, Thalamocortical response transformation in the rat vibrissa/barrel system, J. Neurophysiol. 61 (1989) 311–330.
[23] R. Sosnik, S. Haidarliu, E. Ahissar, Temporal frequency of whisker movement. I. Representations in brain stem and thalamus, J. Neurophysiol. 86 (2001) 339–353.
[24] A. Volny-Luraghi, R. Maex, B. Vos, E. de Schutter, Peripheral stimuli excite coronal beams of Golgi cells in rat cerebellar cortex, Neuroscience 113 (2002) 363–373.
[25] B.P. Vos, R. Maex, A. Volny-Luraghi, E. de Schutter, Parallel fibers synchronize spontaneous activity in cerebellar Golgi cells, J. Neurosci. 19 (1999) RC6, 1–5.
[26] B.P. Vos, A. Volny-Luraghi, E. De Schutter, Cerebellar Golgi cells in the rat: receptive fields and timing of responses to facial stimulation, Eur. J. Neurosci. 11 (1999) 2621–2634.
[27] B.P. Vos, A. Volny-Luraghi, R. Maex, E. de Schutter, Precise spike timing of tactile-evoked cerebellar Golgi cell responses: a reflection of combined mossy fiber and parallel fiber activation? Prog. Brain Res. 124 (2000) 95–106.
[28] M.J. Wall, M.M. Usowicz, Development of action potential-dependent and independent spontaneous GABAA receptor-mediated currents in granule cells of postnatal rat cerebellum, Eur. J. Neurosci. 9 (1997) 533–548.
[29] D. Watanabe, S. Nakanishi, mGluR2 postsynaptically senses granule cell inputs at Golgi cell synapses, Neuron 39 (2003) 821–829.