Dynamic Synchronization of Purkinje Cell Simple Spikes

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kinje cells (PCs) integrate all computations performed in the cerebel-
lar cortex to inhibit neurons in the deep cerebellar nuclei (DCN).
Simple spikes recorded in vivo from pairs of PCs separated by <100
μm are known to be synchronized with a sharp peak riding on a broad
peak, but the significance of this finding is unclear. We show that the
sharp peak consists exclusively of simple spikes associated with
pauses in firing. The broader, less precise peak was caused by
firing-rate co-modulation of faster firing spikes. About 13% of all
pauses were synchronized, and these pauses had a median duration of
20 ms. As in vitro studies have reported that synchronous pauses can
reliably trigger spikes in DCN neurons, we suggest that the subgroup
of spikes causing the sharp peak is important for precise temporal
coding in the cerebellum.

INTRODUCTION

Purkinje cells (PCs) generate two types of spikes, simple
spikes (SSs) and complex spikes (CSs). High-frequency SSs
are generally assumed to be driven by parallel fibers (PFs) (but
see Loewenstein et al. 2005), whereas CSs are generated by
climbing fibers (CFs) originating from the inferior olive (Ito
1984). Despite the large overlap of PF inputs impinging on PCs
lying along the same PF beam (Solinas et al. 2003), SSs do not
show any precise synchronization in PCs separated by >100
μm (Ebner and Bloedel 1981a; Jaeger 2003). Conversely,
precise synchronization of SSs has been reported in pairs of
adjacent PCs, either on the same electrode or on two electrodes
separated by <100 μm (Bell and Grimm 1969; De Zeeuw et al.
1997; Ebner and Bloedel 1981a; Schwarz and Welsh 2001).
Many reported synchronous pairs showed a sharp peak riding
on a broader peak in the cross-correlogram, but whether these
two peaks represent different sources of spikes has not been
investigated. In this study, we could separate the mechanisms
responsible for each peak.

The inhibitory PCs are not final projecting neurons of the
cerebellum but target the real output neurons in the deep
cerebellar nuclei (DCN). In vitro dynamic voltage-clamp ex-
periments showed that ~10% synchronization among PCs
converging onto a DCN neuron increases the reliability of the
neuronal spiking four times more than 1% synchronization
(Gauck and Jaeger 2000). Furthermore, DCN spikes can be
precisely controlled by synaptic input at the millisecond level,
and such spikes mostly followed short decreases in inhibition
lasting ≥15 ms (Gauck and Jaeger 2000). However, it is not
known whether such short pauses in PC SS trains are synchro-
nized in vivo. Pauses in SS trains can be generated by multiple

mechanisms. It has been reported that CSs are always followed
by a pause of variable duration: from several tens of millisec-
onds (Bloedel and Roberts 1971; Granit and Phillips 1956) to
several hundreds of milliseconds (Bell and Grimm 1969; Burg
and Rubia 1972; Latham and Paul 1971; McDevitt et al. 1982;
Murphy and Sabah 1970, 1971; Thach 1967). The CS triggered
pauses can be followed by increased, decreased, or unchanged
SS firing rates (McDevitt et al. 1982; Sato et al. 1992). On the
other hand, pauses can also be generated by intrinsic afterhy-
perpolarization, decreased PF input, and/or increased on beam
or lateral inhibition from interneurons (Cohen and Yarom
2000). Nevertheless, the origin of all these pauses would be
indistinguishable in spike trains recorded from PC axons con-
tacting DCN neurons (Monsivais et al. 2005). Hence we have
defined pauses in this study as all SS ISIs that are indistin-
guishable in duration from the pauses after CSs, as the latter
are uniquely defined. Applying this criterion, we have inves-
tigated the synchronization of SS pauses by analyzing the
cross-correlations of nearby synchronous pairs of PCs.

METHODS

Recording

Forty five male Sprague-Dawley rats (300–400 g, Iffa Credo,
Brussels) were anesthetized with a mixture of ketamine HCl (75
mg/kg; Ketalar, Parke-Davis, Warner Lambert Manufacturing, Dub-
lin), and xylazine HCl (3.9 mg/kg; Rompun, Bayer, Leverkusen,
Germany) in normal saline (0.9% NaCl, Baxter, Lessine, Belgium) by
intraperitoneal injection. A craniotomy exposing Crus II of left cere-
bellar hemisphere was performed (Vos et al. 1999b). Supplemental
doses of the anesthetic (one third initial dose) were given intramus-
cularly to maintain deep anesthesia as evidenced by the lack of a pinch
withdrawal reflex and/or lack of whisking.

A new protocol was developed to record from 38 nearby PCs. One
electrode was inserted perpendicularly to the cerebellar surface. The
second electrode was tilted 58° from a horizontal plane to reduce
dimpling and inserted at a distance from the first electrode so that their
tips converged at the Purkinje cell layer. As control, we also recorded
12 distant pairs that were recorded from two electrodes separated by
≥400 μm. In all cases, the two electrodes were positioned along the
transverse direction relative to each other. Here we describe data from
eight selected pairs of nearby PCs (see RESULTS) and control analysis
based on 39 single-unit recordings of PCs. After recording spontane-
ous activity in four synchronous pairs, tactile stimulus-evoked re-
sponses were recorded. Perioral receptive fields were explored as
reported elsewhere (Vos et al. 1999b). A punctate stimulus was
applied at 0.5 Hz. Electric lesions (15 μA, 8 s, cathodal DC current)
were made to mark the location of the electrodes at the end of five
paired recordings of nearby PCs. The distance between pairs was
estimated from the distance between the centers of the lesion sites.
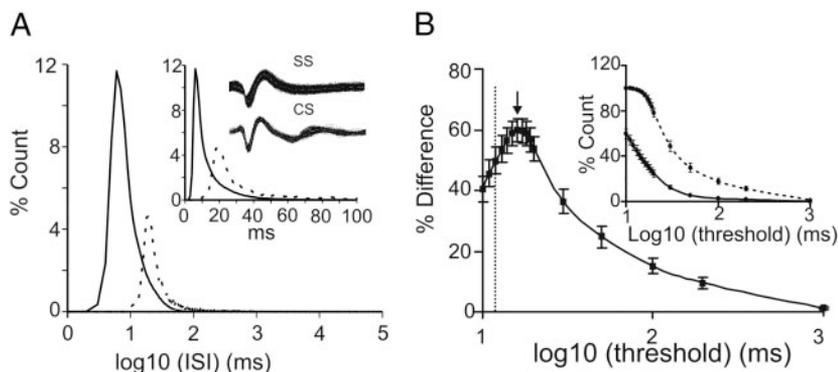

FIG. 1. Defining the threshold for pause detection. *A*: distributions of the 1st simple spike (SS) intervals preceding complex spikes (CSs; —) and of pauses after CSs (· · ·) of pooled interspike intervals (ISIs) from 39 Purkinje cell (PC) recordings in semi-log scale. *Inner inset*: distributions ≤100 ms in linear scale; *outer inset*: example of waveforms of sorted SSs (*top, n* = 11371) and CSs (*bottom, n* = 234). *B*: effect of thresholds for pause detection on the difference between the percentage of pauses after CSs and the percentage of pauses in SS ISIs. ↓ , maximum difference (threshold = 16 ms); · · · , smallest threshold that was not statistically different from the threshold showing maximum difference (threshold = 12 ms). *Inset*: percentage of pauses following a CS (● · · · ●) and percentage of pauses in all ISIs (● — ●) for a given threshold.

Rats received a lethal dose of sodium pentobarbital (120 mg/kg ip; Nembutal, SANTOFI, Libourne, France) at the end of the experiment. The brain was removed and fixed in 4% paraformadehyde. The cerebellum was embedded in paraffin, and 10-μm coronal sections were cut and stained with cresylviolet to visualize the electrolytic lesions. All experimental methods were approved by the ethical commission of the University of Antwerp and conformed to the guidelines of the European Commission.

Data analysis

After recordings of CSs and SSs, they were separated with off-line spike sorting software (Plexon, Austin, TX). Examples of the waveforms of sorted SSs and CSs from one PC are shown in Fig. 1*A, inset*. The off-line analysis of PC firing patterns was carried out using Matlab (The Mathworks, Natick, MA) and Microsoft Excel (Microsoft, Redmond, WA). The mean firing rates of SSs and CSs were 41.8 ± 3.6 and 0.73 ± 0.05 (SE) Hz, respectively, and they were not correlated (R^2 = 0.003). SS trains were constructed by removing all CS spikes from all recordings; any spike occurring within 10 ms of the start of a CS was considered to be part of the CS response as they probably belonged to the CS burst (Monsivais et al. 2005).

The strength of synchrony of SSs between pairs of PCs was measured using a standard score, the *Z* score of the cross-correlogram (Vos et al. 1999a) (bin = 2 ms). $Z = (N_c - N_e)/(SD_e)$, with N_c the number of spikes of the central bin, N_e the mean number of spikes per bin computed in a 2-s window between −1 and 1 s, and SD_e the SD of spikes per bin computed in the same window. Only pairs with *Z* scores of ≥3 were considered as synchronized pairs. The broadness of synchronization was measured with the full width at half-height (half-width). To assess the effect of firing rate co-modulation, shuffled spike trains were generated by randomly shuffling the ISIs within blocks of five consecutive ISIs. All *P* values refer to Student's paired or unpaired *t*-test, unless otherwise specified.

Pauses were defined by setting a threshold for ISI duration, any ISI equal or larger than the threshold was considered a pause. A threshold was chosen based on previous reports that CSs are followed by pauses. The distribution of the first SS ISIs preceding CSs was compared with that of pauses after CSs, where the SS ISIs and pauses after CSs were pooled from 39 PC SS trains (Fig. 1*A*). The median, 10 percentile and 90 percentile of SS ISIs (pauses after CSs) were 10.0 ms (CS: 31.0 ms), 5.9 ms (CS: 17.9 ms), and 29.9 ms (CS: 617.5 ms) respectively. We selected a threshold that would optimally detect the pauses after CSs if the two distributions were mixed as is the case in the PC axon because CS bursts are not transmitted faithfully (Monsivais et al. 2005). This was achieved by computing the proportion of ISIs categorized as pauses for different thresholds (set at 10, 11, 12, . . ., 20, 30, 50, 100, 200, or 1,000 ms) for all first SS ISIs preceding CSs and for all pauses after a CS (Fig. 1*B, inset*). The value that maximized the difference between these two proportions was considered as the optimal threshold, 16 ms (Fig. 1*B*). The smallest threshold which was not significantly different from the optimal, 12 ms, was used as threshold in this study. This threshold included more

pauses caused by CSs, resulting in correct detection of 99.3 ± 0.2% of the pauses after CS (compared with 95.4 ± 1.2% for a 16-ms threshold).

Using this definition of pauses, spikes were classified as either pause times (Pt, spikes beginning or ending a pause) or nonpause times (Nt, the rest of the spikes; Fig. 2*A*). The sensitivity of *Z* score and HW to the pause threshold was tested by computing these values for different thresholds. Measurements from each threshold were compared with those measured in overall spikes with the Wilcoxon signed-rank test. To avoid bias caused by small sample sizes, we excluded all recordings with a sample size <200. The sample sizes of Pt and Nt at different thresholds are summarized in supplemental Table 1.[1]

Responses to punctate tactile stimulation were measured by estimating the firing rates in 200 ms windows before and after stimulation. The significance of responses was determined by comparing numbers of spikes per bin after stimulation with averaged number of spikes per bin over the 100 ms window before stimulation in the peri-stimulus time histogram (bin = 20 ms).

RESULTS

In total, 21 stable recordings of transverse PC pairs were obtained. In 3 of 21 pairs a comparison of their auto- and cross-correlograms indicated that the pairs were probably recorded from the same PC (supplemental Fig. 1). Of the 18

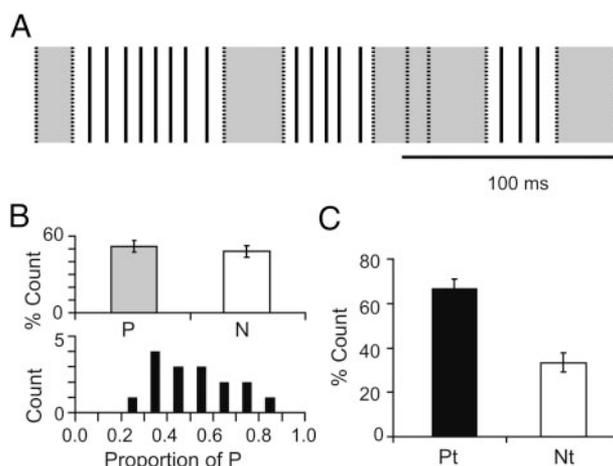[1] The online version of this article contains supplemental material.

FIG. 2. Pauses and pause times. *A*: illustration of pauses (P, ▨) defined as the intervals ≥12 ms, nonpauses (N, □) which are the remaining ISIs, pause times (Pt, · · ·) occurring at the start or the end of pauses, and nonpause times (Nt, —), which are all other spikes in a part of an actual SS train. *B*: average percentage of SS ISIs belonging to N and P (*top*) and proportion distribution of Ps (*bottom*) in 8 pairs of PCs. *C*: average percentage of spike times belonging to Pt and Nt in 8 pairs.

remaining pairs, 13 pairs showed statistically significant central peaks in their cross-correlograms (Z scores for the amplitude of the central peak of ≥ 3) (Vos et al. 1999a), which were not obtained in any distant pairs (3 pairs: $2 < Z$ score < 3; 9 pairs: Z score < 1). The rest of five adjacent pairs showed Z scores of either between 2 and 3 ($n = 3$) or <1 ($n = 2$). For the rest of this study, the eight pairs with Z scores >4 were further analyzed. They were separated by 69.8 ± 9.4 μm (range: $50–100$ μm, Fig. 3A) and revealed a tight synchronization (Z score 8.2 ± 0.9; half-width 17.5 ± 7.9 ms; Fig. 3, B and C,
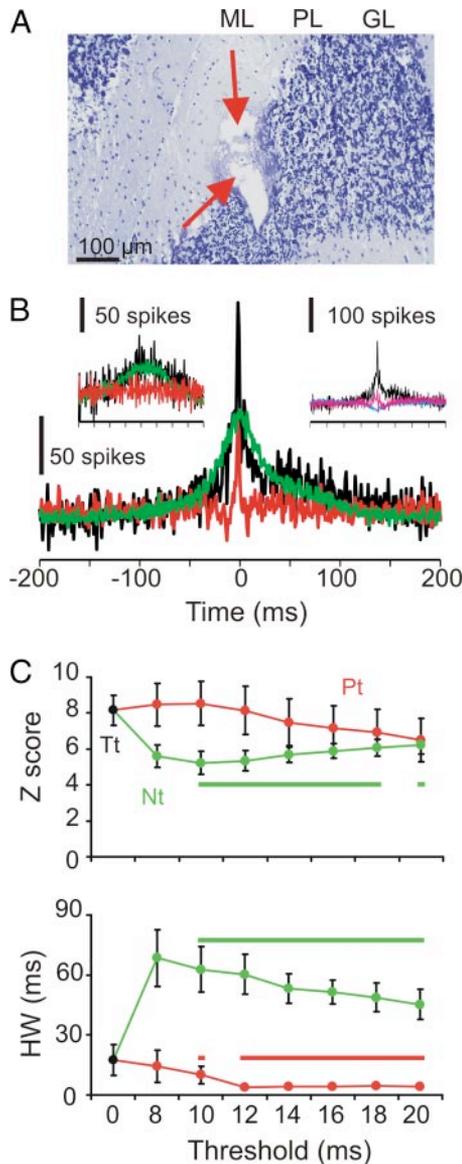

FIG. 3. Synchronized pairs of PCs. *A*: 2 lesions at sites of electrodes (red arrows) where 2 PCs were recorded. GL, granule cell layer; PL, Purkinje cell layer; ML, molecular layer. *B*: cross-correlograms calculated with all spikes (black) or with Nt (green) or with Pt (red) from a representative PC pair. The mean number of spikes was subtracted in each plot. *Left inset*: cross-correlograms calculated from shuffled spike trains (identical color coding). *Right inset*: cross-correlograms between Pt of 1 PC (reference) and Nt of the other PC (magenta dotted line) and Nt of one PC (reference) and Pt of the other PC (cyan dotted line) compared with that with all spikes (black). *C*: mean $\pm$ SE of Z scores and the full widths at half height (HW) of 8 synchronized PCs at different thresholds for pause detection; lines show significant points with $P <$ 0.05, Wilcoxon signed-rank test (control: Tt).

black), which was not correlated to differences in firing rates between the two PCs. On average $34.6 \pm 2.1\%$ (range: $19.5–49.6\%$) of SSs were precisely synchronized (central window of ± 2 ms). In all cases, the peak on the cross-correlograms consisted of a sharp peak (precise synchronization) riding on top of a broader central peak (loose synchronization) as has been reported for nearby PCs in previous studies (Bell and Grimm 1969; Ebner and Bloedel 1981b). Furthermore, spontaneously synchronous pairs responded to sensory tactile stimulation by significantly increasing their firing rate after stimulation from 49.5 ± 7.6 to 71.5 ± 7.1 Hz ($n = 8$, $P < 0.02$, Wilcoxon signed-rank test; Fig. 4B). However, their strength of synchronization (Z score $= 8.1 \pm 1.2$) was not significantly different from that for spontaneous activity (Z score $= 7.9 \pm 1.3$, $P > 0.99$, Wilcoxon signed-rank test).

To study if there is any difference in the precision of synchronization among SSs depending on whether they are related to pauses or not, we developed a spike separation method based on the ISI duration. This method allowed SS intervals to be categorized into pause (P) and nonpause (N) ISIs by a threshold of 12 ms (see METHODS and Fig. 1). In the eight PC pairs, on average $52.5 \pm 4.5\%$ of ISIs were pauses (Fig. 2B, *top*), which was not different from single-cell recordings or the other 10 PC pairs ($P > 0.7$). The proportion of pauses in each SS train was quite different from PC to PC, resulting in a wide distribution (Fig. 2B, *bottom*). As the correlation between the proportion of pauses for each PC of a pair was not high (correlation coefficient of 0.54), this wide distribution seems to be due to genuine differences between the recorded PCs as opposed to differences between rats or experiments.

Using the definition of pauses, spikes were classified as either pause times (Pt) or nonpause times (Nt; METHODS and Fig. 2A). On average, $73.2 \pm 4.1\%$ of spikes were classified as Pt (Fig. 2C). This classification was used to recompute the cross-correlogram for either all Pt (Fig. 3, B and C, red) or all Nt (green), taken from both cells of the pairs. The sharp peak of the cross-correlogram consisted exclusively of Pt (sample size: 8763 ± 714, Z score 8.1 ± 1.3, range: $3.9–15.9$; half-width 4.0 ± 0.7 ms, range: $2–8$ ms), whereas the Nt caused the broad peak (sample size: $3,859 \pm 840$, Z score 5.3 ± 0.6, range: $3.1–7.2$; half-width 60.3 ± 10.0 ms, range: $36–110$ ms). These observations were robust for the threshold selected (Fig. 3C). For thresholds between 12 and 18 ms, Pt had narrower half-widths and higher Z score than Nt did ($P < 0.03$, Wilcoxon signed-rank test). Conversely, smaller thresholds gradually destroyed the difference in precision between Pt and Nt (Fig. 3C). The higher synchronization of Pt was not caused by the different sample size as it persisted when the sample sizes for both Pt and Nt were equivalent (threshold of 16 ms, supplemental Table 1).

These results indicate that mainly the spikes around pauses were precisely synchronized. Conversely, two facts suggest that the broad peak consisting of Nt spikes was caused by firing rate co-modulation, a mechanism that causes spurious synchronization between two neurons which have firing rates that co-vary in time (Eggermont and Smith 1995; Maex et al. 2000). First, in properly shuffled spike trains the broad peak was conserved for Nt but not for Pt (Fig. 3B, *left inset*). Second, precision of synchronization correlated with firing rate as Nt occurring in the central 2-ms window of the broad peak
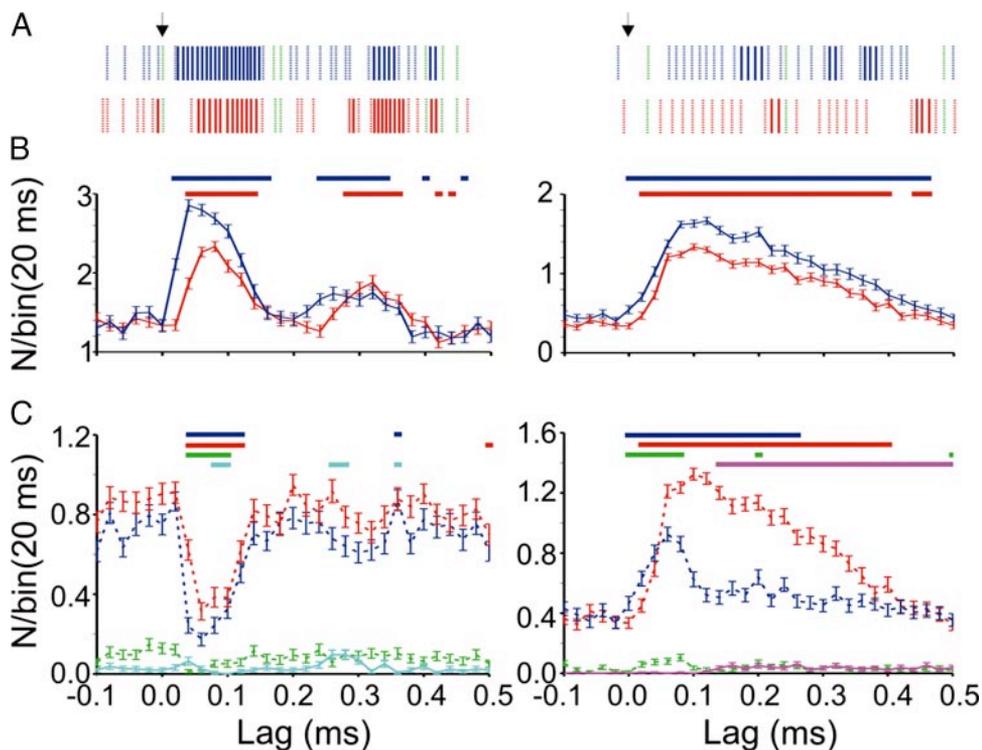
FIG. 4. Response patterns of pairs to sensory stimulation (2 representative examples). *A*: representative raster plot of SS firing during a trial of punctuation tactile stimulation, arrow indicates stimulating time. Red and blue represent respective SS trains of the pair. Dashed lines indicate all pause times. Green dashed lines show synchronous pause times. *B*: peristimulus time histograms calculated with all SSs (red and blue lines indicate each SS train of pairs). *C*: peristimulus time histograms obtained from all pause times (red and blue dashed lines), synchronous pause times (green dashed lines), and all CSs (cyan is a CS train recorded together with the blue SS train and magenta is that recorded together with the red SS train.). Lines above traces indicate bins showing significant difference from a 100-ms window before stimulation ($P < 0.05$).

($18.3 \pm 3.3\%$ of all nonpause ISIs) corresponded to ISIs that were significantly shorter than overall Nt (Fig. 5).

As a control, we correlated the Pt of one PC with the Nt of the other PC. These spikes showed either no synchronization or a small negative correlation and therefore did not make any contribution to the short- or long-duration correlation of the overall cross-correlogram (Fig. 3*B, right inset,* magenta and cyan dotted lines).

Our analysis thus suggests that Pt or Nt of one PC tended to be correlated with corresponding spikes of the other PC. Pt can be related to a pause in three ways: they can start a pause (sPt), end it (ePt), or be interspersed between two consecutive pauses (iPt). We checked whether any of these categories of Pt was preferentially synchronized by correlating only sPt, ePt, or iPt of one PC with the corresponding spikes of the other PC. These specific correlations did not show significant differences compared with each other (Fig. 6, $P > 0.4$, Wilcoxon signed-rank test), indicating that the accurately synchronized spikes did not have a preferential timing relative to the pause. Next, we investigated whether pauses after CSs were responsible for the synchronization observed. The correlation of SS trains where the first SS after a CS was removed for all CSs in one of the SS

trains ($n = 4$, mean CS firing rate $= 0.9 \pm 0.1$) did not show any significant changes in the peak Z score (9.4 ± 1.4) or half-width (30.0 ± 18.7) of the central peak ($P > 0.6$, Wilcoxon paired rank test), suggesting that the precise synchronization of Pt was not caused by CSs.

We analyzed whether tactile stimulation changed the proportion of Pt in the SS trains by constructing peristimulus time histograms using only Pt (Fig. 4*C*, red and blue). On average, there was no significant difference in the mean firing rates of Pt between 200-ms windows before and after stimulation, 34.3 ± 3.8 to 37.0 ± 2.8 Hz, respectively ($P > 0.6$, Wilcoxon signed-rank test). However, this average reflects a large variability in responses between different PCs, which could decrease (Fig. 4*C, left*) or increase (Fig. 4*C, right*) their proportion of pauses during the response to the stimulus.

The analysis presented in Figs. 3, 4*C,* and 6 was based on cross-correlation of specific categories of spikes. This does not guarantee that pauses were highly synchronized because a spike at the start of a pause in one PC can be synchronous with a spike at the end of a pause in the other PC. Therefore in a final analysis, we selected all Pt synchronous within ± 2 ms and checked whether the corresponding pauses where also synchronized. The pauses starting together (S-sP) represented 4.0% of overall pauses. Similar proportions were found for pauses ending together (S-eP, 3.6%) or followed by another pause (S-iP, 5.4%; Fig. 7*A*). If this synchrony was caused by chance, these proportions would be similar to the values obtained from randomly shuffled spike trains. However, all cases were significantly more synchronous than chance level ($P < 0.002$, Wilcoxon signed-rank test). Interestingly, the synchronous pauses (SM-P, the sum of S-sP, S-eP, and S-iP) had a significantly longer duration (median: 19.9 ± 0.6 ms) than the averaged duration of all pauses (Fig. 7*B*, $P < 0.03$, Wilcoxon signed-rank test). But, the durations of SM-P were
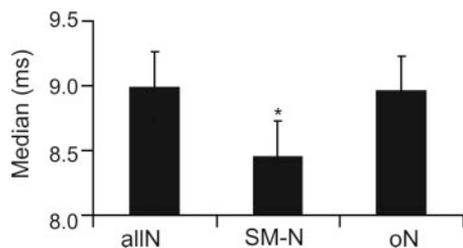

FIG. 5. Synchronous nonpause ISIs. The median durations of all nonpause ISIs (allN) compared with that of synchronous nonpause ISIs (SM-N) and that of all other nonpause ISIs (oN). SM-N was significantly shorter ($P < 0.001$, Wilcoxon signed-rank test), while oN was not different from allN ($P > 0.8$).
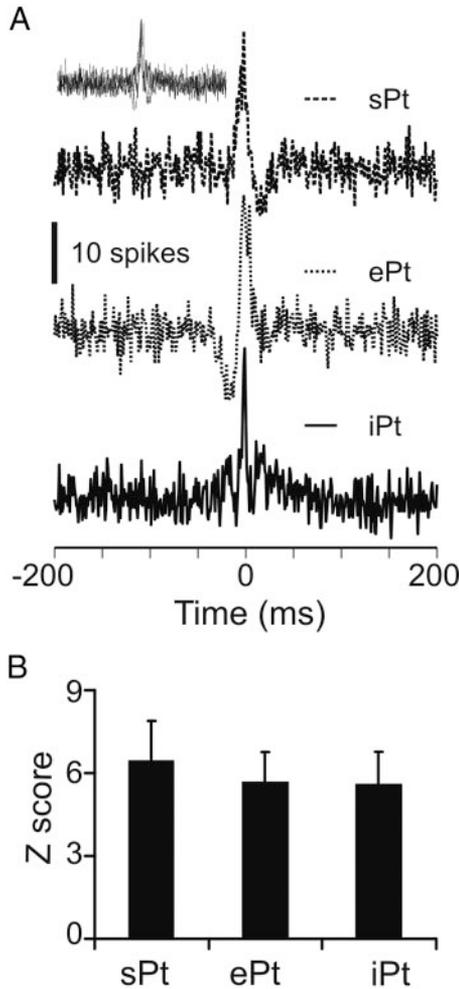
FIG. 6. Cross-correlation of spikes starting (sPt), ending (ePt) or being interspersed (iPt) between pauses. *A*: cross-correlograms calculated with spike times starting (- - -), ending (· · ·), or surrounded by (—) pauses in a representative PC pair. *Inset*: same but superimposed plots. *B*: mean ± SE of Z scores in 8 pairs. $P > 0.4$ (Wilcoxon signed-rank test).

not correlated in paired PCs (correlation coefficient = 0.14 ± 0.04).

Significant changes of synchronous pauses after stimulation were found only during limited time windows, mostly just after stimulation (Fig. 4*C*, green). Whether SM-P increased or decreased after stimulation was strongly related to how the proportion of Pt changed in the PCs (supplemental Fig. 2, $P < 0.05$, Student's *t*-test), indicating that the overall level of synchronization of pauses was not affected by tactile stimulation. Indeed, over longer time scales (500 ms before/after stimulus) we did not find any significant change in the proportion of synchronous pauses (before stimulation: 14.0 ± 1.5%, after stimulation: 12.7 ± 1.7%, $P > 0.09$, Wilcoxon signed-rank test). As the CS response after stimulation showed a different temporal profile (Fig. 4*C*, cyan and magenta), it is unlikely that SM-Ps during the response were caused by CSs. This is also supported by the flat cross-correlogram between CSs and SM-Ps (supplemental Fig. 3).

Taken together, in this study, we have shown that around half of SS intervals are pauses defined as ISIs of ≥12 ms; in transverse pairs of PCs separated by <100 μm, ~35% of spikes are precisely synchronized and ~13% of the pauses

occur synchronously, either starting or ending precisely together; CSs do not cause the precise synchronization of pauses; and coincident pauses are longer than other pauses, but their durations are not correlated.

DISCUSSION

The cerebellum plays an important role in motor coordination by controlling the relative timing of muscle activation (Berardelli et al. 1996) and also in other tasks requiring the precise representation of temporal information (Ivry and Spencer 2004; Koekkoek et al. 2003). Although PCs can generate accurately timed spikes (Mittmann et al. 2005), this accuracy has to be preserved at the level of targeted DCN neurons. In vitro dynamic current-clamping experiments have demonstrated that synchronized pauses in firing of converging PCs can induce DCN neurons, to spike in a reliable fashion (Gauck and Jaeger 2000). This makes DCN neurons quite different from cerebral cortical neurons, the precise spike timing of which is controlled by increased depolarization (Mainen and Sejnowski 1995). Here, we have shown that ~13% of all pauses are synchronized in nearby PC pairs in vivo. Synchronized pauses had a median ISI of ~20 ms which is longer than the minimum duration of 15 ms reported by Gauck et al. (2000) to reliably evoke a DCN spike, it is also similar to the duration of the IPSC recorded in DCN neurons in vitro (Pedroarena and Schwarz 2003; Telgkamp and Raman 2002).

If we assume that the recorded PC pairs are random samples of the ~1,000 PCs converging onto a DCN neuron in rats (Chan-Palay 1977), we expect that statistically a similar percentage of spikes and pauses will be synchronized among PCs converging on a DCN. The DCN spikes generated during the decrease of inhibition caused by synchronous pauses are most probably postinhibitory rebound spikes (Llinas and Muhlethaler 1988). Other in vitro experiments have shown that DCN neurons generate pronounced postinhibitory rebound spikes with a delay of ~30 ms to the onset of the last inhibitory postsynaptic potential (IPSP) (Aizenman and Linden 1999). Such rebound spikes, which rely on the strength and duration of preceding hyperpolarization (Aizenman and Linden 1999), are a good candidate cerebellar timing signal (Kistler and De Zeeuw 2002; Koekkoek et al. 2005).

What causes the precise synchronization of pauses in nearby pairs of PCs receiving common PF input remains unclear, but
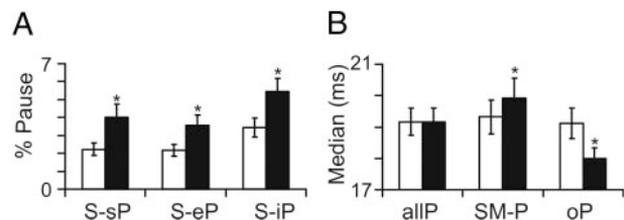

FIG. 7. Synchronous pauses. *A*: percentages of pauses synchronously starting (S-sP), ending (S-eP), and continued by another pause (S-iP) (■) compared with corresponding categories estimated from regenerated spike trains after randomly shuffling ISIs of 1 PC in the pair (□). *, $P < 0.002$, Wilcoxon signed-rank test. *B*: median durations of different categories of pauses (■) compared with those from shuffled spike trains (□). Three categories were compared: all pauses (allP), synchronous pauses (SM-P, sum of S-sP, S-eP, and S-iP), and nonsynchronized pauses (oP). *, $P < 0.05$, Wilcoxon signed-rank test. In the case of shuffled spike trains, allP, SM-P and oP were not different ($P > 0.09$).

we could exclude an important contribution of CSs. It has recently been suggested that CFs may evoke transitions between up and down states in PCs in vivo (Loewenstein et al. 2005; but see also Schonewille et al. 2006). It is unlikely that the synchronized pauses we observed correspond to quiescent down states because the latter last on average several seconds (Loewenstein et al. 2005). Moreover we did not find any evidence for a causal relationship between CF occurrence and transitions between high and low firing rate regimes (S. L. Shin, F. E. Hoebeek, M. Schonewille, A. Aertsen, and E. De Schutter, unpublished data). Indirect effects of CFs on molecular layer interneurons or of mossy fiber branching are also unlikely as these are expected to affect PC synchronization over much longer distances (Shinoda et al. 2000; Welsh et al. 1995; Woolston et al. 1981) than observed here.

A more likely mechanism for the pauses is synchronized IPSPs caused by feed-forward inhibition, which is an important regulator of the timing of the neuronal responses of cerebellar PCs and molecular layer interneurons (MLIs) (Cohen and Yarom 1998; Eccles et al. 1967; Mittmann et al. 2005; Solinas et al. 2006). The role of common feed-forward inhibition in the precise synchronization is supported by the facts that the sharp synchrony has been observed only in very close pairs (Jaeger 2003) and that interneurons have short axonal collaterals (Palay and Chan-Palay 1974; Solinas et al. 2003; Sultan and Bower 1998). However, feed-forward inhibition is more likely responsible for the synchronization of pauses, not for the synchronous spikes that may have been generated by parallel fiber inputs. As not all excitatory PF inputs trigger feed-forward inhibition (Mittmann et al. 2005), carefully designed follow-up studies will be required to understand their exact contribution to the observed synchrony. Another possible cause of the synchronous pauses is intrinsic afterhyperpolarization, which may be modified by learning processes (V. Steuber, W. Mittmann, M. Häusser, and E. De Schutter, unpublished results). An intrinsic origin of pauses could explain the large variation between PCs (Fig. 2B, bottom) as PCs show a large variability in the expression of voltage gated channels (Achard and De Schutter 2006; Swensen and Bean 2005). Although CSs were not directly responsible for the synchronous pauses, this does not exclude that previous learning processes induced by CSs modified dendritic excitability (Schreurs et al. 1998).

Not only precisely synchronized Pt but also the loosely synchronized Nt may be important for controlling DCN neurons. The latter may regulate DCN baseline firing rates (S. L. Shin, F. E. Hoebeek, M. Schonewille, A. Aertsen, and E. De Schutter, unpublished data), which are linearly correlated to the input frequencies of converging PCs (Gauck and Jaeger 2000). In this study we have shown that fast firing spikes tend to be only loosely synchronized. This will raise the actual firing rate of the converged high-frequency input, resulting in more efficient suppression of the DCN neurons.

Our estimate of 35 and 13% of synchronization of simple spikes and pauses, respectively, were made during spontaneous activity under anesthesia. Although it was recently reported that there was no difference in the occurrence of pauses of 50 ms or longer between anesthetized and awake mice (Schonewille et al. 2006), we do not know whether this also applies to synchrony of pauses. We did not find a strong effect of tactile stimulation, but this may have been due to the lack of a behavioral context in the anesthetized preparation (Krupa et al. 2004). Indeed, similar spike correlations with different degrees of temporal precision were observed in motor cortex of behaving monkeys (Riehle et al. 1997). In the motor cortex, precise synchronization without rate modulation was observed during internal (expectation) events. To understand the functional significance of dynamic synchronization in cerebellar PCs experiments in awake, behaving animals will be needed, but under these conditions, recording from nearby PCs on different electrodes will be a technical challenge. Nevertheless, at this time we can propose that the dynamic synchronization of PC SSs may be important for fine control of the target neurons in DCN neurons and that, especially, the precisely synchronized pauses may serve to control the timely generation of rebound spikes in DCN neurons.

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