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Effect of simple spike firing mode on complex spike firing rate and waveform in cerebellar Purkinje cells in non-anesthetized mice

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Abstract

Cerebellar Purkinje cells receive two different excitatory inputs from parallel and climbing fibers, causing simple and complex spikes, respectively. Purkinje cells present three modes of simple spike firing, namely tonic, silent and bursting. The influence of complex spike firing on simple spike firing has been extensively studied. However, it is unknown whether and how the simple spike firing mode may influence complex spike waveform and firing rate in vivo. We studied complex spike firing during tonic and silent mode periods in non-anesthetized mice. We found that complex spike firing rate is not influenced by simple spike firing modes, but that the complex spike waveform is altered following high frequency simple spike firing. This alteration is a specific decrement of the second depolarizing component of the complex spike. We demonstrate that the amplitude of the second depolarizing component is inversely proportional to the simple spike firing rate preceding the complex spike and that this amplitude is independent of previous complex spike firing. This waveform modulation is different from previously reported modulation in paired-pulse depression and refractoriness.

Keywords: Cerebellar Purkinje cell; Climbing fiber; Complex spike; Simple spike

Purkinje cells spontaneously generate complex and simple spikes. Simple spikes are somatic Na^+-related potentials generated by intrinsic conductances [9]. Their firing rate is increased by parallel fiber input. In mice, the frequency of tonic simple spike firing is around 50 Hz [4]. In addition to the tonic mode of simple spike firing, Purkinje cells may also present bursting and silent mode [18].

Complex spikes are caused by climbing fiber activation. Spontaneous complex spikes occur at an average firing rate of 1 Hz [13]. Simple spike firing has been reported to partially predict the occurrence of complex spikes during tonic mode periods [14]. However, to our knowledge, there are no publications concerning variations in complex spike activity related to simple spike firing modes. Complex spike waveform reflects the compound excitation in the proximal Purkinje cell dendrite by the climbing fiber and is classically considered as invariant in vivo for a given Purkinje cell [11]. It remains controversial whether mossy fiber excitation may modulate this waveform in vivo [2,6].

Long- and short-term plasticity have been described in vitro at the climbing fiber–Purkinje cell synapse [7,8]. They cause specific modifications in the complex spike waveform: the amplitude of the second depolarizing component is decreased in long term depression [7], while paired-pulse depression specifically alters complex spike duration and decreases the number of secondary spikes [8].

In order to better understand how complex spike firing rate and waveform may be modulated by simple spike firing in vivo, we compared complex spike activity during silent and tonic mode periods in non-anesthetized mice.

We performed a posterior craniotomy in 10 C57BLC6J wild type mice aged 10–13 months anesthetized with

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xylido-dihydrothiazin (Rompun®, Bayer, 10 mg/kg) and ketamine (Ketalar®, Pfizer, 100 mg/kg). A supplemental dose of xylido-dihydrothiazin 3 mg/kg and ketamine 30 mg/kg was administered if the animal presented agitation or markedly increased respiration or heart rate during the procedure. In addition, local anesthesia with 0.5 ml Lidocaine 20 mg/ml + Adrenaline 1:80,000 (Xylocaine®, Astra Zeneca) was administered subcutaneously during soft tissue removal. Two small bolts were cemented to the skull to immobilize the head during the recording sessions and a silver reference electrode was placed on the surface of the parietal cortex. An acrylic recording chamber was constructed around the craniostomy, covered by a thin layer of bone wax (ethicon) before and between recording sessions. Twenty-four hours after anesthesia, mice were immobilized for the recording session. We paid particular attention to avoid any additional sensory stimulation. Data recorded while the animal was presenting orofacial movements were discarded. The dura mater was removed locally above the vermis and recordings were performed by optimal positioning of a glass micropipette (1.5–5 MΩ of impedance) in order to record simultaneously simple and complex spikes (Fig. 1A). A neural signal was considered as originating from a Purkinje cell if it presented two types of spiking activities: simple spikes characterized by single depolarizations (300–800 μs) occurring at high frequency (∼50 Hz) and complex spikes characterized by an initial fast depolarization (300–600 μs) followed by smaller and relatively constant components. We considered that simple and complex spikes originated from the same Purkinje cell when a transient pause (∼20 ms) in simple spike firing followed each complex spike (Fig. 1A). Secondary spikes were counted if their amplitude reached at least two times the maximal amplitude of the background signal. The duration of a complex spike was defined as the time between the beginning of the first depolarization and the end of the last secondary spike.

All recordings were performed with an amplification of 1000 and a bandwidth of 0.01–10 kHz. They were stored on 4 mm digital audio tapes (Sony PCM-R500) and transferred to a Pentium III personal computer with analog-to-digital converter boards (Power 1401, *CED*). The recorded data were digitized continuously at 100 kHz.

Off-line analysis and illustrations were performed using the Spike 2 CED software. The complex spike waveforms of Purkinje cells were analyzed if the three following conditions were fulfilled: (1) Presence of at least one period longer than 10 s with complex spike and without simple spike firing (silent mode period); (2) Presence of periods of simple and complex spike firing before and after the silent mode period (tonic mode periods); (3) Absence of significant differences in simple spike amplitude during consecutive tonic mode periods. This procedure was adopted in order to record complex spikes in tonic and silent mode periods for a given Purkinje cell, and to ensure that the entire recording was performed at the somatic level, as simple spikes do not backfire into the dendritic tree [16]. Fourteen cells recorded in six

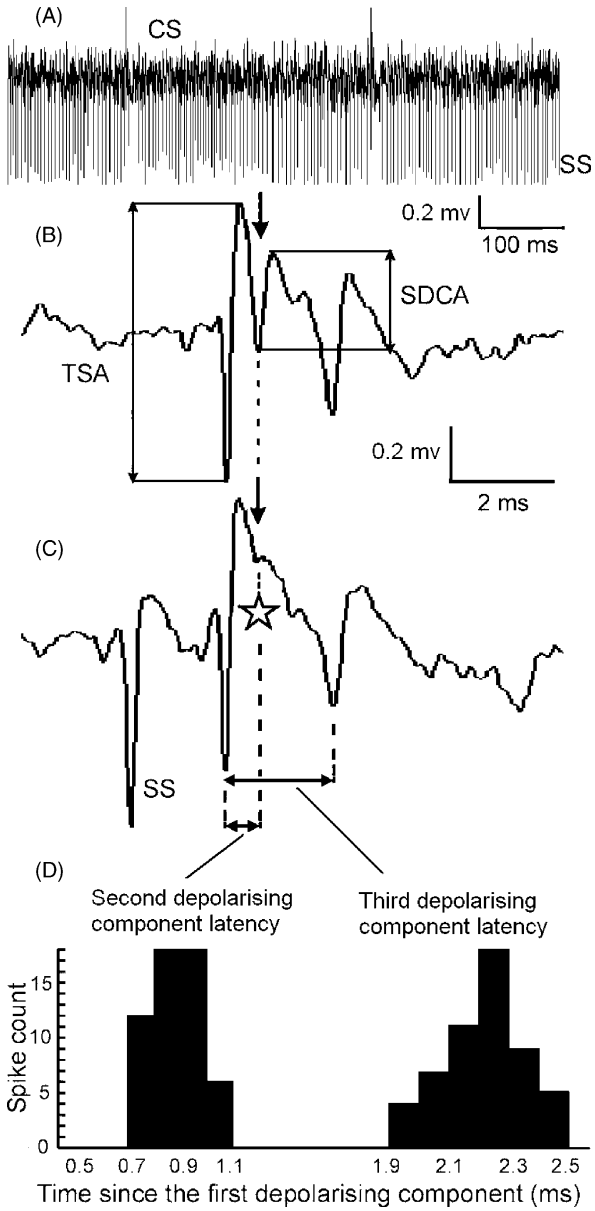

Fig. 1. SDCA/TSA decreases during tonic mode periods. (A) Recording of the spontaneous Purkinje cell activity. (B) Complex spike recorded during a silent mode period. (C) Complex spike of the same Purkinje cell during a tonic mode period. (D) Distribution histogram of the time intervals between first and second depolarizing components (left) and between first and third depolarizing components (right) in complex spikes recorded in the cell illustrated in A–C ($n = 53$).

mice met these restrictive criteria for analysis. We compared the firing rate, amplitude, duration and waveform (amplitude and latency of the first three depolarizing components and number of secondary spikes) of complex spikes during the tonic and silent mode periods.

All measurements were performed on individual complex spikes. The mean values were compared using the ANOVA test. *t*-tests for dependent samples were used to compare complex spikes in silent versus tonic mode periods. Linear correlation tests were used to correlate simple spike firing

rates or pause duration and the ratio between second and first depolarizing components. Results are expressed and illustrated as mean ± S.D. and were considered significant if $P < 0.05$. All analysis were performed on Statistica 6.0.

The mice were treated according to international guiding principles for research involving animals and human beings and guidelines established by the ethical committee of the UMH for the care and use of laboratory animals.

We found that complex spike waveforms were markedly altered in the tonic compared to the silent mode period, with a significant decrement of the second depolarizing component (Fig. 1B and C). In order to ascertain whether the comparison of complex spike waveform could be undertaken by comparing latencies and amplitudes of the depolarizing and repolarizing components, we verified that there was no overlapping between the latencies of the first three depolarizing components in the different complex spikes of a same Purkinje cell (Fig. 1D). In order to rule out that the change in complex spike waveform was due to a change in background noise, we measured the noise in tonic and silent mode periods during three recordings of Purkinje cell. This was done by measuring the maximal amplitude and the S.D. of voltage values during the pause following each complex spike in the tonic mode period. The voltage variation during this time interval is indeed only produced by background noise. The averaged duration of the pause was 14.2 ± 5.9 ms. Consequently we performed the same measure during the same period of time after each complex spike during the silent mode period. There was no difference between the total amplitude of noise and the S.D. of voltage values between tonic and silent mode periods (0.11 ± 0.03 mV versus 0.10 ± 0.02 mV and 0.026 ± 0.006 mV versus 0.023 ± 0.006 mV, respectively). Actually, theses values remained largely constant through a given recording.

In order to quantify the modulation of complex spike waveforms, we averaged the ratio between the second depolarizing component and the total spike amplitude (SDCA/TSA) (Fig. 1B) of complex spike in the silent and in the tonic mode period. We found that this ratio was decreased by about 50–75% in all cells during the tonic mode period (Fig. 2A). In contrast, we did not find any other significant difference in complex spike waveforms between tonic and silent mode periods. When Purkinje cells switched from tonic to silent mode period, the recovery of SDCA was progressively achieved in several seconds (Fig. 3A). During the same period of time, the background noise, quantified by the maximum amplitude of voltage variation and the standard deviation of voltage remained constant, which proves that the progressive recovery of the waveform is not due to a progressive change in the background noise.

In order to determine the factors associated with SDCA/TSA modulation, we measured this ratio on all recorded complex spikes in three Purkinje cells for which the recording was the longest and the most stable. Simple spike firing rate before each complex spike was calculated on a 6-s period (approximately recovery time). Because

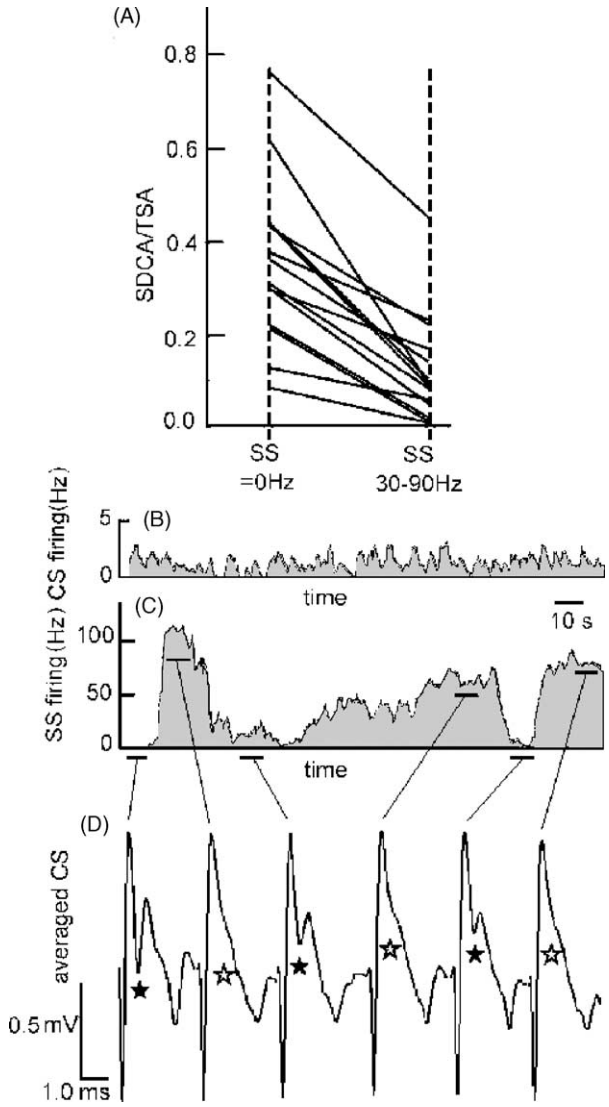

Fig. 2. SDCA/TSA is closely linked to simple spike activity. (A) Mean of SDCA/TSA of complex spikes occurring during tonic and silent mode periods in 14 Purkinje cells. (B) Complex and (C) simple spike firing rates (bin of 1 s). (D) Three-second averaging of complex spikes during periods of constant simple spike firing.

of the irregular pattern of simple spike firing during the tonic mode period, we also measured simple spike firing rates during shorter periods ranging from 0.2 to 4.0 s. We correlated SDCA/TSA with the frequency of simple spike firing during the preceding 0.2, 2.0, 4.0 and 6.0 s periods for each complex spike. For each cell, simple spike firing rate was significantly and linearly correlated with SDCA/TSA (Fig. 3B) whatever the period during which it was measured. The higher r values were obtained in each Purkinje cell from simple spike firing rates calculated during a 6 s period (r values ranging from −0.71 to −0.77), while the lower r values were obtained for simple spike firing rate calculated over a 0.2-s period (r values ranging from 0.51 to 0.66). In contrast, no significant relationship was found between SDCA/TSA and the interval between the complex spike and the last complex spike ($r < 0.2$ for each cell, Fig. 3C).

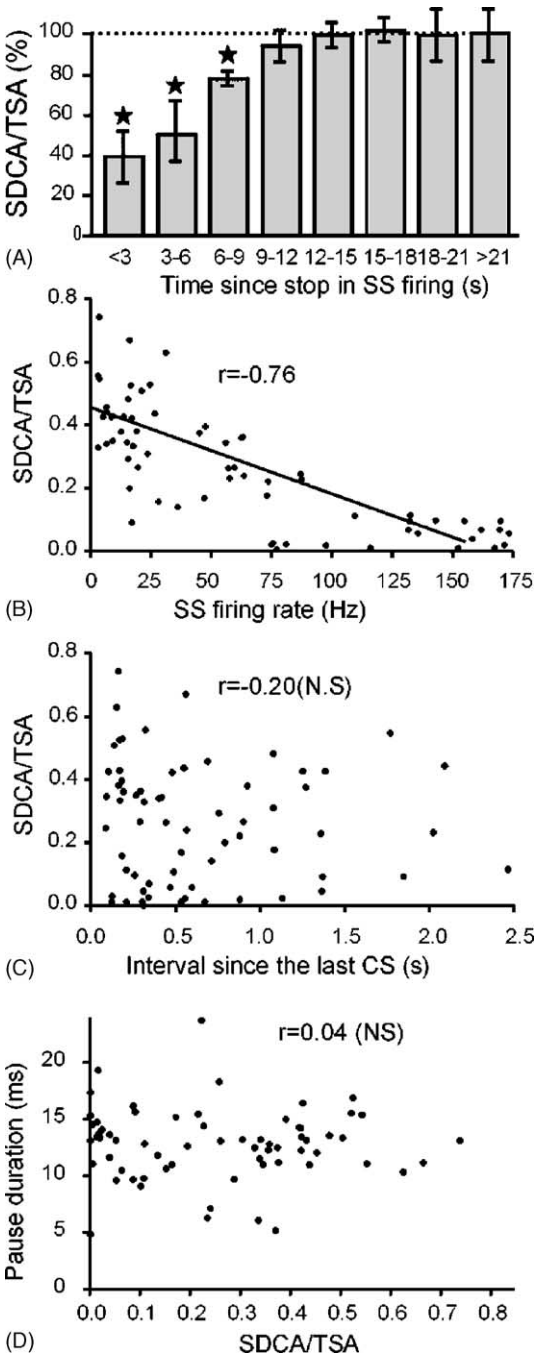

Fig. 3. (A) Linear relationship between SDCA/TSA and time between the complex spike and the onset of silent mode period (three episodes of switch in mode of firing, $n = 37$). SDCA/TSA is expressed in % of mean amplitude in silent mode periods. Stars indicate significant differences with isolated complex spikes. (B–D) Plotted value of SDCA/TSA and frequency of simple spike firing 6 s before each complex spike (B) interval between the complex spike and the last complex spike (C) pause duration (D) ($n = 67$).

During high frequency simple spike firing, there is a high probability that complex spike follows shortly after a simple spike. According to Eccles et al. [5], the refractoriness of a Purkinje cell following a parallel fiber volley is ended around 3 ms. Therefore we compared complex spikes occurring 0–3 and 3–6 ms after the last simple spike during tonic mode periods to complex spikes occurring during silent mode periods. The amplitude of the first depolarization was reduced to $68 \pm 7\%$ in complex spikes occurring 0–3 ms after a simple spike and was unchanged in complex spike occurring 3–6 ms after a simple spike. The amplitude of the following repolarization peak remained unchanged. Conversely, SDCA was reduced to $24 \pm 22\%$ in complex spikes occurring 0–3 ms and to $47 \pm 26\%$ in complex spikes occurring 3–6 ms after the last simple spike. This difference remained significant for the SDCA/TSA ratio ($28 \pm 29\%$ of values of complex spikes occurring during silent mode periods versus $48 \pm 28\%$ for all cells). This confirms that the decrease in SDCA/TSA for complex spikes occurring 3–6 ms after the last simple spike is not due to Purkinje cell refractoriness.

The pause in simple spike firing following the complex spike is the most obvious and studied consequence of complex spike. Therefore, the relationship between the modulation of the second depolarizing component and the subsequent pause in simple spike firing caused by each individual complex spike was examined. For each complex spike, we plotted the values of SDCA/TSA and the duration of the subsequent pause. No significant relationship was found ($r < 0.25$) in any of the studied Purkinje cells. Fig. 3D illustrates this for one cell.

Simple spike firing could facilitate the occurrence of the complex spike [14]. It could then be expected that complex spike firing rates would decrease during silent mode periods. However, in contrast with the marked difference of complex spike waveform between tonic and silent mode periods, we did not find any significant differences in complex spike firing rates ($P > 0.1$) (0.51 ± 0.03 Hz and 0.49 ± 0.02 Hz).

Complex spike waveform may also be modulated by previous complex spike activity [2,7]. In vitro, complex spikes occurring less than 500 ms after the previous one present a decreased number of secondary spikes [8]. In vivo, Campbell and Hesslow [2] have described an increased number of secondary spikes in complex spikes elicited less than 100 ms after the previous complex spike. Therefore, we studied the influence of complex spike firing on the complex spike waveform. To isolate the role of previous climbing fiber activity, we compared complex spike waveform during silent mode periods in four cells. Two-hundred and eleven complex spikes were analyzed and classified according to the time interval with the last complex spike: <100 ms ($n = 6$), between 100 and 500 ms ($n = 63$, further called paired-pulse depressed), between 500 and 750 ms ($n = 34$) and >750 ms ($n = 108$, further called non-depressed). Because of their rarity, complex spikes occurring less than 100 ms after the previous one were discarded. For each cell, we compared the number of secondary spikes, the complex spike duration and the SDCA/TSA ratio. Fig. 4 illustrates examples of paired-pulse depressed and non-depressed complex spikes. For each cell, the number of secondary spikes of paired-pulse depressed complex spikes (Fig. 4B) was lower than the number of secondary spikes of non-depressed complex spikes

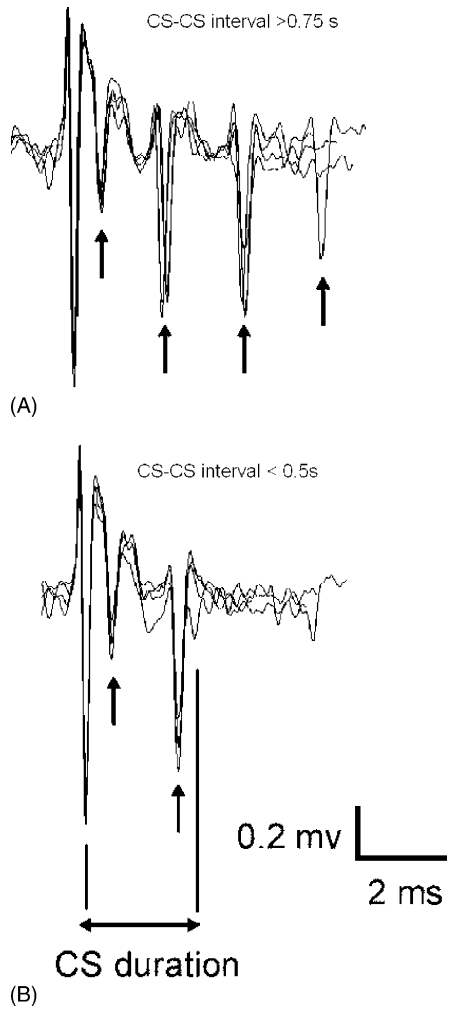

Fig. 4. Complex spikes undergo paired-pulse depression characterized by a decrement of their duration and of the number of their secondary spikes. Superposition of four non-depressed (A) and four paired-pulse depressed (B) complex spikes during a silent mode period. Arrows indicate secondary spikes.

(Fig. 4A) (2.5 ± 0.3 and 3.6 ± 0.4 for all cells). In all but one cell, paired-pulse depressed complex spikes were significantly shorter than non-depressed complex spikes (9.1 ± 0.5 and 10.9 ± 0.6 for all cells). In contrast, there was no difference in SDCA/TSA between paired-pulse depressed and non-depressed complex spikes in each cell (0.52 ± 0.19 and 0.47 ± 0.21 for all cells).

These results demonstrate that the complex spike waveform is not invariant but correlates with previous simple spike firing. Specifically, the amplitude of the second depolarizing component is inversely proportional to the previous simple spike firing rate. In contrast, non-depressed complex spikes occurring during silent mode periods show remarkable similarities in their three first depolarizing and repolarizing components. Even if complex spikes waveforms and durations have already been studied in vivo [2–4,6], it could be argued that non-specific background noise would alter complex spike waveform. Therefore, data recorded during facial or whisker movements were discarded. During silent mode periods, the remarkable reproducibility of non-depressed complex spikes demonstrates that background noise has few, if any consequences on the first three components. The specificity of the second depolarizing component alteration, the persistence of this alteration on averaged waveform (Fig. 2D) and the slow increment of SDCA/TSA after the onset of silent mode periods (Fig. 3A) further assess the physiological nature of the modulation of complex spike waveform by previous simple spike firing. A possible cause of this modulation could be the activation of molecular layer interneurons by the parallel fibers. Indeed, off-beam stimulation of parallel fibers has been shown to decrease the secondary spikes of the complex spike [2], and direct or disynaptic stellate activation decreases Ca^{2+} entry during a complex spike [1]. However, it must be noted that in this latter report, somatic recording of complex spikes in slice preparation was unchanged despite Ca^{2+} entry decrement. Another explanation could be that, when complex spikes are closely preceded by a series of simple spikes, the integrated depolarization renders voltage-sensitive Ca^{2+} channels in the Purkinje cell inactive, thus suppressing the second depolarizing component. Theoretically, both mechanisms could also be elicited by climbing fiber activation but, surprisingly, previous complex spike activity did not modify the complex spike waveform as did previous simple spike activity. This may be due to the difference in the frequency of both signals. Moreover, the interval between two complex spikes is rarely shorter than 100 ms, while in the tonic mode period, complex spikes are closely preceded by a large number of simple spikes.

Paired-pulse depression is a widely studied type of short-term plasticity. Its physiological relevance is suggested by its presence in various central synapses and by the physiological aspect of its triggering, i.e. the occurrence of a complex spike less than 500 ms after the previous one. We demonstrate that in vivo paired-pulse depression has a specific effect on the number of secondary spikes and complex spike duration, as previously demonstrated in vitro [8]. In contrast, we show that paired-pulse depression has no effect on SDCA/TSA. A similar specific decrement of the secondary depolarizing component has been described in slice preparation during LTD at the climbing fiber–Purkinje cell synapse [7], and is associated with a decrement in Ca^{2+} entry during the complex spike [17]. For this reason, this LTD might have important physiological consequences, as Ca^{2+} entry during the complex spike is associated with various regulating pathways, as up-regulation of $GABA_A$ receptor [12] and LTD at parallel fiber–Purkinje cell synapse [10].

The number of secondary spikes transmitted through the axon is by far inferior to the total number of simple spikes during tonic mode periods. Therefore, the physiological meaning of the change in complex spike waveform must be found in the change in ionic currents in the Purkinje cell during the complex spike. Another possibility could be related to an intrinsic code supported by the very high

frequency inside the burst of spikes in the complex spikes. Every secondary spike may be transmitted through the axon [11] but this transmission is not constant [3]. According to Ito and Simpson [11], it is the second spike of the triplet recorded in the Deiters nucleus that specifically disappears when the triplet response to vermal cortex stimulation becomes a doublet. This decreases by 50% the intraburst frequency. The amplitude decrement of a somatic secondary spike may lead to a decrease probability for this spike to be transmitted through the axon, thereby modifying the neural code of the burst discharge.

Even if some reports suggest that in vitro and in vivo recordings of the complex spike are comparable [15], the association of in vivo decrement of complex spike secondary depolarizing peak and a decrease in Ca^{2+} entry during the complex spike remains to be demonstrated. If this association, described in vitro may be confirmed in vivo, this will indicate that parallel fiber pathways realize a feed forward control of its firing through Ca^{2+} entry modulation, which would be of crucial importance in the understanding of cerebellar function.

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