Efficacy and Short-Term Plasticity at GABAergic Synapses Between Purkinje and Cerebellar Nuclei Neurons

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Pedroarena, Christine M. and Cornelius Schwarz. Efficacy and short-term plasticity at GABAergic synapses between Purkinje and cerebellar nuclei neurons. J Neurophysiol 89: 704–715, 2003; 10.1152/jn.00558.2002. Although the entire output of the cerebellar cortex is conveyed to the deep cerebellar nuclei neurons (DCNs) via the GABAergic synapses established by Purkinje cells (PCs), very little is known about the strength and dynamic properties of PC-DCN connections. Here we show that activation of PC-DCN unitary connections induces large conductance changes (11.7 nS) in DCNs recorded in whole cell patch configuration in acute slices, suggesting that activity of single PCs might significantly affect the output of its target neurons. Based on the large unitary quantal content (18) inferred from calculations of PC-DCN quantal size (0.65 nS) and the near absence of failures in synaptic transmission during control conditions, we conclude that PC-DCN connections are highly multi-sited. The analysis of dynamic properties of PC-DCN synapses demonstrated remarkable paired pulse depression (PPD), maximal at short intervals (paired pulse ratio of 0.15 at 7-ms interval). We provide evidence that PPD is presynaptic in origin and release-independent. In addition, multiple pulse stimulation revealed that PC-DCN synapses exhibited larger sensitivity to dynamic than to steady signals. We postulate that the, otherwise paradoxical, combination of marked short-term depression with strong multi-sited connections is optimal for transfer dynamic information at unitary level by performing spatial average of release probability across the numerous release sites. This feature could enable these synapses to encode presynaptic time-varying signals of single PCs as moment-to-moment changes in synaptic strength, a capacity well suited to the postulated role of cerebellum in control of temporal aspects of motor or cognitive behaviors.

INTRODUCTION

The output of the cerebellar cortex is conveyed exclusively by the axons of the inhibitory Purkinje cells (PCs) (Ito et al. 1970) to their main target, the deep cerebellar nuclei neurons (DCNs), which in turn constitute the principal output of the cerebellum. The GABAergic synapses from PCs represent almost 75% of the total synaptic inputs to DCNs (De Zeeuw and Berrebi 1995; Palkowitz et al. 1977). The remainder of the afferent nuclear innervation originates in collaterals of the excitatory mossy and climbing fibers which finally project to the cerebellar cortex (Mihailoff 1993; Shinoda et al. 2000). It is clear from this brief recount that PC-DCN synapses are in a key position in the cerebellar circuit, and it is therefore usually assumed that they play an important role in cerebellar function. However, the ways inhibitory projections transfer information are still largely unknown. In particular, at PC-DCN inhibitory synapses, this issue remains a matter of conjecture and subject of diverging hypotheses (Braitenberg and Preissl 1992; Eccles 1973; Llina`rs and M¨uhlethaler 1988; Mauk 1997). Important clues needed to understand how and which information is transferred by PC-DCN synapses are their strength and dynamic properties. These parameters are considered to be fundamental elements in the processing performed by neural systems and determinants of the type of presynaptic signals that are transferred and finally control postsynaptic activity (Abbot et al. 1997; Galarreta and Hestrin 1998; Thomson et al. 1994; Tsoylyks and Markram 1997). Although, long-term changes of PC-DCN synaptic efficacy have been described in detail (Aizenman et al. 1998; Morishita and Sastry 1996; Ouardouz and Sastry 2000), very little is known about the short-term dynamics associated to the physiological patterns of activation of PC-DCN synapses (Morishita and Sastry 1995; Mougnot and G´awiler 1996) and to our knowledge, no previous studies on unitary-PC-DCN synapses have been published. In vivo studies showed that PCs typically discharge at high frequencies. Simple spikes occur spontaneously at frequencies between 30 and 100 Hz (McDevitt et al. 1987; Thach 1968) and at frequencies ≤300 Hz during movements (Thach 1970) (R. Haas and P. Thier, personal communication). Furthermore, climbing fiber inputs evoke complex spikes in PCs, which on the PC axons appear as a burst of action potentials at frequencies of ≤500 Hz (Ito and Simpson 1971).

To assess the strength of unitary connections and the input-output function of PC-DCN synapses under high-frequency activation, we recorded synaptic currents in DCNs elicited by activation of multiple or single PC axons. Our results demonstrate strong PC-DCN unitary synaptic connections and remarkable short-term depression at high frequencies, which originates in a presynaptic, release-independent mechanism. As a result of their filtering properties, PC-DCN synapses exhibit larger sensitivity to dynamic than to steady PC activation.

METHODS

Slice preparation

Cerebellar slices were prepared as described by Czubayko et al. (2001). Briefly, Sprague-Dawley rats (12–21 days old) were anesthe-
tized with ketamine and tranascendally perfused with ice-cold modified artificial cerebrospinal fluid (ACSF) in which NaCl had been replaced by sucrose. Cerebellum was removed and placed in ice-cold modified ACSF. Modified ACSF contained (in mM) 125 sucrose, 2.5 KCl, 1.25 NaH2PO4, 3 MgCl2, 26 NaHCO3, 0.1 CaCl2, and 20 D-glucose, and was oxygenated with 95% O2-5% CO2. Parasagittal or coronal slices (250–300 μm) were prepared using a microrotome (Leica, Bensheim, Germany) and stored in modified ACSF at room temperature for 30 min. After this period, the solution was slowly (1 h) replaced with normal ACSF containing (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 1.5 MgCl2, 26 NaHCO3, 2.5 CaCl2, and 20 D-glucose, and when bubbled with 95% O2-5% CO2 pH was 7.4. Slices were stored for 1–6 h before being transferred to a submerged recording chamber. During recording, slices were continuously superfused with normal ACSF at room temperature (recording chamber temperature: 25–29°C).

Patch-clamp recordings and cell identification

Whole cell patch-clamp recordings were made from DCNs located in the lateral nuclei. The position of the lateral nuclei was identified in the slices using a ×4 objective. Three main classes of neurons may be distinguished in the cerebellar nuclei: large (approximately 20 μm) excitatory projecting neurons, a group of smaller (approximately 10 μm) GABAergic neurons that project to the inferior olive, and small local inhibitory interneurons, some of them colocalizing GABA and glycine (Batini et al. 1992; De Zeeuw and Berrebi 1995). In the present study, neurons with diameters larger than 15 μm were selected for recording by visual inspection using infrared videomicroscopy. Hence, according to the anatomical data, most of the recorded neurons were glutamatergic projecting neurons. Recordings were performed using a patch-clamp amplifier (Axopatch 1-D, Axon Instruments, Foster City, CA). Patch pipettes were filled, unless specified, with a solution containing (in mM) 130 CsCl, 5.6 NaCl, 10 K-HEPES, 5 EGTA, 2 K-ATP, 0.3 Na-GTP, 0.5 CaCl2, 2 MgCl2, and 5 QX-314, adjusted to pH 7.3 with CsOH. Series resistance ranged between 10 and 25 MΩ and was compensated (40–90%), lag approximately 80 μs. Series resistance and input resistance were monitored throughout the experiment. Recordings were discontinued if changes in series resistance were larger than 20%. Holding potential was −70 ± 5 mV unless specified. To estimate the input resistance, −5 mV steps from the holding potential were applied. The average input resistance was 769 ± 178 MΩ (n = 75).

Data were collected in the presence of kynurenic acid (2.5 mM, Sigma) or DNQX (25 μM, Tocris) plus D-APV (30 μM, Tocris) to block ionotropic glutamate receptors. To study miniature IPSCs (mIPSCs), tetrodotoxin (TTX, 1 μM) was applied to the bath. In some experiments, the probability of release was reduced by the addition of low concentrations of CdCl2 (2–30 μM). The following drugs were applied during some experiments to the perfusing medium: bicuculline methiodide (Sigma) to block GABA<sub>B</sub> receptors; 2-OH Saclofen (Tocris) or CGP 55845 (Tocris) to block GABA<sub>B</sub> receptors; RS-MCPG (Tocris) to block mGluR receptors; t-ACPD (Tocris) to activate presynaptic GABA<sub>B</sub> receptors (the effects induced by activation of postsynaptic GABA<sub>B</sub> receptors were prevented by the presence of CdCl<sub>2</sub> and QX-314 in the intracellular solution (Nathan et al. 1990; Otis et al. 1993). Recordings were digitized (12.5 KHz) and stored using programmable software (Spike 2, Cambridge Electronic Design, Cambridge, UK).

Extracellular stimulation

A pair of tungsten microelectrodes (Frederick Haer, Bowdoinham, ME) glued together side by side were used to apply single, pairs, or multiple current pulses in a bipolar configuration (typical current pulses: 100 μs, 10–30 μA, at 0.02–0.1 Hz unless specified). Stimuli were applied using a constant current unit (Stimulus isolator, World Precision Instruments, Sarasota, FL), which was triggered using Spike 2 software. The stimulating electrodes were located in the white matter surrounding the dorsal or lateral aspect of the lateral nuclei or along the main axes of the adjacent folia to optimize the activation of PCs fibers and prevent activation of efferent axons of DCNs, which exit the lateral nucleus through the ventral and medial border (Chan-Palay 1977; Matsushita and Iwashori 1971).

Single axon stimulation

To study IPSCs induced by the activation of single Purkinje cell axons (unitary IPSCs), we used the technique of minimal stimulation (Allen and Stevens 1994; Dobrunz and Stevens 1997; Raastad et al. 1992; Stevens and Wang 1995). To search for unitary IPSCs, stimulus intensity was increased until a response was detected. Afterwards, the stimulus intensity was decreased and increased in steps of 5% of the threshold intensity, and the responses to >25 pulses were recorded for each step. The criteria for single axon stimulation were as follows: 1) 5% decrements or increments of stimulus intensity did not change the average amplitude of successful responses, 2) evoked IPSCs did not exhibit changes in latency and/or shape, and 3) decrements of the stimulus intensity larger than 5% resulted first in larger percentage of failures without changes in the average amplitude of successful IPSCs and further decrements of stimulus intensity in complete failure of evoked responses. These criteria were met in <10% of the cases (see Fig. 1, B and C, and RESULTS for further information).

Analysis

Short-term plasticity of compound IPSCs was estimated by the peak amplitude ratio between the conditioned and control responses. Peak amplitude of responses was measured on averages of 20–30 single traces as shown in Fig. 1B, inset. Data were analyzed using Igor programmable software (Wavemetrics, Lake Oswego, OR) and Sigma Plot (Jandel Scientific, San Rafael, CA).

To calculate the inverse squared coefficient of variation (CV<sup>-2</sup> = mean<sup>2</sup>/variance), we determined the mean peak amplitude and variance about mean of control and conditioned responses from 60 to 250 single traces. Extended and continuous paired pulse activation (>10 min) of PC-DCN synapses at low frequency (<0.1 Hz) often resulted in strong and prolonged depression of IPSCs. To prevent such trends in the basal probability of release during the acquisition of the number of traces necessary for CV<sup>-2</sup> analysis, paired-stimulation was applied intermittently, making 3-min pauses every 25–30 pairs of stimuli (7–to 100-ms interpulse intervals, delivered at 0.1 Hz). Data were pooled together and averaged if no trend in IPSC amplitude was found. The analysis was performed using Minianalysis (Synaptosoft) and Sigma Plot (Jandel Scientific).

The same software was used for analysis of unitary-, miniature-, and quantal-evoked IPSCs. The peak conductance of unitary IPSCs was calculated for each experiment by dividing the peak amplitude of unitary IPSC by the corresponding driving force (holding potential — the reversal potential). The reversal potential was calculated in each experiment from the current voltage relationship of unitary IPSCs and was on average −2.7 ± 3.1 mV (n = 9). For the analysis of failures in synaptic transmission, paired unitary IPSCs were evoked using intermittent paired pulse stimulation (see previous paragraph). Failures in synaptic transmission were detected by visual inspection of single traces. Data are presented throughout the text as mean ± SE.

RESULTS

Properties of PC-DCN synapses were examined using whole cell voltage-clamp recordings of DCNs and extracellular stim-
ulation of PC axons in the white matter in the presence of blockers of excitatory amino acids.

Properties of compound, unitary, and quantal PC-DCN IPSCs

Recorded postsynaptic responses consisted in short-latency GABAergic IPSCs that could be blocked completely by bicuculline (20 μM). Waveform parameters of the evoked compound IPSCs were as follows (n = 52): 10–90% rise time (RT), 1.2 ± 0.53 ms; half-width (HW), 13.3 ± 0.01 ms; and time constant of decay (τd), 16 ± 7.20 ms. To characterize unitary PC-DCN IPSCs (uIPSCs), we used minimal stimulation of PC axons (Allen and Stevens 1994; Dobrunz and Stevens 1997; Raastad et al. 1992; Stevens and Wang 1995). Figure 1A illustrates an example of evoked uIPSCs. The typical relationship between the average peak amplitude of presumed unitary PC-DCN IPSCs and near-threshold intensity of stimulation is exemplified in Fig. 1B. In this example, a "plateau" in the amplitude of the evoked responses was observed with stimulus intensities ranging from 17 to 19.5 μA. Increasing the stimulus intensity from 17 to 18 μA resulted in a decrease of failure rate of transmission from 60% to 10% without change in the mean amplitude of successful IPSCs, and decreasing the intensity of stimulation from 17 to 16 μA resulted in complete failure of the responses. These data are consistent with the idea that stimulation intensities between 17 and 19.5 μA activated a single fiber, first (17 μA) close to the axon threshold and then suprathreshold (18 μA) (Allen and Stevens 1994). The low percentage of failures observed at suprathreshold intensities is in agreement with the idea that PC-DCN connections present multiple release sites (Palkovits et al. 1977). Low failure rate of synaptic transmission of multisited connections was previously observed in other preparations using minimal stimulation (Gil et al. 1999) or double-patch recordings (Cox et al. 1997; Kraushaar and Jonas 2000). Unitary PC-DCN IPSCs (n = 9) waveforms were similar to compound PC-DCN IPSCs: RT, 1.1 ± 0.48 ms; HW, 11.1 ± 4.39 ms; and τd, 13.5 ± 5.29 ms. The mean unitary IPSC peak amplitude was 780 ± 150 pA (n = 9) at −70 ± 5 mV holding potential, which corresponds

FIG. 1. Purkinje cell (PC)-cerebellar nuclei neuron (DCN) unitary and quantal inhibitory postsynaptic currents (IPSCs). A: example of IPSC evoked by minimal stimulation of PC axons. Trace illustrates the average of 25 single traces. B: minimal stimulation method. Responses evoked in a DCN by stimulation in the white matter using near-threshold intensities. ▲, average peak current of successful trials (left ordinate); ◦, failure rate in percentage (right ordinate); both as a function of the stimulation intensity. Horizontal line represents the range over which no change in average amplitude of responses was detected (see METHODS and RESULTS for details). C: responses evoked in a DCN by minimal stimulation within the deep cerebellar nuclei with intensities close to threshold (see RESULTS). D: 2 examples of recordings showing spontaneous IPSCs (top) and miniature IPSCs (mIPSCs) (bottom) recorded before and after application of TTX (1 μM). E1, amplitude distribution of mIPSCs recorded from a DCN during Na⁺ channels blockade with TTX (1 μM). Data taken from 1,090 IPSCs. Inset: average of mIPSCs aligned by their rising phase. E2, amplitude distribution of quantal IPSCs evoked in a DCN by minimal stimulation during application of the calcium channels blocker, Cd²⁺ (10 μM). Data taken from 136 IPSCs. Inset: average of quantal evoked IPSCs (no failures included).
to a large peak conductance change (see METHODS): 11.7 ± 2.3 nS, ranging from 2.9 to 22.7. Two other observations support that the large amplitude of these IPSCs is a genuine characteristic of unitary PC-DCN events. First, TTX sensitive spontaneous IPSCs, and therefore presumably unitary events, could display similar large amplitudes (Fig. 1D). Second, using minimal stimulation, we could evoke lower amplitude IPSCs when the stimulating electrodes were placed within the cerebellar nuclei (Fig. 1C). These IPSCs probably resulted from the activation of DCNs or of severed branches of PC axons. These data show that our estimation of PC-DCN uIPSCs amplitude was not biased by low sensitivity.

We investigated the quantal content of PC-DCN unitary IPSCs, which is generally assumed to indicate the number of functional sites releasing neurotransmitter per action potential. Assuming that each quantum added linearly to the total unitary conductance, the mean quantal content of unitary connections was calculated by the ratio between unitary and quantal conductance. Two approaches were used to determine the PC-DCN quantal conductance: first, the mean amplitude of miniature IPSCs (mIPSCs) were obtained from recordings obtained under blockade of sodium currents (TTX, 1 μM) and excitatory aminoacid receptors (kynurenic acid, 2.5 mM). The remaining IPSCs could be blocked entirely by bicuculline indicating that they were GABAergic in accord with earlier reports (Anchisi et al. 2001; Pedroarena et al., 2001). Since most of the GABAergic terminals on DCNs originate from PCs (approximately 80%) (De Zeeuw and Berrebi 1995), the amplitude of mIPSCs recorded under the latter conditions should roughly correspond to the amplitude of PC-DCN quantal events. In accordance with this idea, the analysis of mIPSCs waveform parameters yielded similar values to those obtained from unitary or compound PC-DCN IPSCs: RT, 1.2 ± 0.77; HW, 13.1 ± 3.33; tau_d, 11.3 ± 1.92. The quantal conductance estimated from mIPSCs measurements was 0.56 ± 0.08 nS (n = 6). In a second approach, the PC-DCN quantal conductance was evaluated from recordings of mini-evoked unitary IPSCs obtained after decreasing the probability of release to low levels by addition of CdCl_2 (10–25 μM) (Edwards et al. 1990). The mean conductance of successfully evoked IPSCs under these conditions was 0.72 ± 0.04 nS (n = 3), a value reasonably close to the values obtained from the mIPSCs. Figure 1E shows examples of the amplitude distribution of mIPSCs in TTX and kynurenic acid and the amplitude distribution of evoked unitary IPSCs in CdCl_2 (15 μM). Taking the mean quantal conductance resulting from the two approaches, the average quantal content of uIPSCs was 18, as estimated from the ratio 11.7/0.65 nS, and ranged between 4.5 (2.9/0.65) and 34 (22.7/0.65) nS. In conclusion, these values indicate that PC-DCN unitary connections present a large number of functional synaptic release sites.

**Paired pulse depression**

DCN responses to paired stimulation showed a clear depression of the second IPSC in all cases examined (n = 84; Fig. 2A). Paired pulse depression (PPD) was quantified by calculating the ratio p_2/p_1, where p_2 and p_1 are the average peak amplitudes of the IPSCs evoked by the second and first stimulus, respectively. The results from 24 different experiments were averaged and plotted in Fig. 2, B and C. Maximal depression was observed at the shortest intervals analyzed (p_2/p_1 = 0.15 ± 0.05 at 7 ms) and then decayed gradually for longer intervals. The decay of depression was best fitted using a double exponential with time constants of 32 (73% of total amplitude) and 5,000 ms.

We considered the possibility that the observed PPD, especially when evoked using short time intervals, could be a spurious result arising from specific conditions of the experimental approach itself. We then conducted a series of experiments to test these possibilities (Fig. 3). First, although high-frequency bursts of PCs action potentials (named complex spikes) are known to be faithfully conducted through their axons in vivo (Ito and Simpson 1971), we contemplated the possibility that p_2/p_1 ratios smaller than 1 resulted from failures in triggering pairs of action potentials with extracellular stimulation at short intervals. To explore this possibility, we performed whole cell patch recordings of PC somas in current-clamp mode to monitor their antidromic invasion when paired stimulation was applied in the white matter. In the presence of blockers of excitatory and inhibitory aminoacids (kynurenic acid, 2.5 mM; bicuculline methiodide, 25 μM) pairs of antidromic spikes were securely evoked by paired stimulation with interpulse intervals as short as 7 ms (Fig. 3A). When the intervals were shorter (2.5–5 ms), occasional failures in the antidromic invasion were observed. Failures of antidromic somatodendritic invasion when using short stimulus intervals could be attributed to decreased somatic excitability due to afterhyperpolarization or decreased axonal excitability. Although the first possibility is more plausible (Allen and Stevens 1994; Brock et al. 1953), we restricted the range of interpulse...
During partial block of the evoked current with 2/C_m and IPSC (bottom). Both traces are depicted superimposed after normalization to the 1st (25 consecutive recordings). The trace corresponds to the average of the magnitude of somatic, remote voltage escapes were assessed by comparing amplitude of paired IPSCs at short intervals could be a reduction unless specified. A second possibility for the decrease in am-intervals used in this study to values equal or longer than 7 ms, unless specified. A second possibility for the decrease in am-pair of IPSCs evoked in a DCN previous (top) and during application of bicuculline (2 μM) (middle). Both traces are depicted superimposed after normalization to the 1st IPSC (bottom). C. effect of a jump in the voltage command on responses evoked by paired PC stimulation. 1: control protocol: after a jump in the voltage command (VC) a stimulus (P) was applied through the extracellular stimulating electrode (S). 2: conditioned protocol: 2 extracellular stimuli were applied, the 1st before and the 2nd after the VC jump. The interval between pulses was 50 ms. 2: synaptic current evoked by the conditioned protocol superimposed onto the current obtained during the VC jump alone. 3: peak amplitude of responses evoked during control and conditioned protocol were compared. To measure the peak amplitude of IPSCs, the current responses evoked by the VC jump applied in isolation was subtracted from both responses. The resulting traces are shown superimposed.

FIG. 3. Experimental conditions and p2/p1 ratio. A: example of somatic antidromic invasion of PCs evoked by paired pulse stimulation in the white matter with 7-ms interpulse interval. The trace corresponds to the average of 25 consecutive recordings. B: pairs of IPSCs evoked in a DCN previous (top) and during application of bicuculline (2 μM) (middle). Both traces are depicted superimposed after normalization to the 1st IPSC (bottom). C: effect of a jump in the voltage command on responses evoked by paired PC stimulation. 1: control protocol: after a jump in the voltage command (VC) a stimulus (P) was applied through the extracellular stimulating electrode (S). 2: conditioned protocol: 2 extracellular stimuli were applied, the 1st before and the 2nd after the VC jump. The interval between pulses was 50 ms. 2: synaptic current evoked by the conditioned protocol superimposed onto the current obtained during the VC jump alone. 3: peak amplitude of responses evoked during control and conditioned protocol were compared. To measure the peak amplitude of IPSCs, the current responses evoked by the VC jump applied in isolation was subtracted from both responses. The resulting traces are shown superimposed.

intervals used in this study to values equal or longer than 7 ms, unless specified. A second possibility for the decrease in amplitude of paired IPSCs at short intervals could be a reduction in the driving force, caused by voltage escapes induced by the first synaptic event. Although a large fraction of PC inputs are somatic, remote voltage escapes were assessed by comparing the magnitude of p2/p1 ratio induced in control conditions and during partial block of the evoked current with 2 μM of bicuculline. Space clamp errors should be proportional to the current flow along the dendritic tree; a decrease in the magnitude of the evoked synaptic current should therefore result in a decrease in the magnitude of error. The advantage of this strategy was that the same individual synapses were activated in both conditions, while the mean amplitude of evoked current in the presence of bicuculline was reduced to 20–30% of the control. PPD under control conditions and in the presence of bicuculline was not different (range of intervals explored: 7–100 ms, n = 5). This indicates that the robust depression observed at short intervals cannot be due to space clamp errors. An example of these experiments is illustrated in Fig. 3B. Finally, short-term depression of GABAergic IPSCs might arise from modifications of the intracellular concentration of chloride ions caused by the first synaptic event and the consequent reductions in driving force for following events. This effect might be particularly pronounced during voltage-clamp experiments. We investigated this alternative using a procedure that combined paired pulse stimulation with a step in the voltage command such that the first response was evoked at a membrane potential below and the second response at a potential above the equilibrium potential for chloride ions (Fig. 3C). If changes in the chemical gradient were the cause for the short-term depression, we would anticipate a reduction of depression or even a facilitation on application of the voltage step. However, the results from three experiments showed that using this procedure PPD was not different from control (PPD values obtained using the voltage step vs. control conditions for each experiment were 0.77 vs. 0.75; 0.78 vs. 0.76; 0.65 vs. 0.63), indicating that changes in the intracellular chloride concentration are not the basis for the depression of synaptic responses.

Origin of paired pulse depression

Multiple pre- or postsynaptic mechanisms may cause short-term depression of transmitter release (for review see Zucker and Regehr 2002). Changes in the variability of synaptic currents have been used to determine the locus of origin of synaptic modulation (Auerbach and Betz 1971; Bekkers and Stevens 1990; Kuno and Weakly 1972; Malinow and Tsien 1990; but see limits in Faber and Korn 1991). To determine whether pre- or postsynaptic mechanisms were involved in PC-DCN PPD, we compared changes of the inverse squared coefficient of variation (CV−2) of IPSCs to changes in corresponding means (Bekkers and Stevens, 1990; Malinow and Tsien 1990). In a first step, we showed that CV−2 analysis did indeed distinguish between pre- and postsynaptic loci of modulation at PC-DCN synapses (Fig. 4A); reductions of the probability of release by application of the calcium channel blocker, Cd2+ (3–15 μM), or an agonist of GABA B receptors, baclofen (50 μM) (Mauginot and Gähwiler 1996; Morishita and Sastry 1995), resulted in a proportionally larger decrease of CV−2 than of the corresponding mean as expected for a presynaptic mechanism (Bekkers and Stevens 1990). On the contrary, decreasing the postsynaptic response by application of low doses of bicuculline, an antagonist of GABA A receptors, led to a larger decrease of the mean than the corresponding CV−2 consistent with a postsynaptic locus of action. We then proceeded to analyze data obtained with paired pulse stimulation (Fig. 4B). In all cases, CV−2 of the second IPSC decreased, with respect to the values of the first IPSC, proportionally more than the corresponding mean, indicating that PC-DCN PPD is of presynaptic origin.

We further explored the idea of a presynaptic origin of PPD by analyzing the differences in the number of failures in responses to the first versus the second stimulus in unitary recordings. To this end, we recorded IPSCs evoked in DCNs by minimal stimulation. The rational to use minimal stimulation was to maximize the chances to detect failures. It should be noted, however, that the unitary nature of the recording is not a prerequisite for the conclusions drawn from these experiments. Unitary IPSCs were evoked by pairs of stimuli with 50-ms interpulse intervals and the percentage of failures in the first and second responses were calculated. The results are summarized in Fig. 4C. An increase in the percentage of failures to the second stimulus
FIG. 4. Locus of PC-DCN PPD is presynaptic. A: plot of CV \(^{-2}\) against mean amplitude of IPSCs obtained during several manipulations: application of Cd\(^{2+}\) (2–10 \(\mu\)M), a calcium channel blocker (○), of baclofen, an agonist of GABA\(_B\) receptors (□); and of bicuculline (2–5 \(\mu\)M) (△). Data were normalized to CV \(^{-2}\) and peak amplitudes obtained during control conditions. Note that the 1st 2 manipulations, thought to decrease the probability of release, decreased CV \(^{-2}\) more than the mean, as expected for a presynaptic manipulation. On the contrary, postsynaptic blockade of GABA\(_A\) receptors by bicuculline, decreased the mean more than the CV \(^{-2}\). B: CV \(^{-2}\) analysis of PPD. Plot of CV \(^{-2}\) against mean amplitude of p\(_2\), after being normalized to the corresponding values of p\(_1\). Data correspond to interpulse intervals: 100 ms (n = 2), 50 ms (n = 9), 30 ms (n = 4), and 7 ms (n = 2). C: unitary recordings: probability of failures of unitary IPSCs associated to paired pulse stimulation. The bars represent the average percentage of failures in synaptic transmission for the 1st and 2nd stimulus (n = 7). Paired pulse interval: 50 ms. Inset: 10 consecutive recordings of unitary IPSCs evoked by paired PC axon stimulation in a DCN.

was found in 4 of 7 unitary recordings. On average, the percentage of failures was 0.2 ± 0.14 and 28.8 ± 15.60 for the first and second responses, respectively. In summary, both criteria, CV \(^{-2}\) analysis and percentage of failures, consistently support the notion that PPD originates mainly from a presynaptic mechanism.

Investigation of presynaptic mechanisms of PPD

Depletion of neurotransmitter is the most often postulated mechanism for PPD (Debanne et al. 1996; Dobrutz and Stevens 1997; Liley and North 1953; Rosenmund and Stevens 1996). If this is the case at PC-DCN synapses, then a decrease in the probability of release should decrease the magnitude of depression. We therefore investigated whether PPD was affected by application of Cd\(^{2+}\), a blocker of voltage-gated calcium currents. This strategy was applied because addition of Cd\(^{2+}\) at micromolar concentrations (2–15 \(\mu\)M) to the perfusing medium has minimal effects on surface charge and hence on the excitability of presynaptic fibers (Brody and Yue 2000). During this treatment, the amplitude of IPSCs was reduced to values ranging from 10% to 40% of control values. The plot in Fig. 5C illustrates the effects of this procedure on the ratio p\(_2\)/p\(_1\) for different interpulse intervals. A significant decrease in PPD was observed only for interpulse intervals between 30 and 200 ms (Kolgomorov-Smirnov test, \(P = 0.002, n = 5\)) but not for intervals outside this range. Similar results were obtained when the Ca\(^{2+}/Mg\(^{2+}\) ratio was decreased (n = 3, data not shown). Due to the heterogeneous results for different time intervals, we reinvestigated the idea of vesicle depletion by analyzing, in individual traces, whether the variations of p\(_2\) were inversely correlated to variations of p\(_1\), as one would expect if depression were depletion-dependent (Kim and Alger 2001; Kraushaar and Jonas 2000; Kuno and Weakly 1972; Waldeck et al. 2000). Figure 5D illustrates the results from three typical experiments (interpulse interval: 50 ms). The amplitude of p\(_2\) was not correlated to the amplitude of p\(_1\) (\(r^2 = 0.003\)). Similar results were found in five other experiments. Therefore our data do not support the idea that depression is release-dependent, but rather indicate that changes in the probability of release modify the short-term dynamics at these

FIG. 5. Evidence indicates that PPD is release independent. A: plot of p\(_2\)/p\(_1\) ratio of compound IPSCs against interpulse interval. No significant difference is observed in data obtained from 1 DCN before (□) and during blockade of presynaptic GABA\(_B\) receptors with saclofen (400 \(\mu\)M) (●). B: plot of p\(_2\)/p\(_1\) ratio of compound IPSCs against interpulse interval. Data obtained before (□) and during blockade of metabotropic glutamate receptors with MCPG (1 mM) (●). C: plot of p\(_2\)/p\(_1\) ratio against interpulse interval shown at 2 different time scales. Data obtained before (●) and during partial blockade of Ca\(^{2+}\) channels with Cd\(^{2+}\) (2–10 \(\mu\)M; n = 5) (○). Inset: averages of compound IPSCs evoked by paired stimulation before and during Cd\(^{2+}\) application. D: plot of p\(_2\) against p\(_1\) from single traces of compound IPSCs. Data from 3 different experiments are plotted together. Amplitudes of p\(_1\) and p\(_2\) were normalized ([(amplitude – mean amplitude)/SD]) to the mean amplitude and SD values of p\(_2\) and p\(_1\) for each experiment. The line represents the result of linear regression analysis. No significant correlation was found (\(P = 0.45, \text{slope} = 0.05, \text{and } r^2 = 0.003\)).
synapses. These results could be due to the effect of an undetected facilitatory process, a possibility which was addressed in further experiments (see next paragraph).

It has been shown that glutamate spillover can depress the release of GABA by activating presynaptic metabotropic receptors (Mitchell and Silver 2000; Wittmann et al. 2001). Metabotropic receptors from group I-II but not group III seem to be present in the DCN (Daggett et al. 1995; Ohishi et al. 1993; Phillips et al. 1998), and in our experiments, presynaptic metabotropic receptors could have been activated as a result of the stimulation of mossy and climbing fibers present in the white matter (Shinoda et al. 2000). However, the amplitude of IPSCs was not decreased by the application of a group I-II metabotropic glutamate receptors agonist, t-ACPD, (250 μM; data not shown), and PPD was not significantly altered by the application of the broad-spectrum metabotropic glutamate antagonist, MCPG (1 mM (Fig. 5B), indicating that PPD is not caused by activation of presynaptic metabotropic receptors. In addition, PPD does not result from feedback activation of GABA\textsubscript{B} receptors (Fig. 5A), which is consistent with previous results for a narrower range of intervals (Morishita and Sastry 1995).

**PC-DCN synapses exhibit short-term facilitation**

Under conditions of low probability of release induced by application of Cd\textsuperscript{2+}, the decay of depression showed a complex time course that—different from decay in the control situation—could not be fitted by a double exponential curve (compare Fig. 6A with Fig. 2B). However, the curve could be successfully fitted if it was assumed that synaptic facilitation occurred simultaneously with depression and that the two processes decayed with different time constants (Fig. 6A). The question therefore arose as to whether such unmasking of a possible paired pulse facilitation (PPF) was specific to the treatment with Cd\textsuperscript{2+} or dependent on a decrease of the probability of release in general. To answer this question, we availed ourselves of our discovery that repetitive stimulation at frequencies higher than 0.1 Hz resulted in a steady-state depression of presynaptic origin at these synapses (see next paragraph). In one set of experiments, paired pulse stimulation was delivered at rates higher than 0.1 Hz (0.2–1 Hz). The analysis of the decay of depression revealed a complex time course similar to the one observed under Cd\textsuperscript{2+} (Fig. 6B). Moreover, application of baclofen (50 μM) an agonist of GABA\textsubscript{B} receptors that presynaptically decreases the PC-DCN responses (Mauginot and Gâb威尔ver 1996; Morishita and Sastry 1995) induced similar modifications on the frequency dependence of the p\textsubscript{2}/p\textsubscript{1} ratio (n = 2, data not shown). These data indicated that facilitation might occur or be unveiled by conditions that decreased the probability of neurotransmitter release. Actual PPF (p\textsubscript{2}/p\textsubscript{1} > 1) was observed in only 3 of 52 experiments with lowered probability of release (Fig. 6C). In summary, these results indicate that, although depression was the predominant form of short-term modulation exhibited by these synapses, a mechanism of short-term facilitation also exists, becoming significant whenever the probability of release was decreased. The effect of such mechanism was manifested by a change in the frequency dependence of depression.

**Multiple pulse depression**

PCs in vivo typically exhibit repetitive firing at high frequency, which raises the question of how these patterns of activity modulate PC-DCN synaptic strength. To answer this question, we studied the modulation of PC-DCN IPSCs induced by repetitive stimulation at frequencies between 0.1 and 200 Hz. Figure 7A illustrates a typical example of these experiments. Analysis of individual IPSCs along the train indicated that modulation induced by multiple pulse stimulation (MPS) at frequencies equal and higher than 0.2 Hz was characterized by depression whose time course and extent were frequency dependent (Fig. B and C). Amplitude of single IPSCs decayed along the train with an exponential time course. The rate and degree of multiple pulse depression (MPD) increased with frequencies, i.e., time constants of MPD were 2,000 and 3.1 ms for 1 and 200 Hz, respectively, while steady-state MPD values (p\textsubscript{2}/p\textsubscript{1}) were 0.43 and 0.08 for 1 and 200 Hz.

![Facilitatory process tunes frequency dependence of PC-DCN synapses.](http://jn.physiology.org)
respectively (Fig. B and C). The recovery from MPD was slower than after a single pulse (PPD) and could be fitted with a double exponential (see details in Fig. 7D).

What effect does MPD have on the overall current responses to repetitive stimulation? Inspection of current recordings showed that their waveforms varied with frequency according to the different extent of depression, but also according to the different degree of summation of consecutive IPSCs (Fig. 7A).

Current responses to MPS typically exhibited an early peak, after which current decayed until a steady-state level was reached. At frequencies of stimulation lower than 30 Hz, the early peak corresponded simply to the first IPSC of the train, while at higher frequencies, summation of incompletely depressed IPSCs at the beginning of the train resulted in a larger transitory event. For instance, in the example of Fig. 7A, the early peak amplitude was 40% larger at 100 Hz than at 10 Hz. At steady-state level, however, the level of maximal current attained was similar for different frequencies of stimulation. Over the range of frequencies of stimulation analyzed (0.1–200 Hz) frequencies of stimulation higher than 10 Hz induced similar levels of steady-state inhibitory current. From these observations, we conclude that PC-DCN synapses are sensitive to transitions in PC signaling rather than to average firing rates.

To investigate whether presynaptic or postsynaptic mechanisms were involved in MPD, we analyzed the inverse squared coefficient of variation (CV−2) of responses evoked once the steady state of depression had been reached. The CV−2 and mean were plotted, after being normalized to the values corresponding to the first IPSC of the train (Fig. 7E). Results showed a fractional decrease in CV−2 larger than the corresponding mean, consistent with a presynaptic origin of depression. Results from previous studies indicated that both pre- and postsynaptic GABAB receptors are activated by stimulation of PC axons (Morishita and Sastry 1995; Mouginot and Gähwiler 1996). These results were confirmed in our preparation in a series of control experiments where patch electrodes were filled with a solution containing potassium gluconate instead of cesium chloride and no QX-314 was included (n = 5). In current-clamp mode, MPS of PC axons at 30 Hz resulted in suppression of the spontaneous firing of DCNs. The effect was maximal at the beginning of the train of stimuli as was expected from the voltage-clamp recordings (compare Fig. 7F with Fig. 7A). The recordings in voltage-clamp mode showed

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**FIG. 7**. Multiple pulse depression (MPD) of PC-DCN compound IPSCs. A: IPSCs evoked in a DCN by multiple pulse stimulation with 3 different frequencies: 10, 30, and 100 Hz. Note the increase in depression with increasing frequencies. Also note summation of IPSCs at 30 and 100 Hz. B: plots of \( p/p_0 \) ratio against time for different frequencies, where \( p_0 \) refers to the amplitude of the consecutive IPSCs of the train and \( p_1 \) to the IPSC evoked by the 1st stimulus (n = 4). The curves represent an exponential function fitted to the data. The calculated time constants are noted on each plot. Note decreasing steady-state level of \( p/p_0 \) (\( p/p_1 \)) with increasing frequencies. C: frequency dependence of MPD. Plot of steady-state \( p/p_0 \) ratio against frequency (left). Plot of MPD time constant against frequency (note the logarithmic scale) (n = 4; right). D: recovery from MPD (○) compared with recovery from a single pulse depression (PPD) (□; n = 3). Plot of amplitude ratio against interval of time after a single pulse or the end of a train. The experimental protocol is sketched on top. Multiple pulse stimulation consisted of 10 pulses delivered at 100 Hz. Curves represent a double exponential function fitted to the data. MPD: fast time constant: 83 ms (56%); slow time constant: 6,660 ms. PPD: fast time constant: 38 ms (85%); slow time constant: 5,882 ms. E: plot of CV−2 against mean amplitude of IPSCs obtained after steady-state MPD was reached. Data were normalized to CV−2 and peak amplitude obtained during control conditions. These results support a presynaptic origin of MPD. F: peristimulus histogram illustrating an example of DCN firing change evoked by multiple pulse stimulation (MPS) of PC axons at 30 Hz. Recordings were made in current-clamp mode using K-glucuronate–based intracellular solution. Vertical bars represent the number of spikes per bin normalized to the maximum count. The black horizontal bar indicates the duration of the stimulus train. Note that the MPS suppressed spontaneous firing maximally at the beginning of the stimulus train and approaches prestimulus levels toward the end of stimulation. G: current responses to PC MPS obtained using a K-glucuronate–based intracellular solution before (top) and after blockade by bicuculline (25 μM; bottom).
MPD similar to the one observed in recordings using cesium chloride intracellular solution (Fig. 7G, top). Application of bicuculline (10–30 μM) completely blocked the postsynaptic effects of multiple pulse stimulation, indicating that GABA release by PC terminals does not activate GABA_B postsynaptic receptors (Fig. 7G, bottom). In addition, blockers of GABA_B receptors did not modify MPD, confirming that PC-DCN synapses do not exhibit GABA_B-dependent feedback inhibition (Morishita and Sastry 1995; Mouginot and Gähwiler 1996; data not shown).

**DISCUSSION**

PCs are unusual in the CNS in that their axons constitute a long inhibitory projection pathway. Besides the interesting question raised by this fact with regard to signal transfer using inhibitory synapses, it also constitutes an advantage from the experimental point of view: the homogeneous PC inhibitory input can be evoked in DCNs in isolation by simple extracellular stimulation of multiple or single PC axons after pharmacological blockade of excitatory synapses. Using paired pulse and repetitive stimulation, we found that PC-DCN synapses exhibit robust synaptic depression that was maximal at short intervals. The main locus of this depression was presynaptic, and evidence suggested that PPD is based on a release-independent process. PC-DCN synapses do not convert into classical facilitatory ones under conditions of low probability of release, but a facilitatory process is manifested under this condition by a change in the frequency dependence of PPD. By analyzing unitary PC-DCN connections, we obtained evidence that unitary IPSCs present a relatively large conductance compared with the resting conductance of DCNs, suggesting that single PCs might significantly affect the output of their target neurons.

**Properties of unitary PC-DCN synapses**

It is generally assumed for central synapses that, at most, one quantum can be released per spike and per release site (Korn et al. 1994; Triller and Korn 1982). Following this assumption, the quantal content of unitary PC-DCN IPSCs (mean, 18; range, 4.5–22.7) reflects the average number of sites releasing neurotransmitter per spike and per connection, indicating that PC-DCN synapses present a high number of functional release sites in comparison with other inhibitory synapses (Cox et al. 1997; Kondo and Marty 1998; Kraushaar and Jonas 2000; Tamas et al. 1997; Thomson et al. 1996). Moreover, if the probability of release is less than one, the total number of releasing sites may be even larger. For instance, according to a simple binomial model and assuming a more realistic probability of release of 0.5, the total number of sites would be 36, as calculated from the ratio between quantal content (18) and probability of release (0.5). At the moment, there is no conclusive information about the morphological characteristics of unitary connections between Purkinje and nuclear neurons. Statistical calculations based on Golgi preparations estimated the number of contacts per PC-DCN unit to be about 13, and estimations of the number of boutons made by single PC axons on single DCNs ranged between 1 and 50 (Palkovits et al. 1977), which is close to our calculations.

Our estimation of PC-DCN quantal conductance (0.65 nS) is in agreement with previous data from DCN mIPSCs obtained in younger animals (Ouardouz and Sastry 2000). The average PC-DCN unitary conductance was 11.7 nS using high intracellular Cl− and corresponds to approximately 3.5 nS with a more physiological Cl− activity (10 mM) (Bormann et al. 1987). Comparison of the latter value with the “testing” conductance of DCNs (1.2 nS in our recordings; 1.5–3 nS in patch recordings without K+ channel blockers) (Czubayko et al. 2001; Morishita and Sastry 1995) indicates that single PCs might have a significant influence on DCNs activity. Moreover, synchronized firing of PCs, a phenomenon observed in anesthetized animals (Bell and Grimm 1969; Ebner and Bloedel 1981; Sasaki et al. 1989) as well as during performance of a movement in awake animals (Welsh et al. 1995), might have an enormous effect on DCN activity, in case synchronized PCs converge onto single neurons.

**Short-term depression, locus, and mechanisms**

The question of short-term plasticity of PC-DCN IPSCs at high frequencies was not addressed before. Here we show for the first time that PC-DCN synapses exhibit a remarkable PPD, maximal at short intervals, which recovers substantially in short time, such that, synaptic strength may vary between values as low as 10% at 7-ms and 70% at 50-ms interpulse intervals. These data indicate that the mechanism of short-term depression is maximally sensitive for frequencies equal or higher than 20 Hz, which corresponds to the physiological range of firing frequencies displayed by PCs (McDevitt et al. 1987; Thach 1968, 1970) (R. Haas and P. Thier, personal communication). In a previous study using slices from younger animals (7- to 9-day-old rats), PPD was investigated for intervals equal and longer than 50 ms (Morishita and Sastry 1995). Findings of the latter study show moderate PPD with a maximum at 50–400 ms. Due to the lack of information about PPD at short intervals in this study, conclusions about developmental changes in filtering properties of PC-DCN synapses cannot be drawn. Another study using organotypic cerebellar cultures and current-clamp recordings reported very weak frequency dependence of PC-DCN synapses even at 20-ms intervals (Mouginot and Gähwiler 1996), raising the possibility that dynamic properties of PC-DCN synapses might be different in cultured preparations.

Our results, based on CV−2 analysis and percentage of failures of synaptic transmission consistently indicated that the main locus of depression was presynaptic. Therefore postsynaptic mechanisms of depression, such as desensitization (Jones and Westbrook 1995) or saturation of postsynaptic receptors (Auger et al. 1998; Edwards et al. 1990; Tong and Jahr 1994), are not likely to be the major mechanisms of the depression at these synapses. Depletion of neurotransmitter is often postulated as a presynaptic mechanism for short-term depression (Debanne et al. 1996; Dobrunz and Stevens 1997; Liley and North 1953; Rosenmund and Stevens 1996). Experiments in which the probability of release was decreased to test this idea showed reduced PPD, albeit for intermediate intervals only. One explanation for the heterogeneous results at different intervals could be the coexistence of several mechanisms of depression with different time courses, one of them being release-dependent. However, the lack of negative correlation between amplitudes of first and second IPSCs, as would be
expected for a release-dependent mechanism, renders this mechanism unlikely. Regarding the calcium-dependence of paired pulse ratio at intermediate intervals, our demonstration of PPF points to an alternative scenario, namely that the observed changes in paired pulse ratio are caused by disclosure of facilitation by desaturation of the release process (Zucker and Regehr 2002). In addition, due to the release-independent nature of PPD, it is unlikely that other molecules, eventually co-released with GABA, play a role in PPD. Release-independent depression has been demonstrated recently in other preparations (Kim and Alger 2001; Kraushaar and Jonas 2000; Waldeck et al. 2000). One possible mechanism postulated to explain release-independent depression is the failure in triggering or conducting axonal action potentials (Brody and Yue 2000; Luscher and Schiner 1990). Our findings indicate that PPD does not depend on failures in triggering and conducting spikes along the main axon (Fig. 3A). We cannot exclude failures in the invasion of terminals (but see Cox et al. 2000; Emptage et al. 2001). Alternatively, release-independent depression may be based on the presence of heterosynaptic mechanisms. However, the persistence of PPD when minimal stimulation was applied or when blockers of the most likely involved receptors in a heterosynaptic depression were added (metabotropic glutamate and GABA receptors) renders this possibility unlikely. Other mechanisms postulated for release-independent depression are decreased calcium inflow at the terminals (Forsythe 1998) and modifications of the release machinery (Stevens and Wang 1995; Waldeck et al. 2000).

**Functional significance**

What type of signals may PC-DCN synapses transfer? Our results indicate that PC-DCN inhibition is a nonlinear function of PC firing rate, and it is time dependent, suggesting that information encoded in firing rate cannot be directly and unambiguously transmitted to DCNs. Instead, these synapses seem to be well suited to transfer signals related to transitions in firing rate (Fig. 7, A and F). This idea is in agreement with previous studies showing that systems with depressing synapses display sensitivity to dynamic signals (Abbot et al. 1997; Tsodyks and Markram 1997). Dynamic information is to be transferred in such systems because in response to a sudden change in presynaptic frequency, there is a transitory period during which postsynaptic currents not yet in the new steady state of depression occur at the new frequency, thus generating a transient postsynaptic signal which is proportional to the presynaptic change of activity (Abbot et al. 1997; Tsodyks and Markram 1997; see also Fig. 7A). It is clear that a requirement for transferring dynamic information is that changes in probability of release are reflected “moment-to-moment” in the synaptic output. However, an obstacle to fulfill this requirement is the probabilistic and univisceral character of transmitter release at CNS synapses, which makes the instant synaptic output an unreliable indicator of the actual probability of release. The instantaneous adjustment of the synaptic output, however, could be accomplished by performing an “on-line” averaging. Our results suggest that the characteristics of PC-DCN unitary connections are optimal for this task. The combination of a large number of release sites—a fact usually associated with highly reliable transmission—with a remarkable short-term depression seems at first glance paradoxical. However, this configuration might be useful to deliver information about the varying unitary synaptic efficacy, instantly, whenever a PC spike occurs, by averaging across its numerous release sites. This possibility would be precluded in unitary connections with single or few release sites. For networks with weak synapses, it has been proposed that transfer of dynamic information requires synchronization of presynaptic neurons during the periods of change in presynaptic activity (Abbot et al. 1997; Tsodyks and Markram 1997).

The strong activity-dependent depression of PC-DCN synapses, in addition, may help to sustain the steady firing of DCNs despite the massive inhibitory input that originates from high-frequency firing of PCs (McDevitt et al. 1987; Thach 1970), in conjunction with the effect of intrinsic DCNs properties (Aizenman and Linden 1999; Czubayko et al. 2001; Gardette et al. 1985; Jahnsen 1986a,b; Llinás and Mühlethaler 1988; Raman et al. 2000). Persistent firing would enable DCNs to respond to increasing, as well as decreasing, changes in PC firing rate with immediate modification of their output firing (Gauck and Jaeger 2000; Jahnsen 1986a). The idea that dynamic PC signals may induce parallel changes in DCN firing is supported by our findings showing that the effect of repetitive activation of PC axons on DCN firing is characterized by a transient period of maximal depression at the beginning of the train (Fig. 7F). Potential interaction with other intrinsic properties or synaptic inputs may lead to even more interesting effects on firing.

In conclusion, our results indicate that the inhibitory PC-DCN synapses are well suited to encode the dynamics of PC unitary activity with accurate adjustments of their synaptic strength, which may, in turn, be reflected by immediate changes on DCN firing, a capacity well in agreement with studies showing the involvement of the cerebellum in control of temporal aspects of motor and cognitive behaviors (Diener and Dichgans 1992; Hore et al. 1991; Ivry and Keele 1989; Thier et al. 2000).

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