

## HIGHLIGHTED TOPIC | *The Physiology and Pathophysiology of the Hyperbaric and Diving Environments*

# Differential modulation of cerebellar climbing fiber and parallel fiber synaptic responses at high pressure

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**Etzion Y, Mor A, Grossman Y.** Differential modulation of cerebellar climbing fiber and parallel fiber synaptic responses at high pressure. *J Appl Physiol* 106: 729–736, 2009. First published December 4, 2008; doi:10.1152/jappphysiol.90853.2008.—High pressure, which induces central nervous system (CNS) dysfunction (high-pressure neurological syndrome) depresses synaptic transmission at all synapses examined to date. Several lines of evidence indicate an inhibitory effect of pressure on  $\text{Ca}^{2+}$  entry into the presynaptic terminal. In the present work we studied for the first time the effect of pressure on the cerebellar climbing fiber (CF) synaptic responses. Pressure modulation of cerebellar synaptic plasticity was tested in both the CF and parallel fiber (PF) pathways using paired-pulse protocols. CF synapses, which normally operate at a high baseline release probability, demonstrate paired-pulse depression (PPD). High pressure reduced CF synaptic responses at 5.1 and 10.1 MPa but did not affect its PPD. High extracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_o$ ) could not antagonize the effect of pressure on the CF response, whereas low  $[\text{Ca}^{2+}]_o$ , in contrast to pressure, decreased both the response amplitude and the observed PPD. PF synapses, which usually operate at low release probability, exhibit paired-pulse facilitation (PPF). Pressure increased PF PPF at all interstimulus intervals (ISIs) tested (20–200 ms). Several  $\text{Ca}^{2+}$  channel blockers as well as low  $[\text{Ca}^{2+}]_o$  could mimic the effect of pressure on the PF response but significantly increased the PPF only at the 20-ms ISI. These results, together with previous data, show that the CF synapse is relatively resistant to pressure. The lack of pressure effect on CF PPD is surprising and may suggest that the PPD is not directly linked to synaptic depletion, as generally suggested. The increase in PPF of the PF at pressure, which is mimicked by  $\text{Ca}^{2+}$  channel blockers or low  $[\text{Ca}^{2+}]_o$ , further supports pressure involvement in synaptic release mechanism(s). These results also indicate that pressure effects may be selective for various types of synapses in the CNS.

high-pressure neurological syndrome; hyperbaric pressure; paired-pulse facilitation; paired-pulse depression; synaptic plasticity

HIGH HYDROSTATIC PRESSURE (>1.1 MPa) induces the high-pressure neurological syndrome (HPNS) in animals and human divers (reviewed in Ref. 5). The major signs and symptoms of HPNS are dizziness, nausea, vomiting, muscle twitching, and tremors (7) as well as EEG changes and a reduction in cognitive function (1). The pathological effects of pressure

appear to involve increased neuronal excitability in various structures of the central nervous system (CNS) (2, 9, 22, 23, 41). On the cellular level, decreased slow afterhyperpolarization and increased *N*-methyl-D-aspartate (NMDA) receptor responses (37, 38) may partially contribute to the excitatory state, but otherwise pressure seems to only modestly affect intrinsic neuronal properties (21, 34, 45). In contrast, pressure prominently depresses synaptic transmission at all synapses examined to date (reviewed in Refs. 11, 34; see also Refs. 20, 47). The presynaptic nature of this depression is well established (reviewed in Ref. 34). Pressure has little effect on various postsynaptic receptors with the exception of glycine and NMDA receptors (reviewed in Ref. 11; see also Refs. 37, 38) and depresses transmitter release from isolated synaptosomal terminals (25). In addition, quantal analysis revealed that pressure decreases spontaneous miniature end-plate potential frequency (3) and reduces evoked quantal content and the number of active release sites without affecting the quantal size (3, 27). High pressure may decrease transmitter release by affecting one or more steps known to be important in this process. Several lines of evidence indicate a depressant effect of pressure on  $\text{Ca}^{2+}$  entry into the presynaptic terminal (25, 26, 29), which predominantly involves *N*-type  $\text{Ca}^{2+}$  channels (20, 28).

The excitatory inputs on cerebellar Purkinje neurons are composed of two highly distinctive  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid (AMPA) receptor-mediated glutamatergic responses, the parallel fiber (PF) input and the climbing fiber (CF) input. The PF response is induced by multiple fibers innervating the Purkinje cell dendrites. Therefore, activation of this input exhibits a stimulus strength-dependent response (16). In addition, this response exhibits prominent paired-pulse facilitation (PPF) of presynaptic origin (10, 18). The CF synapse has several unique characteristics: it originates from axons of inferior olivary neurons, which climb along the proximal dendrites of the Purkinje cells where they form extensive synaptic contacts. Each Purkinje cell is innervated by a single CF, which, when activated, induces a prominent “all-or-none” excitatory response triggering a series of action potentials followed by afterhyperpolarization (14). Manipulations of extracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_o$ ) indi-

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cate that this synapse operates at a baseline of high release probability, which normally produces a near-saturation synaptic response (13). In addition, this synapse exhibits prominent paired-pulse depression (PPD). This PPD, which correlates well with the high release probability, is unaffected by postsynaptic factors, indicating its presynaptic origin (13, 30).

We have previously demonstrated that the PF synaptic response exhibits "typical" depression at high pressure; however, it is mostly dependent on high-pressure sensitivity of the N-type calcium channels. The selective blockade of N-type calcium channels also revealed a relatively "pressure-resistant" component of this synaptic response (20).

To further understand the mechanism(s) that may be involved in motor and coordination decrements (observed in HPNS), we evaluated the effect of pressure on single cerebellar CF response and compared its effect on short-term synaptic plasticity of both the CF and the PF synaptic responses.

## MATERIALS AND METHODS

### Brain Slice Preparations

All experiments were approved by the Institutional Ethics Committee, Faculty of Health Sciences, Ben-Gurion University of the Negev (Beer-Sheva, Israel). Sagittal (19, 21) and biplanar (20) cerebellar slices were prepared for studies of CF and PF responses, respectively, as previously described. Briefly, adult male albino guinea pigs (200–650 g) were decapitated under pentobarbital sodium (40 mg/kg) anesthesia. The cerebellum was exposed, and the vermis was isolated. Sagittal slices were obtained in a conventional manner. A modified slicing method was devised to obtain

biplanar cerebellar slices similar to those described by Garthwaite and Batchelor (24). The slices (400  $\mu\text{m}$  thick) were incubated for at least 1 h at room temperature in a standard Ringer solution saturated with a mixture of 95%  $\text{O}_2$ -5%  $\text{CO}_2$ . The standard Ringer solution contained the following (in mM): 124 NaCl, 5 KCl, 1.25  $\text{NaH}_2\text{PO}_4$ , 2  $\text{MgSO}_4$ , 26  $\text{NaHCO}_3$ , 2  $\text{CaCl}_2$ , and 10 glucose.

Single slices were transferred to a perspex recording dish (2 ml vol), fixed to the bottom by nylon mesh glued to a stainless steel ring, and constantly superfused with Ringer solution at a rate of 3–4.5 ml/min. The bath was placed in a hyperbaric chamber (Canty) adapted for electrophysiological recordings. The Ringer solution was oxygenated outside the chamber at normal atmospheric pressure and was introduced into the chamber by means of a high-pressure pump (Minipump LDC Analytical). Temperature was continuously monitored by a thermistor located near the preparation and maintained at 30°C by heating the perfusate entering the recording dish.

### Stimulation and Recording of CF Responses

Stimulation at a frequency of 0.2 Hz was done using a unipolar tungsten electrode located at the central portion of the white matter. Field potential recordings were performed from the proximal molecular (dendritic) layer. Typical CF responses were first sought at the somatic layer. Then the electrode was moved distally to the molecular layer until field recordings with a predominant negative voltage deflection were obtained (Fig. 1). Typical all-or-none CF unitary responses of single Purkinje cells were recorded from the somatic layer using the macropatch clamp technique (Fig. 2) as described previously (19, 21). This method enables recording of the somatic calcium spikes in the cells.

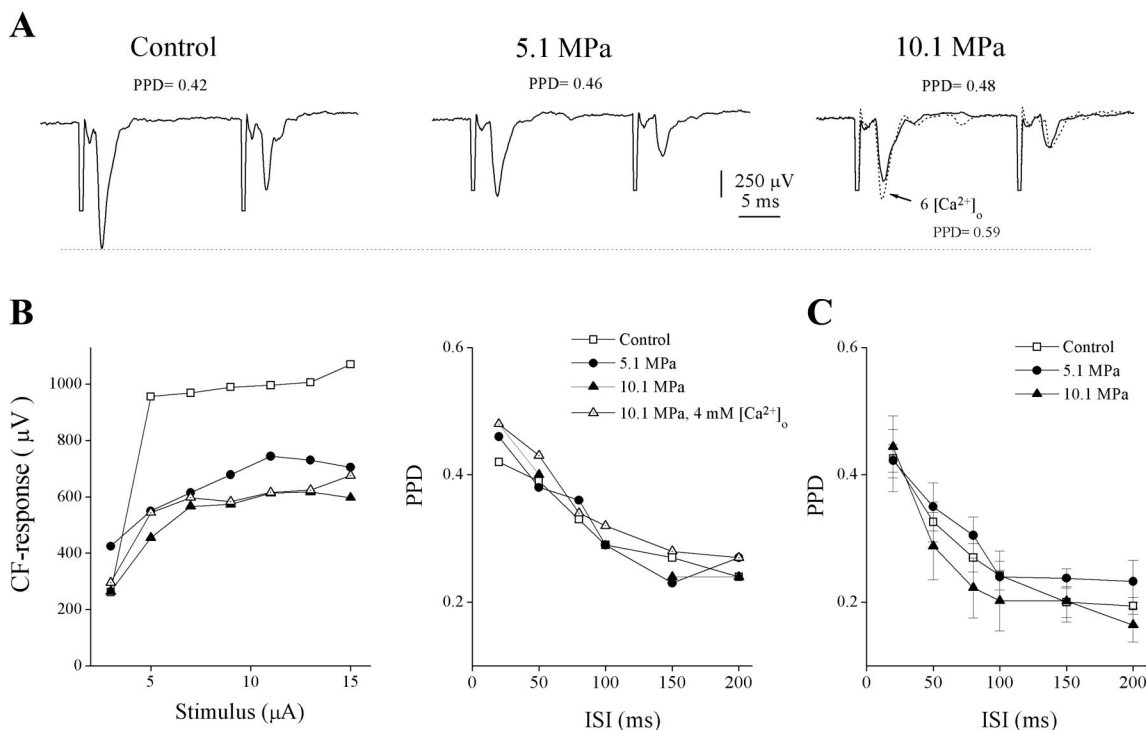


Fig. 1. Dendritic field potential recordings of the climbing fiber (CF) response at pressure. *A*: example of responses to identical stimulus at normal pressure (control), after compression to 5.1 and 10.1 MPa, and following an increase of extracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_o$ ) to 6 mM at maximal pressure (dotted trace). Each recording demonstrates paired-pulse stimulation at an interstimulus interval (ISI) of 20 ms and is an average of 10 similar responses. Calculated paired-pulse depression (PPD) is shown for each condition. *B*: similar to experiment in *A*. *Left*: CF response amplitude as a function of stimulus intensity. *Right*: PPD as a function of ISI. Note that an increase of  $[\text{Ca}^{2+}]_o$  to 4 mM did not affect the amplitude of the response at pressure or the observed PPD. *C*: summary of the effect of pressure on the PPD at all ISIs tested (20–200 ms) in 5 similar experiments. Pressure did not affect the PPD at any of these intervals.

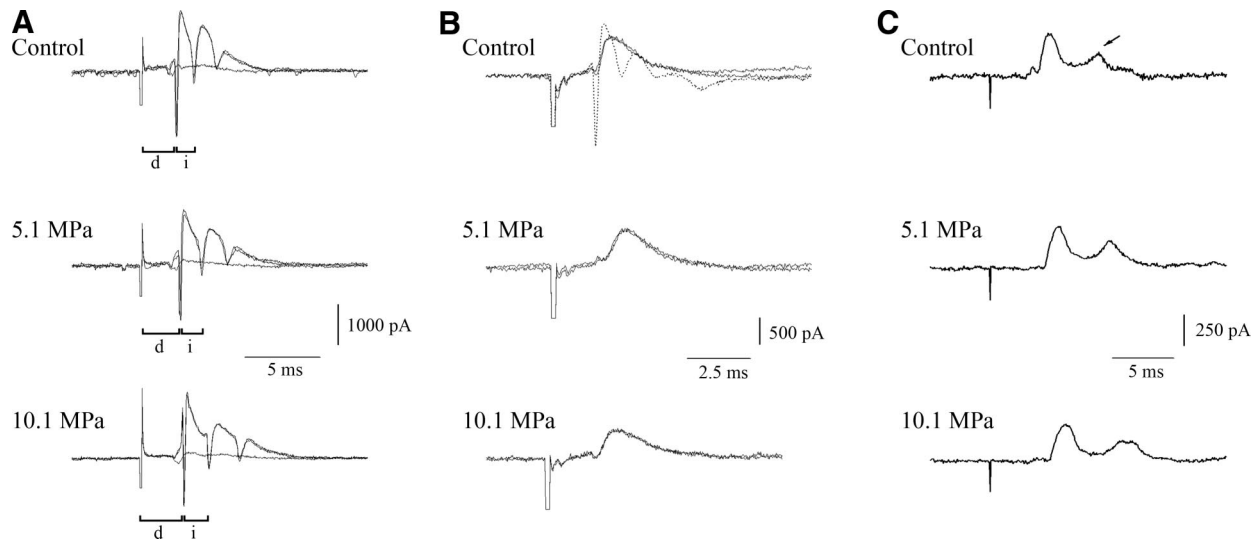


Fig. 2. Macropatch clamp somatic recordings of the CF response. *A*: typical "unit" recordings evoked by threshold stimulus level. Each plot contains superimposed traces of 2 "successes" and 1 "failure," demonstrating the "all-or-none" nature of this response. Note that pressure did not change the number of  $\text{Na}^+$  spikes (fast negative deflections) produced by the response but increased the delay between the stimulus to the first spike (d) and the first ISI (i). Other ISIs were not analyzed in detail (for statistical details of the analysis, see MATERIALS AND METHODS). *B*: similar CF response as in *A* (control, dotted trace), in which the spike initiation mechanism spontaneously deteriorated during normal pressure recordings. Pressure of 10.1 MPa reduced this response by  $\sim 25\%$ . *C*: an experiment in which the  $\text{Na}^+$  spikes overriding the CF response were abolished by the presence of tetrodotoxin (TTX) in the recording electrode. This treatment revealed the synaptic response followed by a dendritic  $\text{Ca}^{2+}$  spike (arrow). Pressure reduced this response by  $\sim 23\%$ . Plots in *C* are averages of 10 similar responses.

#### Stimulation and Recording of PF Responses

The detailed technique is described in Ref. 20. In brief, stimulation was done using a unipolar tungsten electrode located at the bottom of the vertical portion of the biplanar slice at a frequency of 0.2 Hz. Extracellular field potential recordings were made with glass microelectrodes of tip diameter of 10–20  $\mu\text{m}$  filled with Ringer solution.

In both CF and the PF responses, the recording electrode was mounted on a remote-controlled micromanipulator and connected to an Axopatch-1D head-stage (Molecular Devices, Axon Instruments) located outside the pressure chamber. The electrode was placed under visual control through a view port in the pressure chamber.

#### Electrolyte and Drug Application

Increased  $[\text{Ca}^{2+}]_o$  was obtained by addition of 2–4 mM  $\text{CaCl}_2$  to the standard Ringer solution, while lowering of  $[\text{Ca}^{2+}]_o$  was achieved by substitution of  $\text{Ca}^{2+}$  by  $\text{Mg}^{2+}$ .  $\text{Ca}^{2+}$  channel blockers  $\omega$ -conotoxin GVIA ( $\omega$ -CgTx), an N-type blocker, and  $\omega$ -conotoxin MVIIC ( $\omega$ -CTX MVIIC), an N/P/Q-type blocker, were purchased from Alamone Labs (Jerusalem, Israel) and stored in a lyophilized form at  $-20^\circ\text{C}$ . The toxins were dissolved and added to the Ringer solution directly before use, as previously described (20). Briefly, in the experiments in which specific  $\text{Ca}^{2+}$  channel blocker toxins were applied, a closed-loop circle was used to enable working with volumes of 10–20 ml which contained the drugs at the desired concentrations. The perfusion was initially switched to a closed-loop circle with the desired volume of normal Ringer solution, and control recordings were obtained. The drug was then added directly to the reservoir of the closed-loop circle. The use of the closed-loop circle did not by itself have any apparent effect on the recorded responses.

#### Compression and Decompression

Control recordings were taken in the sealed chamber at low helium pressure of 0.1–0.3 MPa. Helium compression up to 10.1 MPa and decompression protocol were described previously (20, 21). Tremor and convulsions thresholds for animals are at 1.1 and 6 MPa for

primates and at 6 and 9 MPa for rats, respectively (8). By assuming that the guinea pig convulsion threshold is similar to that of rats, we chose to compress the preparations up to 10.1 MPa to obtain a full expression of pressure effects. At this stage, only two pressure steps (5.1 and 10.1 MPa) were used to minimize waiting time for equilibration and rundown of the preparation. These pressure steps are being used routinely in our laboratory to reproducibly demonstrate high-pressure effects. Rates of compression/decompression were 0.5–1.0 MPa/min. Decompression was attempted to show reversibility of pressure effects.

#### Data Acquisition and Analysis

Field potential and macropatch clamp recordings were filtered (DC–10 KHz band pass), digitized (National Instruments board; AT-MIO-16F-5), and stored on a personal computer. Acquisition and analysis were performed using self-programmed software (Labview, National Instruments). Eight to fifteen traces were recorded for each stimulus intensity and analyzed separately. Traces shown in figures represent an average of 5–10 single responses each. Data from 5.1 and 10.1 MPa or after application of channels blockers were compared with the control values. Statistical significance was determined by application of Student's paired *t*-test. Data are presented as means  $\pm$  SE unless otherwise noted.  $P < 0.05$  is considered significant. Nonsignificance (NS) is stated for  $P$  values higher than this level.

## RESULTS

### Pressure Moderately Depresses CF Response

*Field potential recordings.* Dendritic field potential recordings were performed from the proximal molecular layer. These recordings demonstrated a large negative initial phase followed occasionally by a smaller positive phase as observed *in vivo* (15, 17) and were completely abolished by CNQX (10  $\mu\text{M}$ ), indicating its excitatory glutamatergic nature. In contrast to the PF response, which gradually increases as stimulus level is increased (20), the CF response was usually increased in a few

distinct steps, indicating the involvement of a limited number of fibers in the generation of the obtained response. Therefore, recordings selected for the study at pressure were made at stimulus strength substantially higher than the last observed step, to exclude changes in the response due to failure at the axonal level (see Fig. 1B). Pressure reduced the CF response amplitude recorded in the dendritic area to  $80.3 \pm 2.7$  and  $70.9 \pm 4.2\%$  at 5.1 and 10.1 MPa, respectively ( $n = 5$ ,  $P < 0.05$  for both; see also Fig. 1).

**“Unit” recordings.** To further investigate the CF response at pressure, typical all-or-none CF responses of single Purkinje cells were evaluated at pressure. Pressure increased the delay to the first spike by  $20.5 \pm 1.2\%$  and the interval between the first consecutive spikes by  $43.9 \pm 2.3\%$  (control vs. 10.1 MPa,  $n = 5$ ,  $P < 0.001$  for both analyses). However, pressure did not change the number of spikes overriding the CF response ( $n = 5$ , NS) or the stimulus threshold for obtaining the unit recordings (Fig. 2A). As seen in Fig. 2A, overriding somatic  $\text{Na}^+$  spikes markedly complicated the shape of the CF synaptic response obtained at the somatic layer (35). This well-known problem can practically be avoided by intracellular recordings from “injured” Purkinje cells with low membrane potential (17) or by active depolarization of the membrane potential to the point of total  $\text{Na}^+$  channel inactivation (13). Although such manipulations could not be employed by the present extracellular methodology used in the pressure chamber, similar suppression of spike initiation was described because of local mechanical injury by the juxtaposed recording electrode (15). Such a response is usually followed by a general deterioration. However, Fig. 2B shows one such recording that was stable

and could be evaluated at pressure. The results demonstrate an  $\sim 25\%$  depression at 10.1 MPa and are consistent with the field potentials recordings above. To suppress the spike initiation more efficiently, an attempt was made to locally apply tetrodotoxin (TTX) through the recording electrode. Several recordings were made in which  $1 \mu\text{M}$  TTX was present in the recording electrode. This manipulation more consistently abolished the  $\text{Na}^+$  spikes shortly after a response was obtained. Unfortunately, in most cases, failures of the response, and finally total block, were usually encountered thereafter, indicating an effect of TTX on the ascending CF itself. Figure 2C demonstrates, however, a single recording in which the stimulated CF was unaffected by TTX, and the response could be examined at pressure. This response was depressed by  $\sim 23\%$ , which also corroborates our finding above. It seems that the CF synapse is less susceptible to pressure than other examined synapses e.g., the  $\sim 50\%$  depression in the single PF response (20).

#### High Pressure Does Not Affect CF PPD

Paired-pulse stimulation of the CF induced the typical PPD of this synapse (13, 30), which was unaffected by  $20 \mu\text{M}$  bicuculline ( $n = 5$ ), confirming no involvement of GABAergic inhibition. While pressure significantly reduced the single CF response amplitude (as above), it did not affect the PPD at all ISIs tested (Fig. 1). Interestingly, doubling the level of  $[\text{Ca}^{2+}]_o$  to 4 mM ( $n = 2$ ) could not antagonize the depression of the CF response at pressure (Fig. 1B). Further increase of  $[\text{Ca}^{2+}]_o$  to 6 mM could slightly antagonize the effect of pressure on the

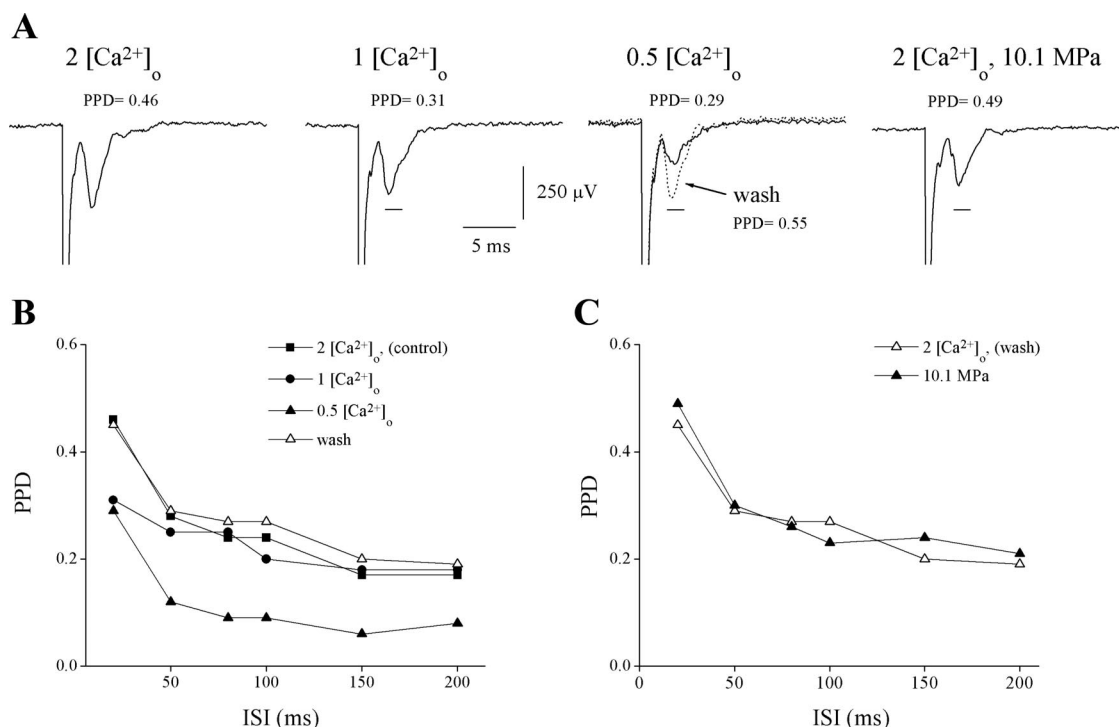


Fig. 3. Comparison between the effects of low  $[\text{Ca}^{2+}]_o$  and high pressure on the same CF response. A: similar representation as in Fig. 1A (single experiment); horizontal lines represent the amplitude of the control response. An experiment in which 1 mM  $[\text{Ca}^{2+}]_o$  and, later on, 1.5 mM  $[\text{Ca}^{2+}]_o$  were substituted by  $\text{Mg}^{2+}$  (1 mM  $[\text{Ca}^{2+}]_o$  and 0.5 mM  $[\text{Ca}^{2+}]_o$ , respectively). High pressure was applied following washout of the  $[\text{Ca}^{2+}]_o$  to 2 mM (wash, dotted trace). B and C: similar experiment as in A. B: the effect of low  $[\text{Ca}^{2+}]_o$  on the PPD at multiple ISIs. C: the effect of pressure on the PPD at multiple ISIs. Note that, in contrast to pressure, low  $[\text{Ca}^{2+}]_o$  reduced the PPD of the CF response.

amplitude of the first response, but this effect was accompanied by increased PPD (Fig. 1A). In another experiment, the effect of low  $[Ca^{2+}]_o$  was compared with that of pressure. Low  $[Ca^{2+}]_o$  (0.5–1 mM) reversibly reduced the CF response as well as the PPD, while pressure reduced the CF response without similar reduction of the PPD (Fig. 3).

#### High-Pressure Modulation of PF PPF

PPF is normally elicited when double-stimulation protocol is applied to the PF synaptic response (4, 16). PPF is defined as the ratio of the amplitude of the second synaptic response over the amplitude of the first synaptic response. Facilitation in various synaptic models and PPF in the CNS are attributed to presynaptic residual  $Ca^{2+}$  that increases the transmitter release in response to the second stimulus given at short time intervals (50). A decrease in transmitter release as a result of reduced presynaptic  $Ca^{2+}$  entry attenuates the postsynaptic response and shifts it away from saturation, leading to a facilitated response to the second pulse of a pair. Therefore, a decreased release probability is expected, in general, to increase the PPF. In the present study, double stimulation at various ISIs (20–200 ms) was performed to compare the effects of pressure (Fig. 4) with those of reduced  $Ca^{2+}$  entry (Fig. 5). The response of the PF was measured in the biplanar slice described in detail in Etzion and Grossman (20). A single response entails a fast, large biphasic deflection that indicates the presynaptic volley of a high number of axons, followed by a slower negative deflection reflecting the synaptic field potential in a number of cells (Fig. 4A). The PPF at 10.1 MPa was significantly increased at the range of 20- to 200-ms ISIs between 155 and 120%, respectively (Fig. 4C). A similar, although less

prominent, effect was also notable for 5.1 MPa (Fig. 4, B and C). Low  $[Ca^{2+}]_o$  (Fig. 5A), N-type (Fig. 5B), and N+P/Q-type (Fig. 5C)  $Ca^{2+}$  channel blockers that reduce presynaptic  $Ca^{2+}$  entry significantly increase the PPF only at 20-ms ISI to 118, 115, and 125%, respectively (Fig. 5, bottom; normalized responses). It is worth noting that they can inhibit the single synaptic response to a similar extent as pressure (20). These results suggest that pressure may reduce  $Ca^{2+}$  entry by blocking additional  $Ca^{2+}$  channels or other presynaptic mechanisms (see DISCUSSION). PF responses were shown to be partially reversible on decompression (data not shown; see Ref. 20).

## DISCUSSION

### CF Response at Pressure

Several reasons led us to select the cerebellar CF response at pressure. Although the cerebellum is not suggested to play a major role in the induction of HPNS convulsions wise (32, 33), it is possibly involved in pressure effects on motor coordination and learning and memory. In view of the possible diversity of pressure effects on various synapses, it is important to study as many as possible CNS structure responses to high pressure. The CF synapse is unique in its characteristic all-or-none near-saturation response, which has never been studied before. Moreover, since the effect of pressure on the more typical PF synaptic response was also described in detail by our group (Ref. 20 and present results), a thorough comparison between these two excitatory pathways to the cerebellar cortex could be obtained.

Our results indicate several important new findings. Both the field potential and the limited number of unit (macropatch)

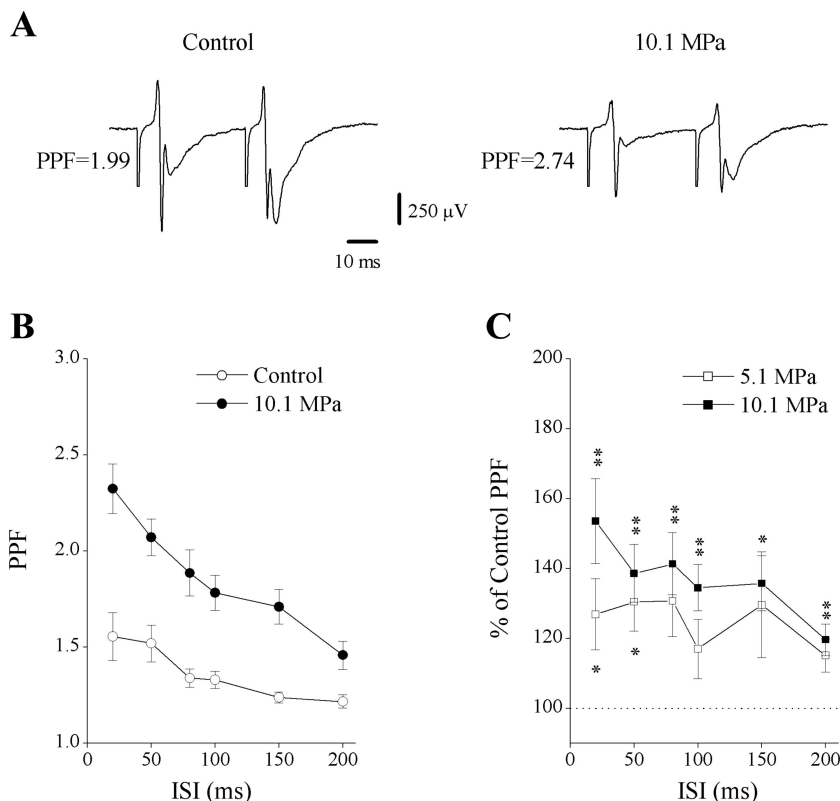


Fig. 4. Effect of pressure on paired-pulse facilitation (PPF) at the parallel fiber (PF) synapses. **A**: an example of paired-pulse stimulation (50-ms ISI) under normal conditions and after compression to 10.1 MPa. The calculated PPF is presented for each condition. **B**: summary of the effect of maximal pressure on the PPF recorded at several ISIs for a single stimulus level. For each interval,  $n = 6-7$  except for 150 ms ( $n = 5$ ). **C**: summary of the effect of 5.1 and 10.1 MPa on the PPF (presented as %control PPF). For 5.1 MPa,  $n = 4-5$  except for 150 ms ( $n = 3$ ). For 10.1 MPa, similar data as in **B**. Note the increased PPF at all ISIs under pressure (although most prominent at 20-ms ISI). \* $P < 0.05$  and \*\* $P < 0.01$ . Statistical analysis, paired  $t$ -test.

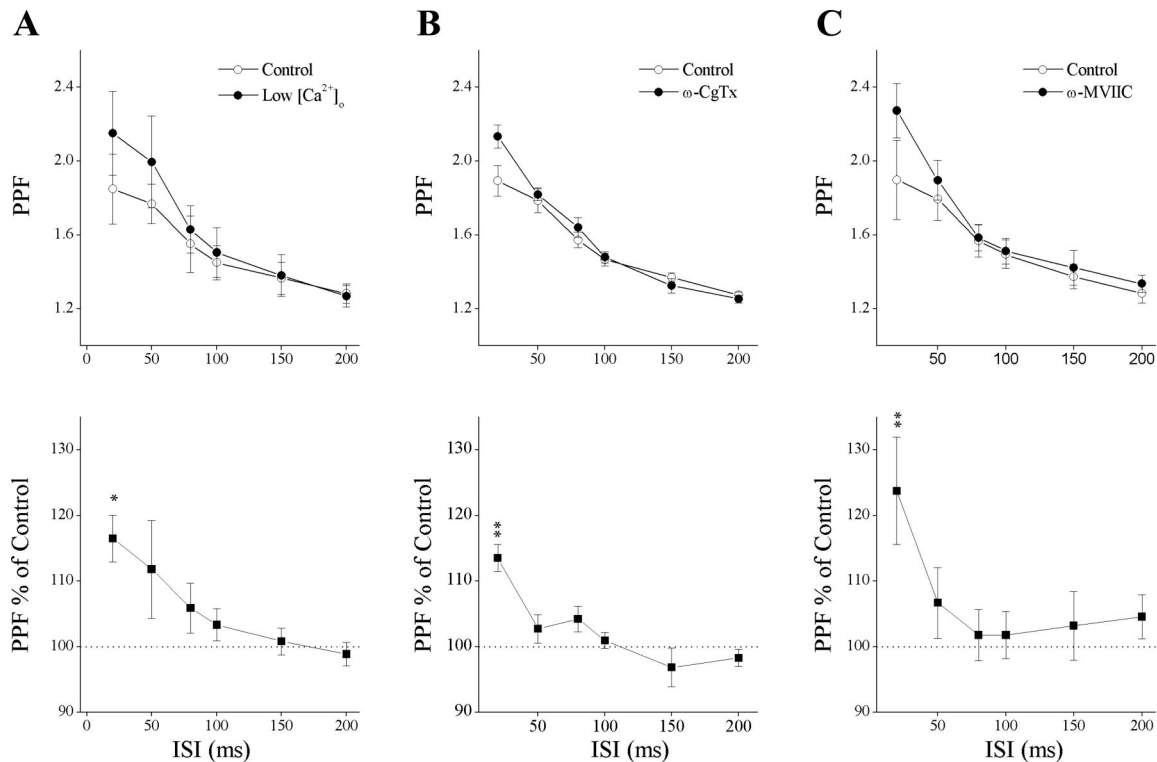


Fig. 5. Effect of low  $[Ca^{2+}]_o$  and  $Ca^{2+}$  channel blockers on the PPF. *Top*: change in PPF values. *Bottom*: normalized data. *A*: summary of the effect of low  $[Ca^{2+}]_o$  on the PPF recorded at several ISIs for a single selected stimulus level. For each ISI,  $n = 4$ . *B*: application of  $\omega$ -CgTx. For each ISI,  $n = 12$  except for 80 ms ( $n = 10$ ). *C*: application of  $\omega$ -CTX-MVIIC. For each ISI,  $n = 7$  except for 150 ms ( $n = 6$ ). Note the significant increase in PPF only at ISI of 20 ms. \* $P < 0.05$  and \*\* $P < 0.01$ . Statistical analysis, paired  $t$ -test.

recordings indicated that the CF response is more resistant to pressure compared with the PF synaptic response (20) as well as other synapses examined at pressure (reviewed in Ref. 34, 47). Consequently, the synaptic input conserved the number of generated somatic action potentials (APs), although their delay is increased and their frequency is reduced. We could not measure clearly the actual synaptic delay in this pathway, but the results indicate that synaptic delay is increased similarly to most invertebrate synapses where it was possible to measure it accurately (reviewed in Refs. 11, 34) and CNS synapses (20, 47). It is important to note that pressure-induced increase in the delay of any response in these preparations is composed of a reduction in AP conduction velocity in addition to an intrinsic delay of the release mechanism caused by an unknown component. The moderate but consistent pressure-induced depression of the CF response did not affect the PPD (see below) and did not change the normally observed saturation, in regard to high  $[Ca^{2+}]_o$ , in contrast to the effect of low  $[Ca^{2+}]_o$  (Figs. 1 and 3) (13, 30).

The validity of the dendritic field potential recordings of the CF synaptic response should be examined. This response may be activated also by nonspecific white matter stimulation that contributes to the observed response. However, such contribution is usually small (13). The difficulty of a small number of fibers involved in the response is avoided by using suprathreshold stimulus intensity above which no further change in the response is observed. Finally, the large CF response normally activates postsynaptic voltage-gated  $Ca^{2+}$  channels, which may contaminate the recorded synaptic response. Such a com-

plication is difficult to avoid unless strong intracellular hyperpolarization is applied (35).

The relatively small pressure-induced depression of the CF response is of special interest. If pressure affects primarily presynaptic  $Ca^{2+}$  entry as previously suggested, then it should be less affected in this synapse. Indeed, the  $Ca^{2+}$  entry in the CF synapse normally produces a near-saturation response (13). Therefore, a reduction of  $Ca^{2+}$  influx by pressure should affect this response less than the response of a nonsaturated synapse such as the PF synapse. In addition, N-type channels, which are probably most sensitive to pressure (20), contribute substantially less to the CF response than to the PF response, at least in the newborn rat (36, 40). Since doubling of  $[Ca^{2+}]_o$  did not antagonize the effect of pressure on the CF synapses (Fig. 1B) as reported for other synapses (26, 29), it may suggest that at least part of the CF depression at pressure results from a process other than presynaptic  $Ca^{2+}$  entry. In this regard, a direct effect of pressure on exocytosis was previously suggested (31).

#### CF and PF Plasticity at Pressure

**PPD.** In contrast to other manipulations that reduce presynaptic release, such as low  $[Ca^{2+}]_o$  (Fig. 3, Ref. 30), pressure seems to be the first "presynaptic inhibitor" that does not affect the PPD of the CF response (Fig. 1). PPD may result from "depletion" of available release sites (13). Therefore, our results suggest that such depletion can occur without an actual release from a site and may involve an upstream step in the

release process. However, it should be noted that further work is needed to fully establish the presynaptic origin of the described effect of pressure on the CF synaptic response. If presynaptic origin is indeed confirmed in future studies, then our present findings may imply that direct modulation of the release process by pressure can serve as a useful and unique tool to study the depletion mechanism of fast synapses.

**PPF.** The effect of pressure on the PPF at short ISIs is in agreement with previous studies and is consistent with a reduction of  $\text{Ca}^{2+}$  entry into the presynaptic terminal (20, 26, 29, 39, 47). However, in the present experiments, pressure increased the PPF also at long ISIs (Fig. 4). Interestingly, application of  $\text{Ca}^{2+}$  channel antagonists increased PPF only at 20-ms ISI (Fig. 5), probably because they block the N- and P/Q-type  $\text{Ca}^{2+}$  channels that may operate in that time window. As expected, pressure increased PF PPF at 20-ms ISI because of its suggested potency in inhibiting N-type  $\text{Ca}^{2+}$  channel (20). However, pressure efficacy also at longer ISIs suggests that other  $\text{Ca}^{2+}$  channels with a longer time course of activity may contribute to the release process or, alternatively, other pressure-sensitive process(es) such as recovery of release sites. Reduced activity of inhibitory pathways may also contribute to such an effect, as suggested for the dentate gyrus synapses (47). However, it seems less likely in the PF, since application of bicuculline (20  $\mu\text{M}$ ,  $n = 3$ ) did not affect the PPF in these synapses (data not shown).

#### *Cerebellum Function at High Pressure*

In view of our present and recent findings, it is worthwhile looking into the general function of the cerebellum under pressure conditions. The Purkinje cell axons are the sole output of the cerebellum, forming GABAergic synapses onto neurons in the cerebellar nuclei. Ignoring mostly inhibitory interneuronal networks, the Purkinje cells receive two major glutamatergic excitatory inputs: the PF and the CF systems (reviewed in Ref. 48). The axons of the PF (originating from the cerebellar granule cells) synapsing on the distal parts of the dendritic tree provide probably tonic and fine tuning of the excitatory state of the cell, since each axon contributes only a small synaptic response. A single axon of the CF (originating from olivary nucleus neuron) synapsing extensively on the proximal part of the dendritic tree exerts a robust, fast, short-lived activation that produces a small number of  $\text{Na}^+$  APs in the soma and a large  $\text{Ca}^{2+}$ -dependent spike in the dendrites (“complex spike,” Ref. 43). The interaction between these two systems has never been studied at pressure, but, based on our present knowledge, we can postulate the final outcome of the network. High pressure depresses single PF synaptic input by 50%, but it could be compensated for by greater PPF (46, 47), which can operate in this system at lower frequencies (wider ISI range) or by increased number of active axons (speculated). Another mechanism, although not predominant in these synapses, could be the proposed increase in NMDA receptor current (12) and NMDA receptor synaptic response (38). Despite increased delay of each of the synaptic potentials, the overall summated tonic input can be preserved. On the other hand, the CF synaptic response is depressed only by ~25% and is capable of maintaining the same number of APs, although they are slightly delayed and their frequency is decreased. Since the shape and amplitude of the somatic  $\text{Na}^+$  APs and  $\text{Ca}^{2+}$  spikes

in the Purkinje cells are not affected by pressure (21) and PPD is preserved, the nature of this fast input is probably unchanged, although the timing of the APs can be distorted, and the spontaneous rhythmic activity observed in some cells is modified (21). Taken together, the excitatory input to the Purkinje cells is probably maintained at normal level or possibly even an overcompensated level if an NMDA receptor component of these synapses (6, 44) is augmented at pressure (37). Since the sole output of the cerebellar system is the GABAergic synapses, it is most likely that their output is also depressed by similar release mechanisms affecting the glutamatergic excitatory synapses, although only limited indirect evidence for such a depression in the CNS is available (42, 46, 49). Thus our overall hypothesis is that the cerebellar cortex preserves most of its “computational” function at high pressure while the final step of inhibition of the cerebellar nuclei is impaired. Since the effects of pressure on several components of this system are still unknown (e.g., local interneurons and forms of long-term plasticity), the general picture we draw is partially speculative. Nevertheless, our present findings should lay the groundwork for detailed studies aimed to resolve the effect of pressure on cerebellar cortex function.

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