LTP Regulates Burst Initiation and Frequency at Mossy Fiber–Granule Cell Synapses of Rat Cerebellum: Experimental Observations and Theoretical Predictions

Thierry Nieus, Elisabetta Sola, Jonathan Mapelli, Elena Saftenku, Paola Rossi, and Egidio D’Angelo

Department of Cellular-Molecular Physiological and Pharmacological Sciences, University of Pavia, Parma, Italy; and Department of General Physiology of Nervous System, A.A. Bogomoletz Institute of Physiology, Kiev, Ukraine

Submitted 1 July 2005; accepted in final form 4 October 2005

Nieus, Thierry, Elisabetta Sola, Jonathan Mapelli, Elena Saftenku, Paola Rossi, and Egidio D’Angelo. LTP regulates burst initiation and frequency at mossy fiber–granule cell synapses of rat cerebellum: experimental observations and theoretical predictions. J Neurophysiol 95: 686–699, 2006. First published October 5, 2005; doi:10.1152/jn.00696.2005. Long-term potentiation (LTP) is a synaptic change supposed to provide the cellular basis for learning and memory in brain neuronal circuits. Although specific LTP expression mechanisms could be critical to determine the dynamics of repetitive neurotransmission, this important issue remained largely unexplored.

In this paper, we have performed whole cell patch-clamp recordings of mossy fiber–granule cell LTP in acute rat cerebellar slices and studied its computational implications with a mathematical model. During LTP, stimulation with short impulse trains at 100 Hz revealed earlier initiation of granule cell spike bursts and a smaller nonsignificant spike frequency increase. In voltage-clamp recordings, short AMPA excitatory postsynaptic current (EPSC) trains showed short-term facilitation and depression and a sustained component probably generated by spillover. During LTP, facilitation disappeared, depression accelerated, and the sustained current increased. The N-methyl-D-aspartate (NMDA) current also increased. In agreement with a presynaptic expression caused by increased release probability, similar changes were observed by raising extracellular [Ca2+]. A mathematical model of mossy fiber–granule cell neurotransmission showed that increasing release probability efficiently modulated the first-spike delay. Glutamate spillover, by causing tonic NMDA and AMPA receptor activation, accelerated excitatory postsynaptic potential (EPSP) temporal summation and maintained a sustained spike discharge. The effect of increasing neurotransmitter release could not be replicated by increasing receptor conductance, which, like postsynaptic manipulations enhancing intrinsic excitability, proved very effective in raising granule cell output frequency. Independent regulation of spike burst initiation and frequency during LTP may provide mechanisms for temporal recoding and gain control of afferent signals at the input stage of cerebellar cortex.

INTRODUCTION

Brain neuronal circuits transmit signals often organized in spikes sequences or bursts (Krahe and Gabbiani 2004; Lisman 1997; Rieke et al. 1996). Bursts can be processed by two forms of short-term synaptic plasticity, facilitation and depression, which reflect the dynamics of synaptic vesicle cycling and regulate excitatory postsynaptic potential (EPSP) temporal summation (Buonomano 2000; O’Donovan and Rinzel 1997). The time-dependent properties of the synapse could be changed by long-term synaptic plasticity (Tsodyks and Markram 1997). Long-term potentiation or depression (LTP or LTD) can alter several functional aspects of the synapse, including neurotransmitter release, neurotransmitter spillover, and postsynaptic receptor gating and expression (Bliss et al. 2003; Kullmann et al. 1996; Lisman 2003; Malenka and Nicoll 1999). Although the particular mechanism of expression could be critical to determining the consequences of LTP and LTD on repetitive neurotransmission at specific synapses, this important issue remained largely unexplored.

Instead of considering synaptic responses to isolated impulses, we studied the response to stimulus trains at the mossy fiber–granule cell relay of cerebellum. Mossy fiber activity in vivo is characterized by repetitive discharges (Chadderton et al. 2004; Kase et al. 1980). Studies in acute cerebellar slices have revealed that repetitive stimulation causes depression in AMPA and summation in N-methyl-D-aspartate (NMDA) receptor-dependent responses (D’Angelo et al. 1995). The AMPA response includes a slow component determined by glutamate spillover, which also plays a major role in NMDA receptor activation (Cathala et al. 2003; DiGregorio et al. 2002; Rossi et al. 2002; Xu-Friedman and Regehr 2003). Interestingly, mossy fiber–granule cell LTP induced by theta-burst stimulation (TBS) is expressed presynaptically through an enhanced neurotransmitter release probability (Sola et al. 2004). Thus the mossy fiber–granule cell relay of cerebellum provides the opportunity for studying the relationship between synaptic mechanisms and the impact of LTP on neurotransmission dynamics.

In this paper, we investigated the effect of release probability changes on mossy fiber–granule cell neurotransmission by combining patch-clamp recordings in cerebellar slices with mathematical modeling. LTP increased the first excitatory postsynaptic current (EPSC) amplitude, accelerated short-term depression and, at the same time, enhanced sustained AMPA and NMDA receptor-mediated currents. As a result, granule cell discharge was initiated earlier while a sustained discharge frequency was maintained. Simulations showed that these effects required glutamate spillover in the cerebellar glomerulus and could not be replicated by postsynaptic changes in

Address for reprint requests and other correspondence: E. D’Angelo, Department of Cellular-Molecular Physiological and Pharmacological Sciences, University of Pavia, Pavia, Italy (E-mail: dangelo@unipv.it).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
METHODS

Patch-clamp recordings in acute cerebellar slices

Patch-clamp recordings in acute cerebellar slices were performed as previously reported (Armano et al. 2000; D’Angelo et al. 1995, 1999). Briefly, slices were cut in the sagittal plane from the cerebellar vermis of 18- to 23-day-old Wistar rats. Recording temperature was maintained at 30°C with a feed-back Peltier device (TC-324B, Warner Instrument Corp., Hamden, CT).

Kreb's solution for slice cutting and recovery contained (in mM) 120 NaCl, 2 KCl, 1.2 MgSO4, 26 NaHCO3, 2 NaH2PO4, 1.0 glucose, 3 ATP-Mg, 0.1 GTP, and 15 HEPES. This solution maintained resting free [Ca2+] at 100 nM, and pH was adjusted to 7.2 with KOH. Patch-clamp pipettes filled with this solution had a resistance of 5–8 MΩ before seal formation. The patch-clamp pipette solution for voltage-clamp recordings contained (in mM): 126 K-gluconate, 4 NaCl, 1.2 KH2PO4, 2 CaCl2, and 11 glucose and was equilibrated with 95% O2-5% CO2 (pH 7.4). Kreb's solutions with different Ca2+ concentrations (from 1 to 4 mM) were prepared maintaining the total concentration of divalent cations ([Ca2+]T) at 30 mM. During recordings, the GABA A receptor blocker, 10 µM bicuculline, was added to the solutions. Local drug perfusion was performed through a multibarrel pipette. Perfusion with control extracellular solution was commenced before seal formation and was maintained until switching to the test solutions. The patch-clamp pipette solution for current-clamp recordings contained (in mM): 126 K-gluconate, 4 NaCl, 1.2 KH2PO4, 0.05 CaCl2, 0.1 BAPTA, 15 glucose, 3 ATP-Mg, 0.1 GTP, and 5 HEPES. This solution maintained resting free [Ca2+] at 100 nM, and pH was adjusted to 7.2 with CsOH. Patch-clamp pipettes filled with this solution had a resistance of 3–5 MΩ before seal formation. All drugs were obtained from Sigma, except BAPTA tetrapotassium salt (Molecular Probes, Eugene, OR), d-2-amino-5-phosphonovaleric acid (APV), 7-Chloro-kynurenic acid (7-Ch-Kyn), and 6-cyano-7-nitroquinolxalene-2,3-dione (CNQX) (Tocris-Cookson, Avonmouth, UK).

Recordings were performed with an Axopatch 200B amplifier, and signals sampled with a Labmaster 1200-B interface (sampling rate = 10 kHz). Current and voltage traces were digitally filtered at 3 kHz and analyzed off-line with P-Clamp (Axon Instruments) and Igor (WaveMetrics) software. Data are reported as means ± SE and, unless otherwise indicated, statistical comparisons are done using paired Student’s t-test and differences are considered statistically not significant for P > 0.05.

Mossy fibers were stimulated with a bipolar tungsten electrode or a patch-pipette through an isolation unit at a basal frequency of 0.3 Hz (Hines and Carnevale 2001). To make model temperature (Tmod) compatible with experiments, rate constants in receptor kinetic schemes and voltage-dependent currents were corrected for the actual experimental temperature (Texp = 30°C) using a Q10 = 3 according to the relation Q10 = (Texp/Tmod)1/10 (D’Angelo et al. 2001).

The cerebellar granule cell patch-clamp recordings with a detailed reconstruction of postsynaptic electrophysiological aspects of neurotransmission derived from past and present measurements. LTP AND BURSTING IN THE CEREBELLUM
S/cm²; J. Magistretti, L. Castelli, and E. D’Angelo, unpublished observations), f_{\text{leak}} density was set at 2.655 × 10⁻⁴ S/cm² (P. Rossi, L. Roggeri, D. Gall, A. de Kerchove d’Exaerde, S. Schifflmann, V. Taglietti, and E. D’Angelo, unpublished observations), and the contribution of GABA-leakage was increased (3 × 10⁻⁷ mS/cm²; Rossi et al. 2002). Because the granule cell is electrotonically compact (Cathala et al. 2003; D’Angelo et al. 1995; Silver et al. 1996b), there was no need to simulate dendrites, and the original monocompartmental structure was maintained. Cerebellar granule cells receive four mossy fiber inputs on average (Eccles et al. 1967), each one impinging on a dendrite placed in a different glomerulus. The glomerulus is formed by a large mossy fiber terminal facing about 50 granule cell dendrites in the rat (Jakab and Hamori 1988). Because glomeruli are physically isolated (they are enveloped into a glial sheet and are spaced by >10 μm) and because EPSCs scale almost linearly during synaptic recruitment (DiGregorio et al. 2002; Sola et al. 2004), the model was implemented with four identical independent synapses.

Simulation of a single EPSC basically needs that a neurotransmitter waveform activates a receptor kinetic scheme (Destexhe et al. 1994). Simulation of an EPSC train is more problematic not only because postsynaptic dynamics must be computed but also because postsynaptic receptor states evolve in time maintaining the history of previous events. The situation is complicated at a multisite synapse by the stochastic activation of different receptor clusters and by neurotransmitter diffusion between sites (Barbour 2001). In principle, if the synaptic structure and diffusion coefficient are known, Monte Carlo simulations with several releasing sites would solve the problem, allowing each receptor to develop its individual time-dependent history. However, the stochastic nature of the output would require repetition over a large number of simulations to obtain average responses making the method impractical for EPSC train fitting. We have therefore adopted the approximation of making neurotransmitter concentration T proportional to a presynaptic variable Y reporting the intensity of neurotransmitter release (Buonomano 2000).

**POSTSYNAPTIC RECEPTOR ACTIVATION.** Granule cell postsynaptic responses are generated both through direct release from active zones onto corresponding postsynaptic receptors and through spillover of glutamate from neighboring releasing sites (Cathala et al. 2003; DiGregorio et al. 2002). For AMPA receptors, which are located into the cleft, glutamate concentration was obtained combining a synaptic pulse (T_p) with a diffusion wave (T_d) Synaptically released glutamate acting on AMPA receptors was generated with a 1-mM, 0.3-ms squared pulse, which was shown to well approximate transmitter action in the cleft (Destexhe et al. 1994). The effective glutamate concentration in the cleft is therefore

\[ T = T_p + T_d = Y \cdot T_{p,\text{max}} + T_{d,\text{max}} \]  

(2)

where \( T_{p,\text{max}} \) and \( T_{d,\text{max}} \) are maximum concentrations. NMDA receptors, which are largely extrasynaptic (Cathala et al. 2003; Rossi et al. 2002), were activated by glutamate diffusion, \( T_g \).

Glutamate binding to postsynaptic receptors activates kinetic schemes governed by microscopic first-order transitions (see Figs. 6 and 7), leading to the open state, \( O(T) \). It follows that

\[ i = g \cdot \Delta V = g_{\text{max}} \cdot O(T) \cdot (V - V_{\text{rev}}) \]  

(3)

where \( V_{\text{rev}} \) is the ionic reversal potential, and \( g_{\text{max}} \) is the maximum synaptic conductance for either NMDA or AMPA channels.

**AMPA RECEPTOR KINETIC SCHEME.** Several investigations have assessed the properties of AMPA receptor–mediated currents in cerebellar granule cells. The time-course of AMPA EPSCs in granule cells and estimates for AMPA channel open probability were reported in situ (DiGregorio et al. 2002; Sola et al. 2004). AMPA receptor desensitization was studied in granule cell patches in culture (Silver et al. 1996a). This allowed us to reconstruct a simple 3-state AMPA receptor kinetic scheme (Fig. 6a) using a parameterization similar to that recently reported by Saffenuk (2005), with \( k_{o+} = 5.4 \text{ ms}^{-1} \), \( k_{o-} = 0.82 \text{ ms}^{-1} \), \( k_{d+} = 1.12 \text{ ms}^{-1} \), \( k_{d-} = 0.013 \text{ ms}^{-1} \), \( k_B = 0.44 \text{ mM} \), and \( S = T^2/(T + k_B)^2 \). Using the PSD conductance (180–267 pS) and receptor open probability (0.4–0.6) measured with nonstationary fluctuation analysis (Silver et al. 1996b; Traynelis et al. 1993), the maximum conductance at a single mossy fiber–granule cell synapse with two to four releasing sites lay between 900 and 1,780 pS. Indeed, with 1,200 pS and \( P = 0.4 \) (Sola et al. 2004), the model correctly simulated unitary EPSC amplitude.

**NMDA RECEPTOR KINETIC SCHEME.** There are no specific kinetic schemes for NMDA receptor–mediated currents in cerebellar granule cells. The time-course of AMPA EPSCs in granule cells and estimates for AMPA channel open probability were reported in situ (DiGregorio et al. 2002; Sola et al. 2004). AMPA receptor desensitization was studied in granule cell patches in culture (Silver et al. 1996a). This allowed us to reconstruct a simple 3-state AMPA receptor kinetic scheme (Fig. 6a) using a parameterization similar to that recently reported by Saffenuk (2005), with \( k_{o+} = 5.4 \text{ ms}^{-1} \), \( k_{o-} = 0.82 \text{ ms}^{-1} \), \( k_{d+} = 1.12 \text{ ms}^{-1} \), \( k_{d-} = 0.013 \text{ ms}^{-1} \), \( k_B = 0.44 \text{ mM} \), and \( S = T^2/(T + k_B)^2 \). Using the PSD conductance (180–267 pS) and receptor open probability (0.4–0.6) measured with nonstationary fluctuation analysis (Silver et al. 1996b; Traynelis et al. 1993), the maximum conductance at a single mossy fiber–granule cell synapse with two to four releasing sites lay between 900 and 1,780 pS. Indeed, with 1,200 pS and \( P = 0.4 \) (Sola et al. 2004), the model correctly simulated unitary EPSC amplitude.

\[ \frac{dX}{dt} = \frac{Z}{\tau_g} - P \cdot X \cdot \delta(t - t_{\text{spike}}) \]

\[ \frac{dY}{dt} = -\frac{Y}{\tau_r} + P \cdot X \cdot \delta(t - t_{\text{spike}}) \]

\[ \frac{dZ}{dt} = Y \cdot Z \]

\[ \frac{dP}{dt} = -\frac{P}{\tau_p} + P(1 - p) \cdot \delta(t - t_{\text{spike}}) \]

(4)

where \( \tau_p \) is the time constant of recovery of releasable transmitter, \( \tau_g \) is the time constant of facilitation, \( \tau_r \) is the time constant of inactivation, \( P \) is release probability, \( p \) is its initial value, and \( \delta \) is Dirac’s delta function. While \( X(t) \), \( Y(t) \), and \( P(t) \) are discontinuous functions, the system is solved analytically by integrating differential equations in intervals \( [t_{\text{spike}}, t_{\text{spike}} + n \cdot \Delta t] \) for initial conditions at time \( t_{\text{spike}} \). At times \( t = t_{\text{spike}} \), the values of functions are changed abruptly by \( pX \) or \( P(1 - p) \). This causes, when a spike arrives, a proportion \( P \) of the resource \( X \) is transferred to \( Y \). Depletion of the resource \( X \) causes synaptic depression (another component depends on postsynaptic receptor desensitization). Synaptic facilitation is governed by \( p \) activity-dependence.
TABLE 1. Neurotransmission parameters obtained from fitting of EPSC trains

<table>
<thead>
<tr>
<th></th>
<th>Regular Train</th>
<th>Random Train</th>
<th>P (Unpaired t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P</strong></td>
<td>0.42 ± 0.05</td>
<td>0.36 ± 0.07</td>
<td>0.71</td>
</tr>
<tr>
<td><strong>τ_f, ms</strong></td>
<td>10.8 ± 0.8</td>
<td>39.4 ± 17.5</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>τ_r, ms</strong></td>
<td>35.1 ± 3.6</td>
<td>16.5 ± 4.5</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>τ_d, ms</strong></td>
<td>1</td>
<td>1</td>
<td>* fixed</td>
</tr>
<tr>
<td><strong>r, μm</strong></td>
<td>1.03 ± 0.10</td>
<td>0.92 ± 0.12</td>
<td>0.69</td>
</tr>
<tr>
<td><strong>M (molecules)</strong></td>
<td>21514.6 ± 3156.8</td>
<td>31688.1 ± 7403.1</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>D_eff, μm^2ms^{-1}</strong></td>
<td>0.22 ± 0.03</td>
<td>0.12 ± 0.01</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Values represent the average of measurements obtained in different cells (n = 7 for regular 100 Hz trains; n = 4 for random Poisson trains with 50 Hz mean frequency). Note that parameters obtained from fittings in regular and random trains are not statistically different.

dependence) and \( \bar{v}_0 = -20 \text{ mV} \) (half-activation potential). By using 376 low open-probability channels with a single channel conductance of 50 pS, as indicated by MK801 blocking kinetics (Rossi et al. 2002), the maximum NMDA EPSC conductance is 18,800 pS per synapse. With this value, activation of the NMDA receptor model (Rosenmund et al. 1998) by using a three-state AMPA scheme and 2D diffusion provided a minimal effective model of repetitive synaptic transmission in the cerebellar glomerulus. A comparison was done using more complex AMPA receptor kinetic schemes. While the Diamond and Jahr (1997) scheme was also effective, the Wadiche and Jahr (2001) and Raman and Trussell (1995) schemes desensitized too fast and failed to reproduce the spillover mediated component in AMPA EPSC trains (data not shown).

EPSC FITTING AND GLUTAMATE DIFFUSION. Because the diffusion space is unknown in principle, its properties were inferred by fitting the model to AMPA EPSCs, which were shown to include a sizeable spillover-mediated component (DiGregorio et al. 2002). Fits were performed using a SIMPLEX routine implemented in NEURON (see Fig. 6C). We preliminary performed AMPA EPSC fits using a diffusion kernel with free geometry (Neher and Sakaba 2001). Iterative adjustment of diffusion parameters fitted the whole EPSC, including both the direct and indirect component, with two-dimensional (2D) geometry. Thus intersite glutamate diffusion (Barbour 2001) was represented as

\[
T_{\text{eff}}(r,t) = \frac{M}{h \cdot 4 \pi D_{\text{eff}}} \exp \left( -\frac{r^2}{4 D_{\text{eff}}} \right)
\]

in which \( M \) is the number of released molecules, \( D_{\text{eff}} \) is the effective diffusion coefficient, \( r \) is the distance from glutamate source, and \( h \) is cleft height (Crank 1975). While \( h \) was fixed at 20 nm, \( D_{\text{eff}}, r, \) and \( M \) were obtained by fitting the first EPSC in a train. Then, \( D_{\text{eff}}, r, \) and \( M \) were fixed, and we obtained \( \tau_R, \tau_F, \) and \( p \) by fitting the whole EPSC train. An example of this procedure is reported in Figs. 5A, 6, and 6, and the corresponding average data are reported in Table 1.

It should be noted that using 2D geometry might lead to an overestimate of spillover glutamate concentration and its effect on desensitization (Xu-Friedman and Regehr 2003), thereby reducing the relative importance of presynaptic vesicle recovery during short-term depression. Although a definite conclusion on the balance between desensitization and vesicle depletion in determining synaptic depression cannot be drawn at this stage, we note that values obtained by fitting were consistent with current knowledge on mossy fiber–granule cell transmission (see Table 1), so that using a three-state AMPA scheme and 2D diffusion provided a minimal effective model of repetitive synaptic transmission in the cerebellar glomerulus. A comparison was done using more complex AMPA receptor kinetic schemes. While the Diamond and Jahr (1997) scheme was also effective, the Wadiche and Jahr (2001) and Raman and Trussell (1995) schemes desensitized too fast and failed to reproduce the spillover mediated component in AMPA EPSC trains (data not shown).

R E S U L T S

In this paper, we studied the impact of LTP on granule cell neurotransmission during repetitive mossy fiber activity.

LTP regulates granule cell burst initiation and frequency

The effect of short stimulus trains on mossy fiber–granule cell responses was initially studied by D’Angelo et al. (1995), who revealed AMPA receptor–mediated EPSP depression and an important contribution of sustained NMDA receptor–mediated responses to EPSPs temporal summation. Here, EPSPs were recorded using minimal stimulation from the initial membrane potential of \(-71.5 \pm 0.5 \text{ mV}\). EPSPs measured 17.8 ± 2.7 mV, peaked in 8.2 ± 1.5 ms, and showed a duration at half-amplitude (HW) of 24.5 ± 4.3 ms (\( n = 10 \)). During a train of 10 impulses at 100 Hz, individual EPSPs showed a marked depression, but their temporal summation caused a progressive depolarization followed by spike discharge (Fig. 1A).

LTP was induced by TBS (see Armano et al. 2000), leading to a 39.2 ± 5.3% EPSP amplitude increase 15 min after induction. At the same time, EPSP temporal summation in response to mossy fiber spike trains was enhanced. The first-spike delay significantly decreased from 41.7 ± 10.4 to 17.3 ± 11.2 ms (\( n = 7 \); \( P < 0.01 \)), and the average firing frequency increased from 20.7 ± 6.7 to 49.9 ± 10.8 Hz (\( n = 7 \); \( P < 0.01 \)). The potential coexistence of persistent changes in intrinsic excitability (Armano et al. 2000) was evaluated by measuring the first-spike delay and spike frequency during injection of depolarizing current pulses (Fig. 1B). These recordings showed that LTP was indeed associated with earlier initiation (16.3 ± 6.2 ms; \( n = 7 \)) and increased frequency (17.4 ± 4.3 Hz; \( n = 7 \)) of spikes independent from synaptic inputs. The changes in delay observed using depolarizing current pulses were significantly smaller than those observed using repetitive mossy fiber stimulation (\( P < 0.01 ; n = 7 \)), while the difference in firing frequency was not statistically significant (Fig. 1C).

This observation suggests a sizeable contribution of synaptic changes to first-spike delay regulation. To determine the underlying synaptic mechanisms, we measured the response to EPSC trains in voltage clamp and reconstructed the voltage response with a mathematical model. Here below we report the analysis of EPSC train dynamics for the AMPA and NMDA currents, which have different receptor gating and kinetics and could give substantially different contribution to synaptic excitation.

Short-term plasticity during LTP: the AMPA EPSCs

AMPA currents were isolated at ~70 mV (Fig. 2A), where the driving force is favorable and NMDA channels are largely blocked by Mg2+ (cf. Fig. 3A). EPSC trains at 100 Hz showed rapid AMPA receptor–mediated transitions sitting over a sustained current most probably corresponding to slow summation of glutamate spillover currents (these parameters are further defined in Methods and are shown in Fig. 2A). AMPA spillover currents can be observed in single EPSCs and are thought to arise from glutamate released from neighboring sites in the glomerulus (DiGregorio et al. 2002; Sola et al. 2004). We recall that glutamate spillover at mossy fiber synapses is particularly intense owing to the special architecture of the cerebellar glomerulus, which is constituted of ~50 closely...
spaced synaptic connections enwrapped into a glial sheet (Eccles et al. 1967; Xu-Friedman and Regehr 2003).

The transient component of AMPA EPSCs showed depression in 75% of cases \( (n = 12; \text{Fig. 2B}) \), whereas an initial transient facilitation followed by depression was observed in the remaining 25% \( (n = 4) \) of cases. In only-depressing trains, the amplitude of AMPA EPSCs decreased nearly exponentially, allowing estimation of the depression time constant, \( \tau_d = 15.4 \pm 2.2 \text{ ms} \) \( (n = 8) \), and the relative steady-state EPSC amplitude, \( A_s/A_0 = 0.25 \pm 0.03 \) \( (n = 8; \text{Fig. 2C}) \).
sustained current increased attaining a steady-state after two to three impulses.

LTP was induced by TBS paired with membrane depolarization at −40 mV to allow Ca2+ influx through NMDA channels (D’Angelo et al. 1999). During LTP, the first AMPA EPSC increased by 43.1 ± 13.9% (n = 16). In the eight depressing synapses, EPSC depression became faster with τd = 10.4 ± 2.2 ms (n = 8; P < 0.01, paired t-test). Moreover, the steady-state A∞ remained almost unchanged causing a significant decrease in the relative steady-state, A∞/A0 = 0.16 ± 0.02 (n = 8; P < 0.01, paired t-test). In the four facilitating synapses, facilitation turned into depression. In all cases, the sustained current showed a significant increase of 22.1 ± 9.7% (n = 16; P < 0.05) at the end of the train. In agreement with the observation reported by Sola et al., (2004), the switch from facilitation to depression and the A∞/A0 and τd changes during LTP are characteristic of an increased presynaptic vesicle turnover (Brenowitz and Trussell 2001; O’Donovan and Rinzel 1997; Tsodyks and Markram 1997), although the changes could also be influenced by postsynaptic receptor desensitization (Xu-Friedman and Regehr 2003).

Short-term plasticity during LTP: the NMDA EPSCs

The NMDA current was isolated at −40 mV, where NMDA channels are unblocked from Mg2+ (Fig. 3A). This choice was further motivated by the observation that the NMDA current in granule cells has voltage-dependent kinetics (NMDA-EPSCs slowdown with depolarization; D’Angelo et al. 1994) and that the membrane potential of −40 mV is critical for NMDA receptor regulation of EPSP temporal summation and spike firing, providing the data used for mathematical modeling.

Figure 3A shows that the sustained component in 100-Hz EPSC trains was similar at −40 and at −70 mV despite the reduction in driving force for AMPA currents, revealing the voltage-dependent contribution of the NMDA current. Subsequent application of 10 μM CNQX, a specific blocker of non-NMDA ionotropic receptors, yielded the NMDA component in isolation. At −40 mV, the NMDA current accounted for most of the sustained current (Fig. 3A, middle right traces; 2.2 ± 0.1 pA at the end of trains, n = 4), showed a marked temporal summation and a smooth time-course. At −70 mV, the NMDA current was negligible (0.5 ± 0.1 pA, n = 4). Accordingly, the current obtained by subtracting the scaled trace at −70 mV from that at −40 mV corresponded precisely to the NMDA current isolated pharmacologically (2.2 ± 0.2 pA at the end of trains, n = 4; P < 10−6). Subsequent addition of 100 μM APV and 50 μM 7Cl-Kyn completely blocked the responses, ruling out any sizeable contribution of reuptake currents.

By applying the subtraction procedure (see METHODS), we could determine the time-course of the NMDA current in the same recordings used to construct Fig. 2 without needing of pharmacological tools. During LTP (Fig. 3B), the first NMDA EPSC increased by 53.3 ± 16.0% (n = 4; P < 0.0007), while the steady-state response did not significantly change (5.2 ± 6.1%, n = 4; not significant). Thus potentiation in NMDA differed from that in AMPA current trains, although both showed LTP on the first EPSC. The apparent saturation of responses during trains could reflect saturation of NMDA receptors because of glutamate accumulation.

Neurotransmission dynamics are regulated by release probability

To understand whether increasing release probability could determine the changes in EPSC trains observed during LTP, release probability was modified with different extracellular Ca2+/Mg2+ ratios (Fig. 4) (Dodge and Rahamimoff 1967; Katz and Miledi 1968). In these recordings, we used 50-Hz EPSC trains, and NMDA receptors were blocked with 100 μM APV and 50 μM 7Cl-Kyn to prevent any NMDA receptor-dependent form of synaptic plasticity. At low Ca2+/Mg2+ ratio (0.5 mM Ca2+, 2.8 mM Mg2+), all granule cells showed a prominent EPSC facilitation. At normal Ca2+/Mg2+ ratio (2 mM Ca2+, 1.2 mM Mg2+) 8 of 11 granule cells showed depression and the remaining showed facilitation. At high Ca2+/Mg2+...
developed a mathematical model (see METHODS) incorporating LTP could explain those in current-clamp recordings, we observed during LTP supporting their presynaptic origin. EPSC trains by raising release probability mimicked those during LTP (Fig. 4). Thus the changes induced in AMPA EPSCs, and were largely blocked at resting membrane potential and unblocked in the threshold region (Fig. 5A). The model fitted both AMPA (Fig. 5A) and the NMDA current (Fig. 5B) during regular 100-Hz trains. Given the stochastic nature of mossy fiber discharge in response to single tactile stimuli (Chadderton et al. 2004), we also tested the ability of the model to fit random spike trains. To this aim, the stimuli were generated according to a Poisson distribution with an average frequency of 50 Hz. Despite the fact that, in these trains, the interstimulus intervals varied from a few to tens of milliseconds, the model could still fit the EPSCs (Fig. 6). Average parameters obtained from fittings were not statistically different when using either regular or random trains (Table 1).

The value of \( \rho \) was close to that estimated using quantal analysis in this same synapse (Sola et al. 2004). Diffusion parameters are in agreement with the analysis reported by Saftenku (2005). The parameter \( M \) indicates the number of molecules diffusing from a distance \( r \). With \( M = 21,500 \) and \( P = 0.42 \), assuming \(-4,000 \) glutamate molecules per synaptic vesicle (SV) (Barbour 2001) and 1 SV/site (Sola et al. 2004), there would be \( M/(4,000 \times \rho) = 12.8 \) releasing sites contributing to spillover. The corresponding density over a circular surface with \( r = 1 \) mm is 3.9 sites/\( \mu m^2 \), in the range of values measured with electron-microscopy (average of 2.9 sites/\( \mu m^2 \)) (Xu-Friedman and Regehr 2003). It should also be noted that \( r \) lays between the intersite distance and the glomerular radius and that the number of releasing sites is within the limits expected for the glomerulus (Xu-Friedman and Regehr 2003; see also Saftenku 2005 for review of literature data). \( D_{eff} \) is very close to the value recently estimated in the cerebellar glomerulus (0.33 mm\(^2\)s\(^{-1}\) at 37°C; Nielsen et al. 2004). There are no previous experimental estimates for \( \tau_f \) and \( \tau_h \) in this synapses, but the values fall in the range reported for other central synapses (Gupta et al. 2000; Tsodyks and Markram 1997).

Thus the model can predict repetitive neurotransmission in the cerebellar glomerulus with physiologically meaningful parameters. Unless differently stated, the values reported in Table

**Modeling EPSC trains and their changes during LTP**

To verify whether the EPSC train changes observed during LTP could explain those in current-clamp recordings, we developed a mathematical model (see METHODS) incorporating the three main factors regulating mossy fiber–granule cell neurotransmission: 1) presynaptic release dynamics, 2) neurotransmitter spillover, and 3) microscopic kinetics of postsynaptic receptors. The model was useful to circumvent a series of experimental obstacles. Most notably, modifications in neurotransmitter release probability obtained by manipulating calcium concentration profoundly alter postsynaptic electroresponsiveness and the coexistence of nonsynaptic plasticity prevents a precise analysis of the contribution of synaptic changes.

In the model, AMPA EPSCs were composed of a fast direct and a slow indirect spillover-mediated component (Fig. 5A; DiGregorio et al. 2002). NMDA EPSCs were activated by glutamate spillover, were small and slow compared with AMPA EPSCs, and were largely blocked at resting membrane potential and unblocked in the threshold region (Fig. 5B) (Cathala et al. 2003; Rossi et al. 2002). The model fitted both changes were not statistically different from those obtained during LTP (Fig. 4C). Thus the changes induced in AMPA EPSC trains by raising release probability mimicked those observed during LTP supporting their presynaptic origin.

**FIG. 4.** \( \text{Ca}^{2+} \)-dependence of short-term plasticity in EPSC trains. A: AMPA EPSC trains elicited in low and normal \( \text{Ca}^{2+} \)/Mg\(^{2+} \) ratio, this was caused by a high initial release probability causing prominent depression. It should also be noted that increasing \( \text{Ca}^{2+}/\text{Mg}^{2+} \) ratio from normal to high caused changes that closely mimicked those observed during LTP. \( A_0 \) showed a significant 39.9 \( \pm \) 7.8% increase (n = 8; \( P < 0.01 \)), \( A_0/A_0 \) showed a reduction from 0.22 \( \pm \) 0.04 to 0.13 \( \pm \) 0.03 (n = 8; \( P < 0.01 \)), and a marked increase in the sustained current (28.2 \( \pm \) 7.5%; n = 8; \( P < 0.01 \)). These changes were not statistically different from those obtained during LTP (Fig. 4). Thus the changes induced in AMPA EPSC trains by raising release probability mimicked those observed during LTP supporting their presynaptic origin.

**FIG. 5.** A: AMPA EPSCs were composed of a fast direct component (A1) and a slow indirect spillover-mediated component (Fig. 5A1). The parameter \( M \) indicates the number of releasing sites within the limits expected for the glomerulus (0.33 mm\(^2\)s\(^{-1}\) at 37°C; Nielsen et al. 2004). There are no previous experimental estimates for \( \tau_f \) and \( \tau_h \) in this synapses, but the values fall in the range reported for other central synapses (Gupta et al. 2000; Tsodyks and Markram 1997).

Thus the model can predict repetitive neurotransmission in the cerebellar glomerulus with physiologically meaningful parameters. Unless differently stated, the values reported in Table

***ratio (4 mM \( \text{Ca}^{2+} \), 0 mM \( \text{Mg}^{2+} \)), all recordings showed depression. Thus when facilitation was not observed at normal \( \text{Ca}^{2+}/\text{Mg}^{2+} \) ratio, this was caused by a high initial release probability causing prominent depression. It should also be noted that increasing \( \text{Ca}^{2+}/\text{Mg}^{2+} \) ratio from normal to high caused changes that closely mimicked those observed during LTP. \( A_0 \) showed a significant 39.9 \( \pm \) 7.8% increase (n = 8; \( P < 0.01 \)), \( A_0/A_0 \) showed a reduction from 0.22 \( \pm \) 0.04 to 0.13 \( \pm \) 0.03 (n = 8; \( P < 0.01 \)), and a marked increase in the sustained current (28.2 \( \pm \) 7.5%; n = 8; \( P < 0.01 \)). These changes were not statistically different from those obtained during LTP (Fig. 4). Thus the changes induced in AMPA EPSC trains by raising release probability mimicked those observed during LTP supporting their presynaptic origin.****
Friedman and Regehr (2003). Raising the initial desensitization, so that EPSC depression during trains depends on sustained current and at the same time accelerates AMPA receptor desensitization. Figure 8 shows the ability of the model to reproduce granule cell intrinsic excitability (D’Angelo et al. 2001), accelerated glutamate depletion and increased glutamate spillover. These changes predicted a series of experimental effects (cf. Figs 1–4): 1) AMPA EPSC synaptic depression accelerated with a marked decrease in the \( A_d/A_0 \) ratio (facilitation emerged at \( P < 0.3 \); data not shown); 2) the sustained AMPA current increased without saturating (Silver et al. 1996b); 3) the NMDA current increased in the first few pulses but then saturated. Quantitatively, the \( p \) increase caused a 35% potentiation in the direct component, a 19% increase in the sustained current, and a 38% decrease in \( A_d/A_0 \), close to experimental observations. Thus the model accounted for the main aspects of glomerular physiology and predicted the changes in EPSC trains occurring during LTP.

Modeling synaptic excitation and its changes during LTP

The model could reproduce granule cell EPSPs and their effect on synaptic excitation. Indeed, coupling AMPA and NMDA synaptic conductances with a detailed reconstruction of granule cell intrinsic excitability (D’Angelo et al. 2001) reproduced the main aspects of granule cell synaptic excitation (Fig. 8, A and B). As reported by D’Angelo et al. (1995), 1) EPSPs generated by synchronous activation of two mossy fibers were slower than single fiber EPSPs because of NMDA current unblock during depolarization; 2) synchronous activation of three mossy fibers was needed to generate an action potential from rest (–70 mV in the model), and a doublet of action potentials was generated with four mossy fibers; and 3) repetitive stimulation generated repetitive spike discharge. Interestingly, despite their small size, NMDA and AMPA spillover currents generated a considerable depolarization, enhancing EPSP temporal summation, EPSP-spike coupling, and firing (Fig. 8B). This effect was caused by the high-input resistance of granule cells (in the gigaohm range), so that even a few pA sustained current generated a remarkable depolarizing action.

The model allowed to dissect the effect of presynaptic changes on firing from those caused by intrinsic electroresponsiveness. Figure 8C shows the ability of the model to reproduce the main effect of LTP on spike trains: by raising \( p \), the first-spike delay decreased and spike frequency was maintained.

Theoretical predictions on the LTP mechanism

The involvement of spillover in LTP expression was tested by restricting the \( p \) change to the direct component \( (p_{dir}) \), while \( p \) of the indirect component \( (p_{ind}) \) was fixed. Clearly, this simulation makes the case of potentiation occurring only in the...
releasing sites facing the granule cell dendrite under study. With \( p_{\text{ind}} = 0.4 \), changing \( p_{\text{dir}} \) did not cause any noticeable modulation in spike delay and frequency (Fig. 9, A and C). Thus probably, the whole glomerulus was potentiated in our experiments.

The classical alternative to a \( p \) raise is an increase in maximum postsynaptic conductance, \( g_{\text{max}} \). By raising \( p \) (Fig. 9, B and C), repetitive activation of one synapse yielded large spike delays and relatively low firing frequencies, as observed in recordings performed with minimal stimulation. Conversely, a proportional change in \( g_{\text{max}} \) (for both AMPA and NMDA receptors) with \( p \) fixed at 0.4 caused poor modulation of spike delay and steep modulation of firing frequency. The difference highlights the effectiveness of presynaptic regulation of neurotransmission dynamics, which allows slow progressive EPSP temporal summation at low \( p \) causing long delays. Thus changing \( p \) or \( g_{\text{max}} \) is not equivalent in terms of synaptic dynamics, although the effect on an isolated EPSP would be indistinguishable.

**Theoretical predictions on the role of nonsynaptic changes**

Simulations shown in Figs. 8C and 9C suggest that earlier burst initiation during LTP is largely caused by enhanced neurotransmitter release and therefore by a presynaptic mechanism. Conversely, the increased firing frequency could be mostly explained by changes in intrinsic excitability and be postsynaptic in nature.

In a series of simulations, we modified intrinsic excitability by changing ionic current density or gating (Fig. 10A). Although we tested just some of the possible mechanisms of regulation (see for review Debanne et al. 2003), these examples have been grounded on the hypothesis proposed by Armano et al. (2000) and help understanding how the changes observed during LTP might originate (see Fig. 10 for details). We have therefore modified the density of the M-current (\( I_{K_M} \)), the density of the persistent Na\(^+\) current (\( I_{Na_p} \)), and the threshold of the transient Na\(^+\) current (\( I_{Na_t} \)). The firing frequency increase was obtained, in order of efficacy, by \( (I_{K_M} \times 0.12) > (I_{Na_p} \times 1.5) \approx (I_{Na_t} \text{ activation - 3 mV}) \). The first-spoke delay reduction was obtained, in order of efficacy, by \( (I_{Na_p} \times 1.5) \approx (I_{Na_t} \text{ activation - 3 mV}) > (I_{K_M} \times 0.12) \). Combinations of these changes proved particularly effective in reproducing the experimental results (Fig. 10B). Among other possible changes, reducing the Ca\(^{2+}\)-dependent current (\( I_{K_{Ca}} \)) was ruled out because it caused repetitive bursting (D’Angelo et
al. 2001), a firing pattern not observed during LTP (data not shown).

When the changes in $I_{K_{M}}$, $I_{Na_{M}}$, and $I_{Na_{P}}$ were associated with a $p$ increase from 0.4 to 0.6, we observed a good matching with experimental measurements obtained during repetitive synaptic transmission (cf. Fig. 1C). Simulations showed that the contribution of pre- and postsynaptic changes was not simply additive. The presynaptic change dominated delay regulation and was modestly improved by changes in intrinsic excitability. Conversely, postsynaptic changes dominated frequency regulation, which was poorly improved by changes in release. These simulations suggest therefore that delay and frequency are to a considerable extent subjected to differential regulation by pre- and postsynaptic mechanisms of potentiation.

**DISCUSSION**

The main result in this paper is that increased release probability during LTP, by regulating short-term facilitation and depression, caused earlier activation of cerebellar granule cell spike bursts elicited by repetitive mossy fiber activity. The presynaptic mechanism coexisted with postsynaptic regulation of ionic channels, which played a major role in determining the granule cell output firing frequency.

**FIG. 8.** Simulation of synaptic excitation and LTP. A: model reproduced activation of EPSPs and EPSP-spike complexes by 1, 2, 3 (dotted trace), or 4 (dashed trace) mossy fibers as in D'Angelo et al. (1995). Note that the single fiber EPSP measured 14 pA, peaked in 8 ms, and lasted 27 ms at half-peak amplitude, very close to experimental estimates. B: response to spike trains at 100 Hz with 2 simultaneously active mossy fibers. Firing was depressed by switching off glutamate spillover currents. Note strong depression in AMPA EPSCs. Bottom traces show corresponding AMPA (gray) and NMDA (black) currents. C: regulation of EPSP temporal summation by a change in $p$ from 0.4 to 0.6 simulating LTP. Note the shorter 1st spike delay at $p = 0.6$.

**FIG. 9.** Simulation of alternative potentiation models. A: when spillover is left unchanged ($P_{na} = 0.4$) and only direct release is changed by 0.25 or 1.5 times ($P_{dir} = 0.2, 0.4, 0.6$), no remarkable modulation in granule cell discharge is observed. B: effects of changing release probability or maximum conductance are compared. Starting from $P = 0.4$, $P$ was either increased by 1.5 times or decreased by 0.25 times. The results are compared with corresponding changes in maximum conductance, $g$. C: dependence of 1st spike delay and average discharge frequency on release probability and postsynaptic conductance. Curves correspond to activation of 1 mossy fiber. Note that, unlike $P$, $g$ has a limited control on delay (beginning at the arrow) but a strong influence on firing frequency. A change in $P_{na}$ causes poor modulation in either delay or frequency.
Repetitive neurotransmission at the mossy fiber–granule cell relay of cerebellum

Repetitive synaptic activity at the cerebellar mossy fiber–granule cell relay determined complex temporal dynamics in EPSC trains. In response to a 100-Hz burst, the most prominent behavior was short-term depression, leading AMPA EPSCs toward a reduced steady state in about five pulses. A transient short-term facilitation affecting the first two to three EPSCs emerged at low release probability. In addition, AMPA and NMDA receptor activation generated a sustained current, which showed marked temporal summation during trains. The sustained current was probably the extension of the spillover currents recorded in single EPSCs (Cathala et al. 2003; DiGregorio et al. 2002; Xu-Friedman and Regehr 2003). These processes provided the key to interpret EPSP trains and spike firing (this paper, see also D’Angelo et al. 1995) and their changes during LTP.

Two aspects indicated that, as reported by Sola et al. (2004), the expression of LTP depended on increased release probability. First, either raising extracellular [Ca$^{2+}$] or eliciting LTP enhanced AMPA EPSC depression, leading to a common steady state ($\Delta s$) (cf. Tsodyks and Markram 1997). Second, raising extracellular [Ca$^{2+}$] or eliciting LTP enhanced the sustained AMPA and NMDA receptor–mediated currents. It should be noted that sustained AMPA and NMDA currents did not show an identical behavior. The sustained AMPA current was potentiated over the entire train, suggesting that AMPA receptors are not saturated, as observed at this (DiGregorio et al. 2002; Silver et al. 1996b) and other central synapses (McAllister and Stevens 2000). The sustained NMDA current showed LTP in the first (e.g., D’Angelo et al. 1999) but not in later responses. Thus NMDA receptors are probably not saturated by a single pulse (Mainen et al. 1999; McAllister and Stevens 2000) but tend to saturate during repetitive stimulation.

Modeling repetitive neurotransmission

Mathematical modeling helped visualizing the interplay of the three main processes supposed to govern neurotransmission dynamics, namely 1) turnover of presynaptic resources (vesicle cycling), 2) glutamate diffusion, and 3) postsynaptic receptor gating.

During trains, presynaptic resource depletion and postsynaptic receptor desensitization caused AMPA EPSC depression, as predicted by Xu-Friedman and Regehr (2003). AMPA, unlike NMDA receptors, did not tend to saturate during trains. Spillover in the model generated sustained AMPA (DiGregorio et al. 2002) and NMDA currents (Cathala et al. 2003). There was no need in the present simulations to account for a second NMDA receptor population, which proved to have a distinct sensitivity to spillover (Rossi et al. 2002). With input bursts longer than those used in the present simulations (>150 ms), the NMDA current may be reduced by slowly developing processes like glutamate reuptake (Overstreet et al. 1999).

By raising $p$, four main experimental observations were predicted (this paper and Sola et al. 2004; see also DiGregorio et al. 2002): simulations of the changes in 1st-spike delay ($\Delta s$) turnover of presynaptic resources (vesicle cycling), 2) glutamate diffusion, and 3) postsynaptic receptor gating.

During synaptic stimulation, the 1st-spike delay and firing frequency increase observed during LTP were reproduced within the experimental error ($\pm SE$ bars are shown only for delay changes during synaptic simulation). Dashed arrows facilitate the comparison of simulated with experimental results. It should be noted that release probability is very effective in regulating delay but poorly effective in regulating firing frequency.

FIG. 10. Simulation of changes in intrinsic excitability. A: action potential firing was simulated by either 100-Hz stimulation of a single synapse or by depolarizing current injection from −71.5 mV (mean holding potential of experiments reported in Fig. 1C). LTP was simulated by raising $p$ and by changing postsynaptic currents ($I_{NMDA}$, $I_{AMPA}$, $I_{Na_p}$), as explained in B. B: simulations of the changes in 1st-spike delay and firing frequency associated with LTP are compared with those observed experimentally. Experimental changes have been obtained from data reported in Fig. 1C. Synaptic transmission was potentiated by raising $p$ from 0.4 to 0.6, and intrinsic excitability was enhanced by reducing $I_{Na}$ (×0.12), raising $I_{Na_p}$ (×1.5), and lowering $I_{Na}$ activation (−3 mV). Changes in postsynaptic currents were also combined, as indicated. The combination ($\Delta s$, $I_{Na}$, $I_{Na_p}$) proved very effective in reproducing changes in intrinsic excitability and was therefore adopted to simulate responses to 100-Hz synaptic stimulation. During synaptic stimulation, the 1st-spike delay and firing frequency increase observed during LTP were reproduced within the experimental error ($\pm SE$ bars are shown only for delay changes during synaptic simulation). Dashed arrows facilitate the comparison of simulated with experimental results. It should be noted that release probability is very effective in regulating delay but poorly effective in regulating firing frequency.
et al. 2002; Xu-Friedman and Regehr 2003): 1) the first EPSC increased, 2) AMPA EPSC depression accelerated, 3) a common steady state was attained, and 4) glutamate spillover increased enhancing the sustained AMPA and NMDA currents. It should be noted that spillover promoted both AMPA receptor activation and desensitization (Fig. 7), preventing de facto a net change with release probability of AMPA EPSCs at steady state. Thus the model behaved as expected from a pure presynaptic mechanism, in which the steady-state EPSC amplitude does not change with release probability (Brenowitz and Trussell 2001; O’Donovan and Rinzel 1997; Tsodyks and Markram 1997).

**Pre- and postsynaptic mechanisms determining mossy fiber–granule cell LTP**

Mossy fiber–granule cell LTP is a composite process involving a presynaptic increase of release probability (Sola et al. 2004) and a postsynaptic increase in intrinsic excitability (Armano et al. 2000). The observation that, during LTP, shortening of first-spike delay was significantly more pronounced with repetitive synaptic activation than with depolarizing current injection, indicates that the pre- and postsynaptic components preferentially control different aspects of granule cell excitation. The analysis of EPSC trains and mathematical modeling revealed the underlying mechanism: EPSP temporal summation is critical for reaching spike threshold but, once firing begins, it is efficiently regulated by postsynaptic ionic conductances. Because of the complexity of the system, it seems worth summarizing current understanding about the main mechanisms contributing to potentiate the response of granule cells during LTP.

Increasing release probability determines an initial EPSC potentiation followed by a protracted spillover current. The AMPA and NMDA currents play distinct roles in this mechanism. The increase of the AMPA current, through its large transient component, would be particularly important to promote coincidence detection of activity in more than one mossy fiber (Cathala et al. 2003). A substantial contribution to EPSP temporal summation, which was revealed by activating single mossy fibers from rest, was determined by spillover-mediated activation of sustained AMPA and NMDA currents. Increased spillover during LTP contributed not just to maintain repetitive spike discharge counteracting synaptic depression, but also to accelerate membrane depolarization and spike activation.

Increasing granule cell intrinsic excitability regulates both granule cell firing initiation and frequency (D’Angelo et al. 2001). During LTP, control on firing frequency is greater than control on delay. This effect could be reproduced by reducing $I_{\text{Na}}$ threshold, raising $I_{\text{Na}}$ and reducing $I_{\text{K-M}}$ density, consistent with the hypothesis proposed by Armano et al. (2000), predicting a raise in $I_{\text{Na}}$ and a decrease in $I_{\text{K-M}}$ currents activating in the just subthreshold region.

**Effect of release probability on glomerular information processing**

A relevant prediction of the model is that changing $p$ favors modulation of burst delay (at least at $P < 0.3$), whereas changing postsynaptic conductance favors modulation of firing frequency. Moreover, the model predicts that potentiation should occur at multiple sites in the mossy fiber terminal to raise spillover and obtain a reliable delay and frequency modulation. This effect is not surprising, because TBS also activates other granule cells impinging on the glomerulus (~50 per mossy terminal in the rat; Jakab and Hámosi 1988). It is therefore possible that LTP in these recordings reflects a collective potentiation, as discussed in Maffei et al. (2003). These results suggest that synapses differing for pre- or postsynaptic LTP expression or for the intensity of spillover (Bliss et al. 2003; Lisman 2003) could differ in their ability to process spike sequences in the time and frequency domain (Rieke et al. 1996; Tsodyks and Markram 1997). For instance, at hippocampal synapses, evidence for postsynaptic expression is compelling (for review, see Malenka and Nicoll 1999) and spillover enhances the NMDA but not the AMPA response (Kullmann et al. 1996), while at central auditory synapses, increasing release enhances EPSC depression without apparent involvement of spillover currents (Brenowitz and Trussell 2001). At hippocampal synapses, LTP preserves the fidelity of postsynaptic responses to presynaptic bursts (Selig et al. 1999), suggesting that there are differences in the way neuronal circuits use modifications in synaptic strength to encode new information.

These results extend the implications for burst processing at central synapses. Bursts have been proposed 1) to provide a safety mechanism against synaptic unreliability, 2) to generate short-term temporal dynamics, and 3) to recode the temporal structure of input spike trains (Krahe and Gabbiani 2004; Lisman 1997). Here we show that burst processing provides an adaptable control over output spike initiation depending on release probability. Although we have limited our analysis to $p$ regulation by LTP, bidirectional plasticity may be a natural extension of these observations toward LTD (Gall et al. 2005).

Regulation of granule cell burst initiation could implement adaptable delay lines affecting downstream activation of the cerebellar circuitry. Precise timing of granule cell spikes is critical to determine parallel fiber–Purkinje cell coincidence detection and hence LTD and LTP (Coemans et al. 2004; Han et al. 2000). Maintenance of a sustained bursting is also critical for inducing parallel fiber–Purkinje cell LTD and LTP (Casado et al. 2002; Wang et al. 2000). Moreover, bursts, by causing facilitation and temporal summation, determine reliable activation of synapses between parallel fibers and their targets, including Purkinje cells (Dittmann et al. 2000), stellate cells (Carter and Regehr 2000), and Golgi cells (Bureau et al. 2000; Watanabe and Nakanishi 2003).

**Conclusions and functional implications**

Regulation of response initiation by changes in release probability is suitable to perform the extensive spatio-temporal recoding of mossy fiber inputs predicted by certain cerebellar theories (Braitenberg 1967; DeSchutter and Bjaalie 2001; Medina and Mauk 2000). For example, setting the appropriate delay in granule cell responses could be critical for eye-blink reflex conditioning. Interestingly, timing of conditioned stimuli carried by mossy fibers is learned with millisecond precision in the time range (<150 ms) characteristic of LTP-dependent delay regulation (Hansel et al. 2001; Koekkoek et al. 2002). Other mechanisms, like regulation of granule cell intrinsic...
excitability or yet undiscovered changes in postsynaptic conductance, could be important to control the gain of the mossy fiber input along specific input lines (Albus 1971; Mitchell and Silver 2003), modulating the frequency of Purkinje cell discharge as in the VOR (van Alphen and De Zeeuw 2002). Implementation of large-scale cerebellar network simulations may be useful to further clarify the computational implications of short- and long-term synaptic plasticity at the mossy fiber–granule cell relay of cerebellum.

GRANTS

This work was supported by projects of the European Community (CEREBELLUM QLG3-CT-2001-02256 and SPIKEFORCE IST-2001-35271), Ministero dell’Istruzione, dell’Università e della Ricerca, and Instituto Nazionale Fisica della Materia of Italy to E. D’Angelo.

REFERENCES


