Long-Term Potentiation of Intrinsic Excitability at the Mossy Fiber–Granule Cell Synapse of Rat Cerebellum

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Synaptic activity can induce persistent modifications in the way a neuron reacts to subsequent inputs by changing either synaptic efficacy or intrinsic excitability. After high-frequency synaptic stimulation, long-term potentiation (LTP) of synaptic efficacy is commonly observed at hippocampal synapses (Bliss and Collingridge, 1993), and potentiation of intrinsic excitability has recently been reported in cerebellar deep nuclear neurons (Aizenmann and Linden, 2000). However, the potential coexistence of these two aspects of plasticity remained unclear. In this paper we have investigated the effect of high-frequency stimulation on synaptic transmission and intrinsic excitability at the mossy fiber–granule cell relay of the cerebellum. High-frequency stimulation, in addition to increasing synaptic conductance (D’Angelo et al., 1999), increased granule cell input resistance and decreased spike threshold. These changes depended on postsynaptic depolarization and NMDA receptor activation and were prevented by inhibitory synaptic activity. Potentiation of intrinsic excitability was induced by relatively weaker inputs than potentiation of synaptic efficacy, whereas with stronger inputs the two aspect of potentiation combined to enhance EPSPs and spike generation. Potentiation of intrinsic excitability may extend the computational capability of the cerebellar mossy fiber–granule cell relay.

Key words: synaptic plasticity; LTP; NMDA receptors; cerebellum; granule cells; intrinsic excitability; E-S potentiation

In addition to causing transient modifications in neuronal potential, synaptic activity can induce changes in the way a neuron responds to subsequent inputs. This property, which is called synaptic plasticity, can entail persistent changes in both synaptic efficacy and intrinsic neuronal excitability. Although these two aspects of plasticity may concur to improve neuron and network computation (Fregnac, 1998), their potential coexistence remained unclear.

A well-known model for synaptic plasticity is long-term potentiation (LTP), which is typically induced by high-frequency activation of NMDA receptors at glutamatergic synapses (Bliss and Collingridge, 1993; Bear and Malenka, 1994). Synaptic efficacy is enhanced during LTP. However, in hippocampal pyramidal cells the probability of action potential activation increases more than expected from potentiation of synaptic efficacy (E-S potentiation) (Bliss and Lømo, 1973; Andersen et al., 1980), suggesting that additional factors are involved. E-S potentiation was usually shown to depend on depression of synaptic inhibition (Abraham et al., 1987; Chavez-Noriega et al., 1990; Breakwell et al., 1996), although in some cases an intrinsic excitability change was suggested (Pugliese et al., 1994; Daoudal et al., 1999).

Activity-dependent changes in intrinsic excitability are common in the developing brain (Spitzer, 1991); their mechanisms have been investigated in cell culture (Turrigiano et al., 1994; Desai et al., 1999), and their computational implications have been predicted by using theoretical models (Stemmler and Koch, 1999).

Recently, an NMDA receptor-dependent potentiation in intrinsic excitability has been observed in cerebellar deep nuclear neurons after high-frequency tetanic stimulation similar to that used to induce LTP, although in the absence of any synaptic efficacy changes (Aizenman and Linden, 2000).

We have investigated whether changes in intrinsic excitability could be induced by high-frequency stimulation of the mossy fiber–granule cell synapse of the cerebellum, at which NMDA receptor-dependent LTP has recently been demonstrated (D’Angelo et al., 1999). After pharmacological blockage of inhibitory synapses, high-frequency stimulation induced an NMDA receptor-dependent potentiation of intrinsic excitability. This depended on a rise in input resistance and a decrease in spike threshold, which enhanced EPSPs and spike firing. Together with potentiation of synaptic conductance, potentiation of intrinsic excitability may play an important role in regulating granule cell synaptic excitation and cerebellar network computation.

MATERIALS AND METHODS

Acute 250-μm-thick cerebellar slices were obtained from 19- to 22-old Wistar rats as reported previously (D’Angelo et al., 1999). The rats were anesthetized with halothane (Aldrich, Milwaukee, WI) before being killed by decapitation. Slices were cut in the sagittal plane from the cerebellar vermis in cold Krebs’ solution and maintained at room temperature before being transferred to a 1.5 ml recording chamber mounted on the stage of an upright microscope (Zeiss Standard-16). The preparations were superfused with Krebs’ solution and maintained at 30°C with a feedback Peltier device (HCC-100A; Dagan Corporation, Minneapolis MN).

The Krebs’ solution contained (in mM): NaCl 120, KCl 2, MgSO4 1.2, NaHCO3 26, KH2PO4 1.2, CaCl2, 2, glucose 11, and was equilibrated with 95% O2 and 5% CO2, pH 7.4. The control and test solutions were applied locally through a multi-barrel pipette. Perfusion of the control solution was commenced before seal formation and was maintained until switching to the test solutions. Unless stated otherwise, the perfused solutions contained the GABA-A receptor blocker 10 μM bicuculline. Nystatin and bicuculline were obtained from Sigma (St. Louis, MO), and the glutamate receptor antagonists 1-2-amino-5-phosphonovaleric acid (APV), 7-chlorokynurenic acid (7-Cl-kyn), and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were obtained from Tocris Cookson (Bristol, UK).

Whole-cell patch-clamp recordings were performed in granule cells using the perforated-patch technique, which prevents cytoplasmic washout (Horn and Marty, 1988; Edwards et al., 1989). The pipette solution contained (in mM): K2SO4 80, NaCl 10, glucose 15, HEPES 5 (pH adjusted to 7.2 with KOH), and nystatin 100 μg/ml. Membrane potential was measured relative to an Ag-AgCl reference electrode (Clark Instruments, Pangbourne, UK) and was not corrected for the Donnan potential developing across the patch (~5 mV inside the cell). Electrical activity was recorded with an Axopatch 200-B amplifier, sampled with a Digidata 1200B interface (500 psec/poin), and analyzed off-line with P-Clamp software (Axon Instruments). The mossy fibers were stimulated with a bipolar tungsten electrode via a stimulation isolation unit.

In a typical experiment, mossy fibers were activated at a frequency of 0.1 Hz, and step current pulses were applied every 5 min from the membrane potential of ~80 mV. High-frequency pulses were delivered 10 min after...
Membrane potential during step current injection was estimated as the average value between 500 and 800 msec. Membrane potential during TBS was estimated as the mean of average values measured in the central 70 msec of each burst (tracings were filtered at 100 Hz). The action potentials consisted of two components, the prepotential and the upstroke (D’Angelo et al., 1998). The threshold of spike prepotential (\( T_{h} \)) was measured at the flexus in the interspike trajectory, whereas that of the upstroke (\( T_{up} \)) was measured at the sharp transition from prepotential to upstroke (see Fig. 5, inset). \( T_{h} \), coincided with the minimum depolarization necessary to activate an action potential, whereas \( T_{up} \) approached the depolarization reached by those prepotentials that did not initiate the ballistic phase of the action potential (examples are shown in Figs. 3B, 4A,B, and 7A). In some cases, threshold identification was aided by taking the first time derivative of the signal (data not shown). Data are reported as mean ± SD, and statistical comparisons were performed using Student’s t test.

Just after obtaining the cell-attached configuration, electrode capacitance was carefully cancelled to allow for electronic compensation of pipette charging during subsequent current-clamp recordings (D’Angelo et al., 1995). The cerebellar granule cell is electrotonically compact and can be treated as a simple RC system, in which relevant parameters can be extracted by analyzing passive current relaxation induced by voltage changes (D’Angelo et al., 1995, 1999; Silver et al., 1996). Monoexponential fitting to current transients elicited by 10 mV hyperpolarizing voltage steps from the holding potential of −80 mV yielded the voltage-clamp time constant, \( \tau_{VC} \). The input capacitance (\( C_{in} \)) was measured from the capacitive charge (the area underlying current transients), and series resistance (\( R_s \)) was calculated as \( R_s = \tau_{VC}/C_{in} \). Input resistance \( R_{in} \) was computed from the steady current flowing after termination of the transient. When the patch perfusion had stabilized, typical granule cell values were obtained [\( C_{in} = 3.1 ± 0.7 \, \text{pF} \), \( R_{in} = 2.3 ± 0.5 \, \Omega \), and \( R_s = 53.4 ± 17.2 \, \Omega \, \text{n} = 18 \) for all measurements].

Current-clamp recordings were performed in the “fast” operating mode to optimize the reaction rate of the amplifier (D’Angelo et al., 1998; Magistretti et al., 1998). The effect of \( R_c \) in current-clamp recordings is inversely proportional to \( R_{in} \). Because \( R_c \) was approximately two orders of magnitude smaller than \( R_{in} \), the effect of \( R_c \) on voltage recordings was negligible (−1%). For this reason, (1) perforated-patch recordings were at no disadvantage to ruptured-patch recordings despite \( R_c \) being nearly 50% higher; (2) bridge balancing was unnecessary, and (3) recording stability was ensured despite changes in \( R_c \) that might occur during prolonged recordings. Recording stability was attested by \( R_{in} \) measurements, as shown in Figure 4.

Although perforated-patch recordings prevent cytoplasmic constituents from being washed out, EPSP size and spike threshold showed a slow time-dependent decrease (see Figs. 2C, 3C, 4C). This may reflect long-term modifications induced by spike discharge (Pockett et al., 1990; Christofi et al., 1993; Aizenmann and Linden, 2000) generated by step current pulses used to monitor granule cell intrinsic excitability.

RESULTS

The effect of high-frequency mossy fiber stimulation on synaptic efficacy and intrinsic granule cell excitability was investigated in rat cerebellar slices at P19–P22, when granule cells show mature synaptic and excitable properties (D’Angelo et al., 1998), using whole-cell perforated-patch recordings (Horn and Marty, 1988).

Preliminary observations

Granule cells are excited by glutamatergic mossy fiber synapses and inhibited by GABAergic Golgi cell synapses (Fig. 1A). EPSPs recorded with 10 μM bicuculline in the bath to block GABA-A receptors (Fig. 1B) measured between 8 and 22 mV in different experiments (compare Fig. 2). Considering that cerebellar granule cells receive four mossy fiber inputs on average (Eccles et al., 1967), each causing an 8–12 mV depolarization (D’Angelo et al., 1995; Silver et al., 1996), one to three mossy fiber synapses should have been activated. Golgi cells can be activated directly by mossy fibers in the glomeruli, as well as by granule cell axons, the parallel fibers (Eccles et al., 1967). Mossy fiber stimulation in bicuculline-free solution (i.e., with unblocked GABA-A receptors) caused strong granule cell inhibition, curtailing EPSPs and preventing spike generation (cf. tracings obtained before and after bicuculline application in Fig. 1B). Because parallel fibers are severed in sagittal slices, Golgi cells should be preferentially activated through their mossy fiber input rather than through the granule cell–parallel fiber recurrent loop. Consistently, inhibition arose quickly (3–5 msec), whereas longer delays would be expected for feedback inhibition (Vos et al., 1999). IPSPs were evident at potentials positive to −64.2 ± 3.3 mV (n = 5) (Fig. 1C), above the chloride equilibrium potential (−66 mV) (see Materials and Methods) and the granule cell resting potential (−63.6 ± 8.9 mV; n = 10). Because GABAergic responses have a depolarizing action below the chloride equilibrium potential, the marked EPSP reduction observed in this membrane potential range indicates that GABA receptors act through a shunting mechanism to decrease granule cell input resistance. GABA-A receptors may also operate through a tonic inhibition mechanism (Brickley et al., 1996), because granule cell input resistance was 28% lower before bicuculline perfusion than after it (1.8 ± 0.7 \( \Omega \) vs 2.5 ± 1.7 \( \Omega \); n = 7).

Mossy fibers convey complex patterns of high-frequency spike bursts during movement (Kase et al., 1980) and can entrain the granular layer to discharge at theta frequency (Pellerin and Lamarre, 1997; Hartmann and Bower, 1998). In preliminary experiments we observed that LTP was similar when it was induced by high-frequency stimulation organized either in repeated bursts (TBS) or in a single continuous burst (CS) (Table 1). TBS was thus preferred because it allowed a closer comparison with previous voltage-clamp experiments (D’Angelo et al., 1999).
In an initial set of experiments, LTP was investigated during GABA-A receptor blockage by 10 μM bicuculline. LTP recordings were grouped depending on membrane depolarization efficiency during TBS, which in turn depended on the initial EPSP size (Fig. 2, Table 2).

In the first group, TBS had a weak depolarizing action \((n = 5)\) (Fig. 2A1,B1). A few spikes could be elicited in the first TBS burst in three of five cells, and depolarization tended to decrease in subsequent bursts (Fig. 2). After TBS, EPSP amplitude increased over control values by 23 ± 14% \((n = 5; p < 0.01)\) in 15 min (Fig. 2A1,C).

In a second group, TBS caused a strong granule cell excitation \((n = 5)\) (Fig. 2A2,B2). Robust action potential discharge was generated in all TBS bursts (Fig. 2). After TBS, EPSPs increased by 84 ± 16% \((p < 0.01; n = 5)\) over control values in −7 min, and most of them then elicited action potentials (Fig. 2A2,C). In a different set of cells showing strong TBS \((n = 8)\), action potentials were occasionally generated by control EPSPs (19.7%) and became more frequent after LTP, precluding EPSP changes from being measured. In these recordings, after TBS, the probability of action potential generation increased by 120 ± 85% over control values in 15 min \((p < 0.01; n = 8)\) (Fig. 2D), and in many cases spikes occurred in doublets or triplets (data not shown).

Thus, although EPSP potentiation was observed in all cases, it was of different magnitude depending on the initial EPSP size and...
Table 2. Effect of TBS strength on EPSP potentiation

<table>
<thead>
<tr>
<th>TBS</th>
<th>n</th>
<th>EPSP change (mV)</th>
<th>m.b.d. (mV)</th>
<th>s.f. (Hz)</th>
<th>LTP (% EPSP change)</th>
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<tr>
<td>Weak TBS</td>
<td>5</td>
<td>9.2 ± 1</td>
<td>-56 ± 6.5</td>
<td>13 ± 15</td>
<td>23 ± 14</td>
</tr>
<tr>
<td>Strong TBS</td>
<td>5</td>
<td>14.6 ± 4.8</td>
<td>-38 ± 4.9</td>
<td>71 ± 14</td>
<td>84 ± 16</td>
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Recordings were parsed according to the efficiency of granule cell synaptic excitation during TBS, which was expressed as mean burst depolarization (m.b.d.) and spike frequency (s.f.). “Weak TBS” corresponds to data in Figure 2, A2, and “strong TBS” corresponds to data in Figure 2, A1 and B2, and average LTP time courses are summarized in Fig. 2C. EPSP potentiation was higher after strong than weak TBS. It should be noted that the initial EPSP size was comparatively smaller when weak rather than strong TBS was observed. Data in the two groups were significantly different (p < 0.01).

Figure 3. Enhanced action potential generation during LTP. Granule cell responses to current injection (bottom tracings, 2 pA/step) from −80 mV are compared in (A) control recordings and in (B) recordings in which LTP was induced (this cell was one of those included in Fig. 2A). Tracings were taken 8 and 25 min after the beginning of recordings. C, Time course of the current needed to fire action potentials (current threshold) in control recordings (○; n = 5) and in recordings in which LTP was induced (●; n = 10). Note the marked decrease in current threshold during LTP.

Figure 4. Increased input resistance during LTP. A, B, Voltage–current plots have been constructed by measuring steady-state depolarization in the tracings of Figure 3, A and B, respectively (○ 8 min and ● 25 min after beginning of recordings). C, Time course of $R_{in}$ in control recordings (dotted line; n = 5; SD was between 0.5 and 0.7 GΩ) and in recordings in which LTP was induced (solid line; n = 7). Note that after LTP induction, $R_{in}$ increased at potentials higher (54) but not lower (55) than −80 mV. In control recordings $R_{in}$ remained stable in both potential ranges.

The excitatory action of TBS (see also below). It should be noted that control EPSPs tended to decrease slightly with time (−17.2 ± 11.4% after 20 min recordings; n = 5) (Fig. 2C), probably reflecting simultaneous synaptic depression (Pockett et al., 1990; Christofi et al., 1993).

Potentiation of intrinsic membrane excitability

Cerebellar granule cells injected with step depolarizing currents showed inward rectification in the subthreshold membrane potential region and, once the threshold was reached, generated a repetitive spike discharge (D’Angelo et al., 1995, 1998) (Fig. 3A). After TBS, action potential generation was enhanced, and enhanced depolarization associated with membrane potential oscillations could often be observed in the threshold region (Figs. 3B, 5B, 7A). The current needed to generate spikes (current threshold) decreased (Fig. 3A, B), becoming significantly smaller than in control recordings (−70 ± 16% 15 min after TBS; n = 10; p < 0.03) (Fig. 3C). Because GABA-A receptors were blocked, the reduction in current threshold reflected a potentiation of intrinsic granule cell excitability. The mechanism of excitability potentiation was further investigated by measuring changes of apparent granule cell input resistance ($R_{in}$) and of action potential threshold in the experiments of Figure 2C (those in Fig. 2D had a similar behavior; data not shown).

$R_{in}$ was measured from membrane potential changes caused by current steps in the 10 mV potential range either below or above −80 mV (Fig. 4A, B). After TBS, $R_{in}$ rapidly increased above −80 mV, whereas $R_{in}$ remained unchanged below −80 mV (Fig. 4C). The $R_{in}$ increase reached 37 ± 33% 15 min after TBS (n = 10; p < 0.03).

Action potential threshold was measured both at the beginning of spike prepotential ($T_{h1}$) and at the beginning of upstroke ($T_{h2}$) (Fig. 5, inset) (D’Angelo et al., 1998). After TBS, both thresholds decreased, becoming significantly lower than in control recordings (n = 10; p < 0.05) (Fig. 5A–C). Because $T_{h1}$ decreased more than $T_{h2}$ (Fig. 5C), the spike prepotential was enhanced (4.1 ± 3.2 mV 15 min after TBS; n = 10; p < 0.03) (Fig. 5D).

These results indicated that an increase in $R_{in}$ and a decrease in spike threshold combined to reduce the current needed to fire...
Figure 5. Decreased spike threshold during LTP. The inset shows where the thresholds of spike prepotential (Th1) and spike upstroke (Th2) were measured. A, B, Spikes in control recordings (A) and in recordings in which LTP was induced (B). Tracings were taken 8 and 25 min after beginning recordings from the same cells shown in Figure 3. C, Time course of threshold changes in control recordings (n = 5; dotted line; the SD was between 4 and 6 mV) and in recordings in which LTP was induced (n = 10). D, Greater decrease in Th1 than Th2 caused an enlargement of spike prepotential during LTP (●; n = 10; control recordings 35; n = 5).

Figure 6. Relationship between EPSP and intrinsic excitability potentiation. A, Plot of EPSP versus Rm changes at potentials higher than −80 mV. The diagonal is the place where EPSP equals Rm changes. ○ corresponds to weak TBS (same experiments as in Fig. 2A,B), and ● corresponds to strong TBS (same experiments as in Fig. 2A,B). B, Plot of EPSP versus Th1 (●, strong TBS, n = 5; □, weak TBS, n = 5) and Th2 (●, strong TBS, n = 5; ○, weak TBS, n = 5) changes. Th1 and Th2, which were measured in the same cells included in Figure 2C and Table 2, were corrected for time-dependent changes in control recordings. Note that changes in Rm, Th1, and Th2 were already appreciable, with relatively small EPSP changes. All data in this figure were recorded 15 min after TBS.

The role of membrane depolarization
Membrane depolarization is a fundamental factor in the induction of synaptic plasticity (Kelso et al., 1986). Indeed, the results re-
ported in Figures 2 and 7 suggest that membrane depolarization during TBS plays an important role in the subsequent potentiation of EPSPs and intrinsic excitability. The effect of membrane depolarization was further investigated by delivering TBS from a hyperpolarized membrane potential (−90 mV) (Fig. 8A). During TBS (Fig. 8B), burst membrane potential settled at −71.1 ± 3.2 mV (n = 6). After TBS, EPSPs tended to decrease (Fig. 8C,D), although the change was not statistically significant (p > 0.3).

When the effect of membrane depolarization in different experimental conditions was considered (Fig. 9A), a direct relationship between mean TBS burst depolarization and the magnitude of EPSP potentiation was observed. Potentiation of intrinsic excitability (Fig. 9B) showed a lower threshold than potentiation of EPSP or synaptic conductance, as also suggested by the plot in Figure 6A. As well as membrane depolarization, the spikes may themselves enhance the induction of synaptic plasticity (Thomas et al., 1998; Linden, 1999). Optimal EPSP potentiation was associated with high spike frequency (Fig. 9A, B), whereas intrinsic excitability was already potentiated to the maximum at low spike frequency. The implications of membrane depolarization and spike discharge for mossy fiber–granule cell plasticity are considered in Discussion.

The role of NMDA receptors

Voltage-dependent NMDA receptor activation is the principal factor responsible for LTP induction (Bliss and Collingridge, 1993). It was therefore interesting to investigate whether potentiation of both EPSP and intrinsic granule cells excitability depended on NMDA receptor activation. Application of the NMDA receptor blockers, 100 μM APV and 50 μM 7-Chkyn (Fig. 10), depressed EPSP temporal summation (Fig. 10B) (D’Angelo et al., 1995). During TBS applied from −70 mV, spike threshold was reached in three of six cells. In the remaining three cells, mean burst depolarization was −57.5 ± 6.5 mV (n = 3). None of these cells showed any potentiation of EPSPs or intrinsic excitability (compare Fig. 9).
Figure 9. LTP dependence on voltage and spike frequency. A1, Magnitude of EPSP amplitude changes as a function of depolarization \( A_1 \) or spike frequency \( A_2 \) during TBS. A, Strong TBS in 10 \( \mu \)M bicuculline (as in Fig. 2A); ⋄, weak TBS in 10 \( \mu \)M bicuculline (as in Fig. 2A); \( \square \), TBS in bicuculline-free solution (as in Fig. 7); \( \triangle \), TBS from \(-90\) mV in 10 \( \mu \)M bicuculline (as in Fig. 8). In A, synaptic current changes in voltage-clamp recordings with pairing at \(-60\) or \(-40\) mV are shown for comparison (●) (data from D’Angelo et al., 1999). B1, B2, Normalized changes in EPSP (solid line), \( R_m \) (dashed line), and spike threshold (\( Th_1 \); dotted line) as a function of depolarization \( B_1 \) or spike frequency \( B_2 \) during TBS. EPSP and \( Th_1 \) data have been adjusted for time-dependent changes in control recordings. Note that changes in \( R_m \) and \( Th_1 \) occur earlier than those in EPSPs. All data in this figure were recorded 15 min after TBS.

Figure 10. NMDA receptor block prevents LTP. Effect of TBS delivered from \(-70\) mV in the presence of 100 \( \mu \)M APV and 50 \( \mu \)M 7-Cl-ky to block NMDA receptors (10 \( \mu \)M bicuculline in the bath). A1, A2, EPSPs recorded before and after TBS shown in B1 and B2. Although a normal TBS was applied in B1, TBS was reinforced in B2 by a 10 pA pulse during synaptic activation. C, Average EPSP changes after TBS in six cells as in A1 and B1 and in four cells as in A2 and B2. D, \( R_m \) changes (both above and below \(-80\) mV) and changes in \( Th_1 \) and \( Th_2 \) adjusted for time-dependent changes in control recordings. All data were recorded 15 min after TBS (same cells as in C).
In an additional four experiments (Fig. 10A.), membrane depolarization was reinforced by associating TBS with depolarizing current pulses (Fig. 10B.). In these experiments neither EPSP nor intrinsic excitability potentiation were induced. The cumulative results of the experiments in Figure 10, A and B, are shown in Figure 10, C and D. These results indicate that although NMDA receptors reinforce membrane depolarization during repetitive stimulation, they are needed to induce potentiation of EPSPs and post synaptic responsiveness through a mechanism that differs from their direct depolarizing action and presumably involves an increase in Ca\(^{2+}\) influx and the consequent activation of Ca\(^{2+}\)-dependent processes.

**DISCUSSION**

This paper demonstrates the potentiation of intrinsic excitability in cerebellar granule cells after high-frequency mossy fiber stimulation. The apparent input resistance (\(R_{in}\)) increased and spike threshold decreased, enhancing granule cell synaptic excitation. Potentiation of intrinsic excitability could coexist with potentiation of synaptic efficacy, and both depended on NMDA receptor activation.

Potentiation of granule cell intrinsic excitability was induced between \(-60 \text{ and } -40 \text{ mV}\). In this potential range, granule cell NMDA receptors activate sizeable conductance (D’Angelo et al., 1995), probably because the NR2C subunit confers low sensitivity to Mg\(^{2+}\) block (Monyer et al., 1994). The main action of NMDA receptors could be that of gating Ca\(^{2+}\) influx (in fact, in whole-cell recordings performed with pipettes containing 10 mM BAPTA, any excitability change was prevented; \(n = 5\) (E. D’Angelo and P. Rossi, unpublished observation). In addition, NMDA receptors enhanced membrane depolarization (Fig. 10).

Synaptic conductance needed stronger depolarization (approximately \(-40 \text{ mV}\)) than intrinsic excitability to be potentiated (see also D’Angelo et al. [1999]). A discriminating factor between these two aspects of potentiation may be the intensity of the NMDA current, which rises steeply between \(-60 \text{ and } -40 \text{ mV}\). Another discriminating factor may be spikes (Thomas et al., 1998; Linden, 1999). Spikes were frequent when LTP included synaptic conductance potentiation, whereas they were rare or absent when potentiation of intrinsic excitability was prominent. Spikes may favor the induction of plasticity through a Na\(^{+}\)-dependent enhancement of the NMDA current (Yu and Salter, 1998) and by activating voltage-dependent Ca\(^{2+}\) channels (Magee and Johnston, 1997; Markram et al., 1997; Aizenmann et al., 1998; Aizenmann and Linden, 2000). It should be noted that spikes alone were not sufficient to induce synaptic plasticity, as demonstrated by recordings in which NMDA receptors were blocked.

The control of granule cell synaptic excitation proved critical in allowing a voltage- and NMDA receptor-dependent regulation of plasticity. On the one hand, granule cell excitation was finely modulated by the intensity and frequency; see D’Angelo et al., 1995) of repetitive mossy fiber discharge. The physiological significance of this mechanism is suggested by the observation that repetitive mossy fiber discharge changes in relation to specific behavioral states in vivo (Kase et al., 1980; Pellerin and Lamarre, 1997; Hartmann and Bower, 1998). On the other hand, granule cell excitation was reduced through both tonic and phasic mechanisms of synaptic inhibition mediated by Golgi cells (Fig. 1) (Brickley et al., 1996). Although Golgi cell inhibition prevented mossy fiber–granule cell plasticity, the efficiency of this process in vivo remains speculative. Golgi cell activity reflects excitation in a large population of mossy and parallel fibers (Eccles et al., 1967; van Kan et al., 1993; Vos et al., 1999), the discharge of which is probably less synchronous than in our experiments (in which the whole afferent fiber bundle was excited). Thus Golgi cells should dynamically modulate mossy fiber–granule cell plasticity rather than exert an all-or-none preventative action. Moreover, local depression of GABA release by high-frequency mossy fiber discharge (Mitchell and Silver, 2000) may favor LTP induction at specific synapses by contrasting background granule cell inhibition (Davies et al., 1991).

A closer understanding of Golgi cell functions seems crucial to clarify the physiological induction mechanism of mossy fiber–granule cell plasticity.

The increase in granule cell input resistance and spike prepotential occurred in a limited membrane potential region, suggesting that voltage-dependent conductances involved in spike generation were changed. Although at present there is no direct evidence to implicate any specific membrane conductance, it should be noted that an increased Na\(^{+}\) current and a decreased K\(^{+}\) current may account for a reduced firing threshold, as demonstrated recently in cell culture (Desai et al., 1999). A Ca\(^{2+}\)-current-dependent effect, which might occur in hippocampal pyramidal neurons (Wathey et al., 1992) or in cerebellar deep nuclear cells (Aizenmann and Linden, 2000), seems less likely to occur in cerebellar granule cells because their Ca\(^{2+}\) currents activate once the spike has already been generated (D’Angelo et al., 1998). It should be noted that persistent depression of synaptic inhibition (as reported in hippocampal E-S potentiation) (Abraham et al., 1987; Chavez-Noriega et al., 1990; Breakwell et al., 1995) did not significantly contribute to enhance granule cell excitation.

Potentiation of intrinsic excitability and synaptic conductance cooperated to strengthen EPSP–spike coupling and to increase the reliability of spike generation during synaptic transmission (Davodal et al., 1999). However, the functional significance of intrinsic excitability potentiation may differ partly from that of synaptic conductance potentiation. First, because potentiation of intrinsic excitability could be achieved at a relatively low threshold, it may have a compensatory effect by restoring granule cell readiness in conditions of weak synaptic excitation (Fregnac, 1998). An increased excitability in turn may facilitate the subsequent induction of synaptic conductance potentiation. Second, because the granule cell has a compact electrotonic structure and a marginal potential loss is expected from dendritic endings to soma (Gabbiani et al., 1994; D’Angelo et al., 1995; Silver et al., 1996), potentiated excitability should affect neuronal responsiveness as a whole. Conversely, synaptic conductance changes are thought to be synapse specific (Bliss and Collingridge, 1993).

By allowing learning and storage of activity patterns at specific synapses while maintaining neuronal firing within an appropriate operating window, the combination of changes in synaptic conductance and intrinsic excitation may optimize information transfer and network computation (Stemmmer and Koch, 1999). Although plasticity at the mossy fiber–granule cell synapse was not included in Marr’s (1969) original model of the cerebellum, it may have important implications for cerebellar control of motor coordination (Arbib et al., 1998; Schweighofer et al., 1998; N. Schweighofer, personal communication).

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