

Pattern-dependent, simultaneous plasticity differentially transforms the input–output relationship of a feedforward circuit

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Memories are believed to be encoded by changes in the synaptic connections between neurons. Although many forms of synaptic plasticity have been identified, it remains unknown how such changes affect local circuits. Feedforward inhibitory networks are a common type of local circuitry and occur when principal neurons and their afferent inhibitory interneurons receive the same input. Using slices of cerebellar cortex, we explored how synaptic plasticity at multiple sites within a feedforward inhibitory network consisting of parallel fibers, interneurons, and Purkinje neurons alters the output of this circuit. We found that stimuli resembling baseline activity potentiated feedforward excitatory and simultaneously depressed feedforward inhibitory pathways. In contrast, stimuli resembling sensory-evoked patterns of firing potentiated both types of feedforward connections. These distinct forms of ensemble plasticity change the way Purkinje neurons subsequently respond to inputs. Such concerted changes in the circuitry of cerebellar cortex may contribute to certain forms of sensorimotor learning.

cerebellum | inhibition | interneuron | Purkinje

Learning and memory in mammalian brains is widely believed to be anchored in long-lasting changes in the strength of synaptic connections between neurons. Despite the identification of dozens of forms of synaptic plasticity, it remains unknown how these phenomena change the response properties of local circuits. We set out to explore how use-dependent changes in synaptic strength within a feedforward circuit affect the transformation from input to output.

Feedforward inhibition is a common configuration for local circuitry incident on principal neurons. In such circuits, a common excitatory pathway synapses on both principal neurons and their afferent inhibitory interneurons (IN). This situation occurs in the cerebral (1) and cerebellar cortices (2), hippocampus (3), thalamus (4), and other brain areas (5, 6). In the cerebellar cortex, granule cell axons bifurcate in the molecular layer to form parallel fibers (PFs). These excitatory inputs synapse on Purkinje (Pjk) neurons and their afferent molecular layer interneurons (Fig. 1A).

Within this simple cerebellar circuit, different forms of synaptic plasticity have been described at various synapses. We explored how multiple types of synaptic plasticity occurring at the same time, hereafter called “simultaneous plasticity,” leads to differential changes in the input–output relationship of the feedforward inhibitory circuit in response to realistic conditioning activity. Because Pkjs fire spontaneously (7, 8), spike outputs in response to inputs are superimposed on a high rate of background activity. This mechanism enables the circuit to reflect changes in inhibition as well as excitation (9). We show that patterns of conditioning activity that resemble baseline firing cause potentiation of direct PF input to Pkjs and depression of PF input to INs, resulting in a potentiation of spike output. In contrast, patterns of conditioning activity that resemble responses to sensory stimuli potentiate both the excitatory and inhibitory arms of the feedforward circuit, resulting in a

more temporally complex transformation of the input–output relationship. The enhancement of temporal contrast caused by conditioning bursts may sensitize the circuit to inputs in pathways encoding salient stimuli, providing well timed disinhibition of the deep cerebellar nuclei, responses known to develop during cerebellar learning (10).

Methods

Brain Slice Preparation. After anesthetization with halothane and decapitation, the cerebella of 14- to 20-day-old rats were removed and immersed in ice cold (4°C) artificial cerebrospinal fluid (aCSF). Transverse slices were cut 300- μ m thick on a vibratome (Leica VT-1000) and transferred to a recovery chamber. After incubation at 36°C for 30 min, slices were stored at room temperature before being transported to a recording chamber. aCSF contained, as follows: 119 mM NaCl/26 mM NaHCO₃/11 mM glucose/2.5 mM KCl/2.5 mM CaCl₂/1.3 mM MgCl₂/1 mM NaH₂PO₄ and was bubbled with 95% O₂/5% CO₂.

Electrophysiology. Whole-cell recordings were performed with glass pipettes (1.1–3.0 Mohm for Pkjs and 3.0–7.0 Mohm for INs) under visual guidance of infrared-DIC optics including a Leica $\times 40$ water-immersion objective, as previously reported in ref. 11. Recordings were performed with either Dagan BVC-700A or Axon Instruments 200B amplifiers. Drugs [(2S)- α -ethylglutamic acid (EGLU), picrotoxin (PTX), and 3-((R,S)-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP); Tocris] were present for at least 10 min before recording and remained in the bath for the duration of the experiment.

A stimulus electrode (a glass theta pipette, 2- μ m tip) was positioned 100–1,000 μ m away. All stimuli were 100- μ s long and 5–22 μ A in amplitude to obtain excitatory postsynaptic currents (EPSCs) of ≈ 100 pA. Because unitary PF-Pjk synapses usually elicit currents < 40 pA (12, 13) and our failure rates were low (0–20%), we estimate that we usually stimulated between 3 and 10 PFs. PFs were stimulated every 5 s with pairs of impulses separated by 50 ms. Stimuli were delivered with Iso-Flex stimulus isolation units and timed by a Master 8 timing device (AMPI, Jerusalem).

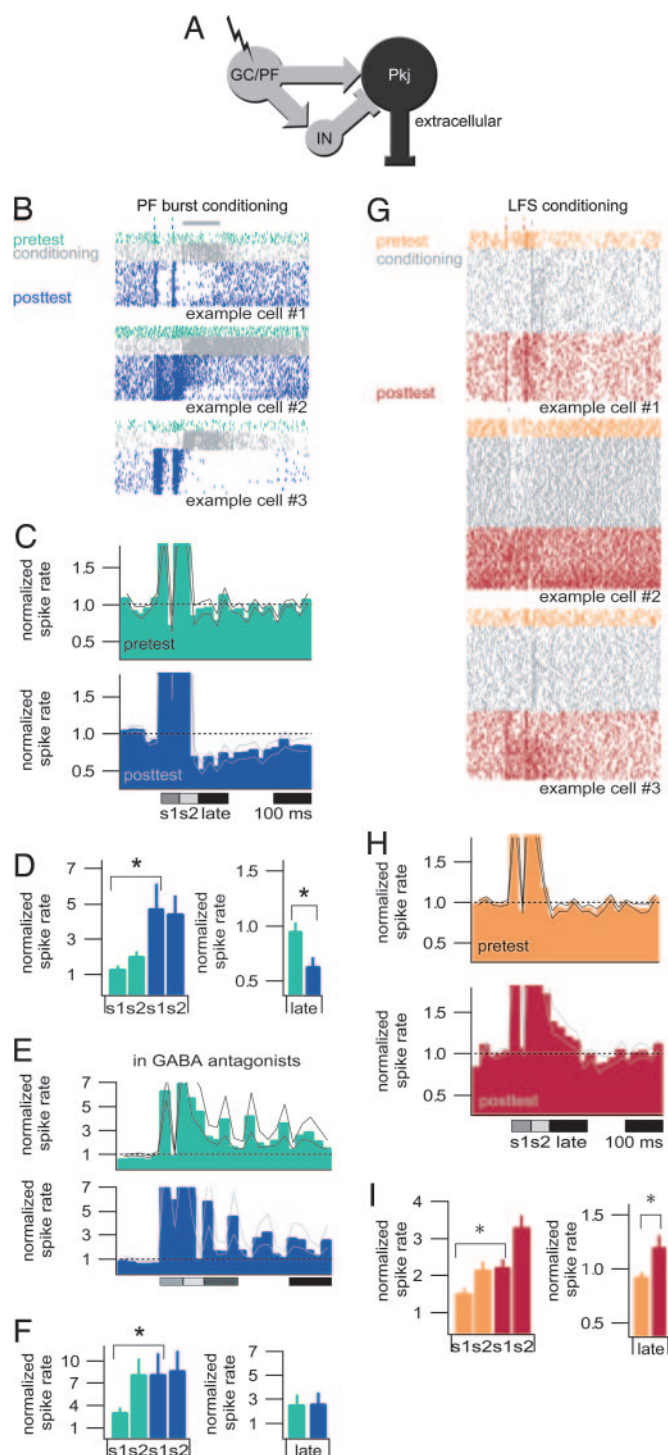
When recording EPSCs and inhibitory postsynaptic currents (IPSCs) in Pkjs, we voltage-clamped the cell at the reversal potentials for IPSCs and EPSCs, respectively. Posttest measurements of EPSCs and IPSCs were taken consecutively. Internal solutions for these experiments contained 110 mM cesium methane sulfonate, 10 mM Hepes, 2 mM MgCl₂, 4 mM ATP-Na, 0.4 mM GTP-Na, 30 mM 1,2-bis(2-aminophenoxy)ethane-

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Abbreviations: CPP, 3-((R,S)-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid; EGLU, (2S)- α -ethylglutamic acid; EPSC, excitatory postsynaptic current; eEPSC, evoked EPSC; IPSC, inhibitory postsynaptic current; eIPSC, evoked IPSC; IN, interneurons; LFS, low-frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; PF, parallel fiber; Pjk, Purkinje; PPF, paired-pulse facilitation; PSTH, peristimulus time histogram.

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N,N,N',N'-tetraacetate (7.15 pH, 280 mosM). The high concentration of 1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetate was used only in these experiments to avoid triggering Ca^{2+} -dependent mechanisms while the neuron was clamped at 0 mV. The internal solution was allowed to dialyze the cell for at least 10 min before clamping Pkjs at 0 mV to measure IPSCs. Uncompensated series resistances were monitored with a conductance pulse, and experiments were terminated if series resistance changed by >15%. These intracellular recordings of Pkjs were performed at 34–36°C.

The pool of INs recorded from was likely dominated by stellate cells, because we deliberately selected small somas in the upper half of the molecular layer. These cells were held in current clamp with 100 μ M PTX in the bath to block spontaneous inhibitory inputs. Neurons were not used if they failed to spontaneously fire spikes that overshoot 0 mV. Current was injected to hold the cells at approximately -80 mV, and a current pulse was used to monitor input resistance. Cells were discarded if the input resistance changed by $>15\%$. The internal solution used for these recordings contained 122 mM potassium methane sulfonate, 10 mM Hepes, 5 mM KCl, 1 mM NaCl, 2 mM MgCl_2 , 2 mM ATP-Na, 0.4 mM GTP-Na, and 1 mM EGTA (7.15 pH, 280 mosM). These intracellular recordings of INs were performed at room temperature.

Extracellular recordings of Pkjs were performed in the loose cell-attached configuration as described in ref. 7. These experiments were performed at 36–38°C.

Data Analysis. All data were filtered at 5 kHz and sampled at 10 kHz with a Digidata 1320 A/D interface and PCLAMP (Molecular Devices) software. Custom programs written for IGOR PRO (including NEUROMATIC by J. Rothman, University College London) were used to analyze the data. The maximum amplitude of postsynaptic potentials and postsynaptic currents were calculated in a 30 ms window after each stimulus and then corrected for baseline on a trace-by-trace basis. When analyzing IPSCs, the baseline was taken to be the minimum membrane voltage in a 250-ms period during the 5-s interstimulus interval. Normalized and averaged data are shown as fold change $\pm$ SEM. Tests of significance were performed by using either the Student *t* test (all intracellular IN data are presented in *Supporting Data*, which is published as supporting information on the PNAS web site) or the Wilcoxon nonparametric test (rest).

Results

Bursting PF Input Induces a Biphasic Spike Output and Low-Frequency PF Activity Induces a Prolonged, Potentiated Spike Output. We used

transverse slices of cerebellum to preserve the feedforward inhibitory circuitry comprised of PFs, INs, and Pkjs (Fig. 1A). To examine the changes in spike output caused by simultaneous plasticity induced by realistic stimuli, we made extracellular recordings of Pkjs before, during, and after conditioning. Pretests and posttests consisted of pairs of stimuli separated by 50 ms and delivered every 5 s. Paired stimuli were used to infer whether changes in response were due to pre- or postsynaptic mechanisms. Before conditioning, pairs of stimuli interacted with the spontaneous firing rate of Pkjs, causing a brief increase

antagonist (PTX, 100 μ m) induces a potentiation of the excitatory response, but it is not followed by an inhibitory period, as is the case in the control experiments. (F) Bar graphs summarize the data in E. (G–I) Sister experiments were performed by using LFS to condition the circuit. (G) Example data from three cells, in this case 300 sweeps of conditioning, are shown. (H) PSTHs of pretest data and posttest data for seven control cells and six cells in EGLU. Lines in the PSTHs indicate the SEM. (I) Summary of averaged data over regions indicated. A significant increase in firing during the late time window is observed in the posttest. *, $P < 0.05$.

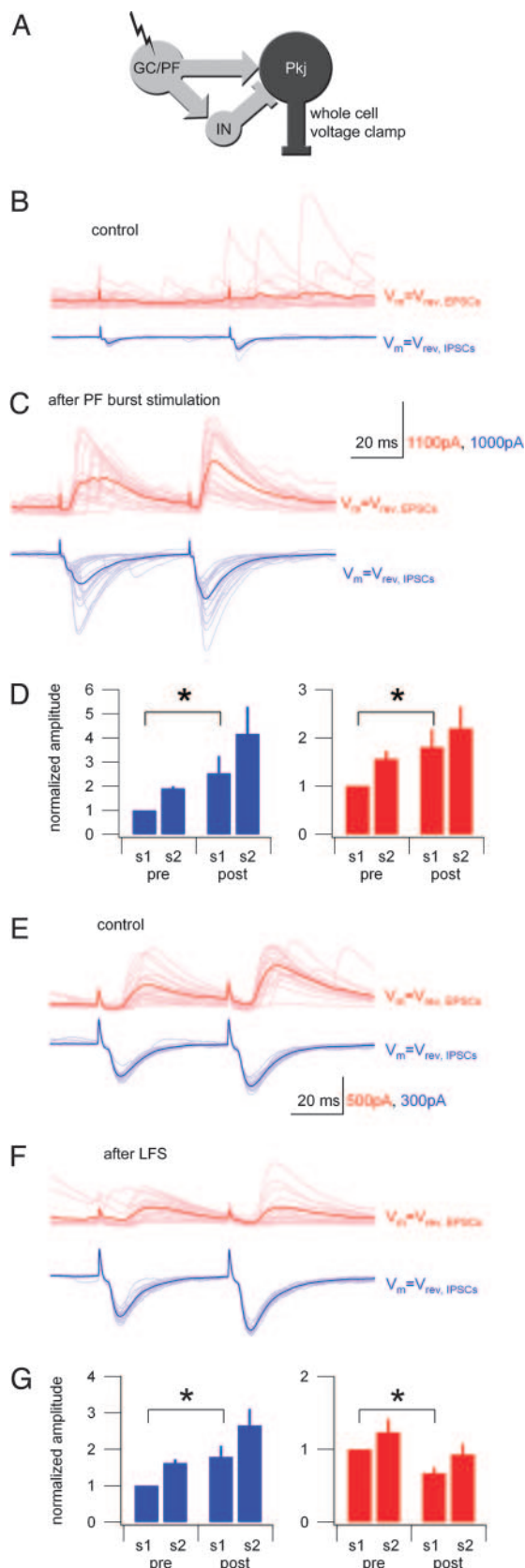


Fig. 2. PF bursts potentiate both monosynaptic eEPSCs and disynaptic eIPSCs, but LFS potentiates monosynaptic eEPSCs and depresses disynaptic eIPSCs. (A) Intracellular recordings of Pkj synaptic input upon stimulation of PFs. (B and C) Pkjs were voltage-clamped at different potentials to record eEPSCs (blue) and eIPSCs (red) before (B) and after (C) PF burst stimulation. The greater delay

in spike rate directly after each stimulus (after first stimulus in a pair, relative to baseline: 2.87 ± 0.37 ; after second stimulus: 4.72 ± 0.58 , $n = 6$, $P < 0.05$, Fig. 1D). Thus, paired-pulse facilitation (PPF) at the PF-Pkj synapse is reflected in the spike rate (1.64 ± 0.41).

After a 5-min control period, one of two conditioning protocols was delivered to the PFs. A high-frequency burst protocol (PF burst) consisted of 10 stimuli delivered at 100 Hz every 3 s for 5 min (14). This conditioning pattern resembles the brief, high frequency bursts observed in granule cells *in vivo* upon whisker deflection (15). Alternatively, circuits were conditioned with a low-frequency, sparse stimulation protocol consisting of single stimuli delivered every second for 5 min (16). This pattern of stimuli resembles spontaneous firing of granule cells *in vivo* in the absence of overt sensory input or movement (15).

PF burst stimulation elicited reliable trains of spikes (Fig. 1B). After PF burst stimulation, a large potentiation of the excitatory response can be seen in all cells. This result represents a significant long-term potentiation (LTP) of spike output in response to PF stimuli compared with pretest (increase in response to the first of a pair of stimuli: 3.99 ± 1.12 , $P < 0.05$, Fig. 1D and *Supporting Data*; also see Fig. 5, which is published as supporting information on the PNAS web site).

A striking observation in these experiments was that after PF burst, a period of inhibition appeared after the potentiated spike response. On average, depressed firing rates were observed for 200 ms after the test stimuli (10-ms bins, $P < 0.05$). To describe these data, we focused on an arbitrarily chosen, 80-ms-long time window (labeled “late” in Fig. 1C) which was held in the same place for analysis on the experiments in Figs. 1 and 4. Averaged over this window of time, the spike rate was significantly inhibited (normalized to baseline firing, pretest: 0.96 ± 0.08 , posttest: 0.64 ± 0.08 , $P < 0.05$, $n = 6$; Fig. 1C and D).

We next set out to test whether the late decrease in firing rate depended on GABA-mediated inhibition by performing experiments in the GABA_A receptor antagonist PTX. Similar to the control situation, conditioning with PF burst stimulation in PTX caused an increase in spiking at a short latency after stimulation (2.04 ± 0.38 , $n = 7$, $P < 0.05$; Fig. 1 *E* and *F*). However, in contrast to control conditions, there was no change in firing during the late time window (1.02 ± 0.32 , $P > 0.05$, $n = 7$; Fig. 1 *E* and *F*). This result demonstrates that the decrease in firing rate during the late time window is due to GABA_A-mediated inhibition and argues that it is not a result of refractory effects or intrinsic hyperpolarizing conductances. Taken together, these findings demonstrate that PF burst stimulation enhances a simple excitatory spike response and adds temporal contrast by potentiating feedforward inhibition.

In contrast to PF burst stimulation, low-frequency stimulation (LFS) had a different effect on the input–output relationship of the molecular layer. Before conditioning, pairs of PF responses were not significantly different from those in previous experiments (after first stimulus in a pair, relative to baseline: 3.16 ± 0.24 , $P < 0.05$; after second stimulus: 4.84 ± 0.44 , $n = 7$; Fig. 1*I*). When PFs were stimulated at a low frequency (LFS), the Pk_j spike response to PF impulses potentiated (relative to the pretest

in the disinaptic pathway is reflected in the eIPSCs. Input strength was tested with pairs of stimuli separated by 50 ms. Approximately 20 min after conditioning the PF input with a burst protocol, the strength of both the excitatory and inhibitory inputs were potentiated simultaneously. The example traces are taken from a single neuron. (D) The changes in eEPSCs (blue) and eIPSCs (red) are summarized with bar graphs. (E–G) Sister experiments were performed by using LFS to condition the circuit instead of PF bursts. Example traces from a single neuron show responses before (E) and after (F) LFS. Conditioning with LFS potentiated eEPSCs but depressed eIPSCs. (G) The changes in eEPSCs and eIPSCs are summarized with bar graphs. $*P < 0.05$.

response: 1.61 ± 0.13 , $P < 0.05$, $n = 7$; Fig. 1I) with no significant change in PPF.

However, unlike PF burst stimulation, LFS did not enhance the late inhibitory response to the PF stimuli. In fact, there was a significant increase in firing rate during this period (normalized to baseline firing, pretest: 0.93 ± 0.04 , posttest: 1.20 ± 0.12 , $P < 0.05$, $n = 7$; Fig. 1I).

PF Bursts and LFS of PFs Differentially Affect EPSCs and IPSCs Recorded in Pkjs. To determine whether changes in spike output of the circuit reflected simultaneous synaptic plasticities, we recorded from Pkjs in the whole-cell voltage clamp configuration. We measured how evoked excitatory and inhibitory postsynaptic currents (eEPSCs and eIPSCs) responded to conditioning by voltage-clamping Pkjs at the reversal potentials for IPSCs and EPSCs, respectively. During preconditioning and postconditioning test periods, PFs were stimulated with pairs of impulses separated by 50 ms. Before conditioning, direct excitatory PF inputs exhibited strong PPF, as expected (Fig. 2B). Disynaptic (PF-IN-Pkj) inhibitory inputs also showed PPF (Fig. 2C). Consistent with previous work (17, 18) we found that IN-Pkj synapses and PF-IN synapses facilitate (Fig. 3A); thus, the net facilitation of the disynaptic circuit likely reflects short-term plasticity at both synapses.

PF burst stimulation simultaneously increased the size of both the eEPSCs and eIPSCs (Fig. 2D, change in eEPSC amplitude: 2.54 ± 0.714 , $P < 0.05$, change in eIPSC amplitude: 1.80 ± 0.374 , $P < 0.05$; $n = 7$). By contrast, Fig. 2G shows that LFS simultaneously increased the size of eEPSCs and decreased the size of eIPSCs (change in eEPSC amplitude: 1.79 ± 0.304 , $P < 0.05$, change in eIPSC amplitude: 0.671 ± 0.0793 , $P < 0.05$; $n = 8$).

These results show that the polarity of synaptic changes within the feedforward inhibitory network differs depending on the pattern of input to the circuit. After PF burst stimulation, both excitatory and inhibitory inputs potentiate simultaneously. By contrast, after LFS stimulation, excitatory inputs potentiate, but inhibitory inputs depress. These changes persist for at least 20 min. Changes in excitatory input are likely due to synaptic plasticity at PF-Pkj synapses. However, the mechanism by which inhibition changes is not as obvious because there are two synapses between the stimulated fibers at the Pkj: the PF-IN synapse and the IN-Pkj synapse.

PF Burst Stimulation Causes NMDA Receptor-Dependent LTP and LFS Causes Group II Metabotropic Glutamate Receptor (mGluR)-Dependent Long-Term Depression (LTD) at PF-IN Synapses. To explore whether the changes in evoked inhibitory input recorded in Pkjs could be explained by changes at the PF-IN synapse, we recorded excitatory postsynaptic potentials from INs in current clamp. Current was injected to hold the cells near -80 mV to prevent these spontaneously active neurons from firing, and PTX was included in the bath to block GABA_A inhibition and isolate PF input.

PF burst stimulation caused an increase in the strength of PF excitatory postsynaptic potentials (1.59 ± 0.17 , $P < 0.05$, $n = 6$; Fig. 3C). This increase was accompanied by a small but not quite significant decrease in PPF (0.82 ± 0.078 , $P > 0.05$, $n = 6$; Fig. 3C). To explore whether LTP at this synapse was sensitive to postsynaptic activity, we also performed experiments with a pairing protocol. Current was injected in the postsynaptic cell to evoke 3–10 action potentials during the train of stimuli to PFs. Pairing postsynaptic action potentials with PF burst stimulation also caused LTP (increase in excitatory postsynaptic potential: 1.29 ± 0.05 , $P < 0.05$, $n = 4$; data not shown) and this pairing-induced LTP was not significantly different from unpaired LTP ($P > 0.1$).

Previous studies of train-induced LTP at the PF-Pkj synapse

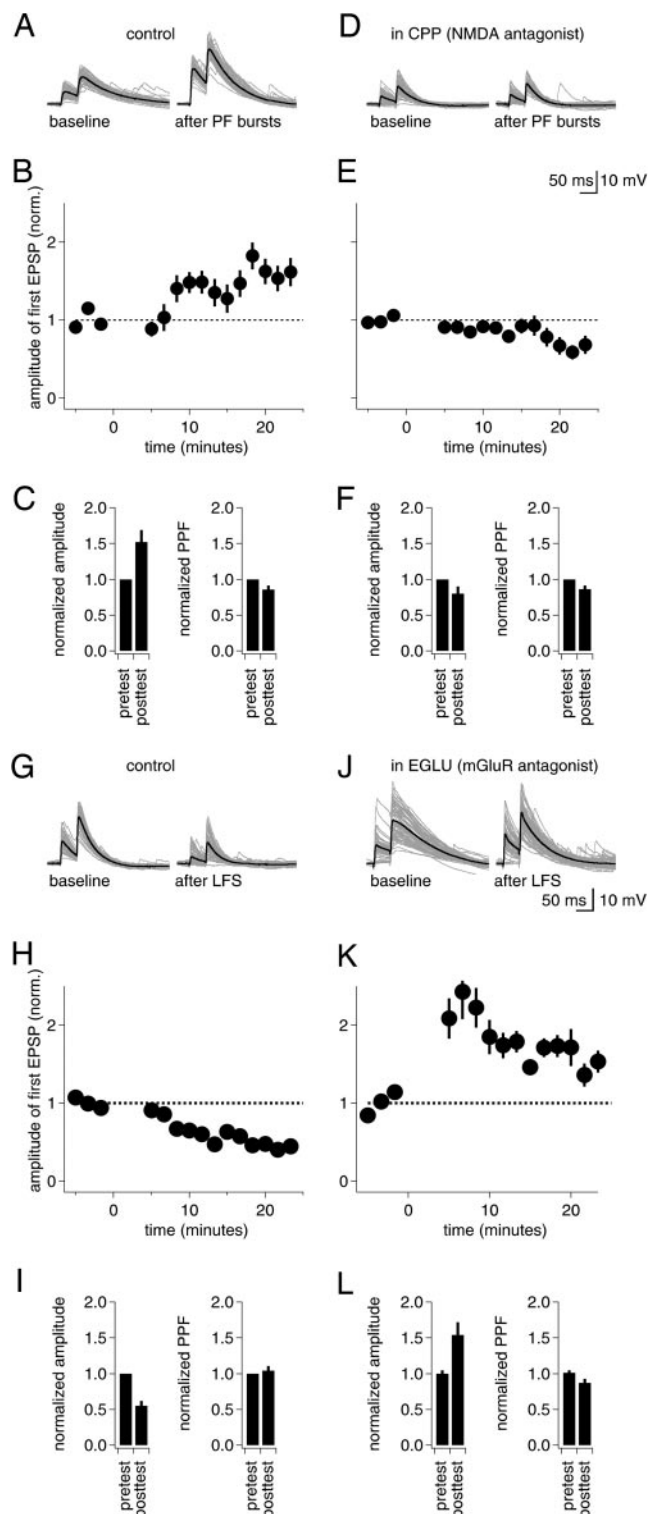


Fig. 3. PF-IN synapses are potentiated by PF burst stimulation through an NMDA-dependent mechanism and are depressed by LFS through a group II mGluR-dependent mechanism. (A–F) Data from control experiments (A–C) and experiments in the presence of $10 \mu\text{M}$ CPP (D–F), an NMDA receptor antagonist, show that conditioning with PF burst induces NMDA-dependent LTP. Results are summarized by example traces (A and D), averaged data (B and E), and summary bar graphs (C and F). (G–L) By contrast, data from control experiments (G–I) and experiments in the presence of $200 \mu\text{M}$ EGLU (J–L), a group II mGluR antagonist, show that conditioning with LFS induces mGluR-dependent LTD. Results are summarized by example traces (G and J), averaged data (H and K), and summary bar graphs (I and L).

(19–21) have concluded that this plasticity was predominately expressed presynaptically. Because LTP at PF-IN synapse has several characteristics in common with this form of LTP (both induced by PF burst, accompanied by a decrease of PPF, and both have the same presynaptic fibers), we further explored whether LTPs at PF-Pkj and PF-IN synapses share similar presynaptic mechanisms. This possibility appears not to be the case as a bath-applied NMDA receptor antagonist, CPP, blocked the LTP observed at the PF-IN synapses (Figs. 3 *E* and *F*), and previous results have shown that NMDA receptor blockade does not affect LTP at the PF-Pkj synapse (19, 20). Also, while discriminating the two forms of LTP, we found that chelating postsynaptic calcium with 30 mM 1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetate blocks the LTP at PF-IN synapses (0.43 ± 0.07 , $P < 0.05$, $n = 4$; data not shown), whereas LTP elicited by LFS at the PF-Pkj synapse has been shown to be facilitated by chelation of postsynaptic calcium (16). These data support the interpretation that PF burst-induced LTP at the PF-IN synapse is mechanistically distinct from both PF burst-induced LTP at the PF-Pkj synapse and LFS-induced LTP at the PF-Pkj synapse.

LFS caused an LTD of the excitatory postsynaptic potential recorded in INs (0.55 ± 0.065 , $P < 0.05$, $n = 6$) which was not accompanied by a change in PPF (1.04 ± 0.096 , $P > 0.5$, $n = 6$; Fig. 3 *H* and *I*). Repeating the experiments in EGLU, an antagonist of group II mGluRs, unmasked a potentiation (1.54 ± 0.18 , $P < 0.05$, $n = 6$) with little change in PPF (0.87 ± 0.057 , $n = 6$, $P > 0.05$; Figs. 3 *K* and *L*). These results are similar to those reported by Rancic and Crepel (22).

Our observations of LTP and LTD at the PF-IN synapse support the hypothesis that the changes in eIPSCs recorded in Pkjs after LFS and PF burst stimulation could be explained by changes at the PF-IN synapse (for more details, see *Supporting Data*; see also Fig. 6 which is published as supporting information on the PNAS web site). Moreover, the residual plasticities in receptor antagonists (e.g., the potentiation seen after LFS in the presence of mGluR antagonists and the depression seen after PF burst in the presence of NMDA receptor antagonists) suggest two competing pathways with overlapping induction mechanisms.

The Late Inhibitory Period Observed with PF Bursts Is NMDA Dependent and the Late Excitatory Period Observed with LFS Is Group II mGluR-Dependent. We hypothesize that the late decrease in firing observed after PF burst conditioning is largely due to an NMDA receptor-dependent form of LTP at PF-IN synapses. Although earlier experiments with PTX (Fig. 1 *E* and *F*) argue against a refractory mechanism for the effect, other mechanisms outside of PF-IN LTP may be playing a role and we sought direct evidence for our hypothesis.

Given that NMDA receptors mediate LTP at the PF-IN synapse (Fig. 3*E*), we reasoned that bath applications of NMDA receptor antagonists could be used to test whether PF-IN LTP was required for this inhibitory phase. Because adult Pkjs lack NMDA receptors (23), this manipulation leaves postsynaptic signaling at the PF-Pkj synapse intact. When NMDA receptors were blocked (with 10 μ M CPP; Fig. 4), pretest responses to PF stimuli were indistinguishable from pretests performed without CPP (after first stimulus in a pair, relative to baseline: 3.00 ± 0.47 , $P < 0.05$; after second stimulus: 4.57 ± 0.84 , $n = 6$; not significantly different from pretests without CPP, $P > 0.05$). However, consistent with our hypothesis, potentiated inhibitory periods were not observed after PF burst stimulation (relative to baseline firing, pretest: 1.03 ± 0.10 , posttest: 1.11 ± 0.08 , $P > 0.05$, $n = 6$; Figs. 4 *C* and *D*). Instead, the potentiated spike response was prolonged in time. This result implies that an enhanced inhibitory response normally truncates the excitatory spike response in addition to causing a late decrease in firing rate. These findings also argue that plasticity at the PF-IN

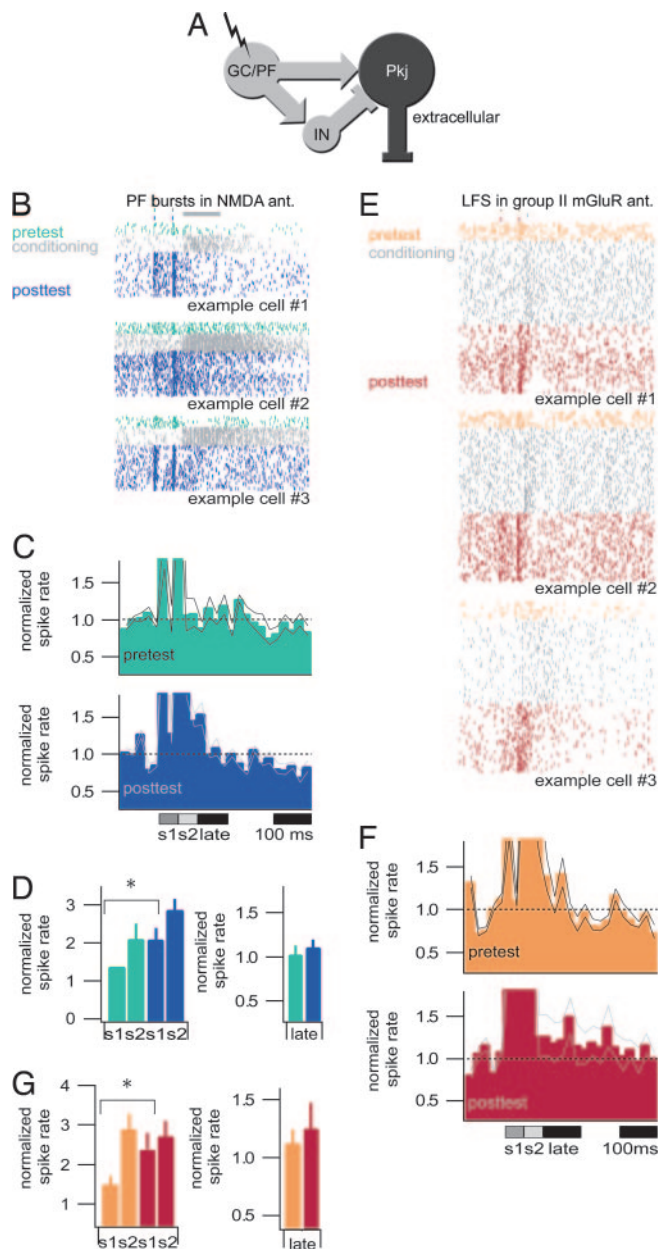


Fig. 4. The late inhibitory period induced by PF bursts depends on NMDA receptors and the prolonged increase in firing induced by LFS depends on group II mGluRs. (A) To explore whether changes at the PF-IN synapse could account for the changes in spike output of the circuit, the extracellular experiments were repeated in the presence of antagonists to block specific forms of PF-IN plasticity. (B–D) Recordings performed in the presence of 10 μ M CPP, an NMDA receptor antagonist that blocks PF-IN LTP. Example cells (B), PSTHs (C), and summary bar graphs (D) show that the late inhibitory period in the PSTH that was observed under control conditions (Fig. 1) is blocked by this drug, which also blocks LTP at the PF-IN synapse (Fig. 3). (E–G) Recordings performed in the presence of 200 μ M EGLU, a group II mGlu receptor antagonist that blocks PF-IN LTD. Example cells (E), PSTHs (F) and summary bar graphs (G) show that the prolonged excitatory response in the PSTH that was observed under control conditions (Fig. 1) is blocked by this drug, which also blocks LTD at the PF-IN synapse (Fig. 3).

synapse can account for the potentiated inhibitory responses after PF burst stimulation.

Next we examined the changes in LFS. To test whether the late excitatory period is due to group II mGluR-mediated LTD at the PF-IN synapse, we repeated the extracellular experi-

ments in the presence of EGLU, a group II mGluR antagonist (Fig. 4). In the presence of EGLU, potentiated responses remained, as expected (1.75 ± 0.21 , $P < 0.05$, $n = 5$; Fig. 4 *F* and *G*). However, the excitatory response was not prolonged in time, as it was in control conditions, so the firing rate had returned to baseline by the late time point (1.12 ± 0.20 , $P > 0.05$, $n = 5$; Fig. 4 *F* and *G*).

This experiment shows that LFS transforms the response of Pkjs to pairs of stimuli in the PFs. Specifically, LFS potentiates the increase in spiking that follows the stimuli. Additionally, LFS increases the firing rate at later times, in a window in which PF burst stimulation was found to cause a decrease in firing rate. This result is likely due to LTD at the PF-IN synapse occurring simultaneously with LTP at PF-Pkj synapse (Supporting Data and Fig. 6).

Discussion

This study demonstrates that simultaneous plasticity at multiple sites differentially alters the input–output function of the cerebellar cortex after conditioning with two physiologically representative patterns of activity. A conditioning pattern resembling granule cell firing in response to sensory stimuli (PF bursts) causes the circuit to produce biphasic spike responses involving an early excitatory phase and a later inhibitory phase. Another conditioning pattern, resembling baseline firing of granule cells (LFS), leads to a prolonged potentiation of excitatory spike output. Our data show that these circuit transformations are due to simultaneous plasticity at two types of synapses in the feedforward inhibitory circuit. LFS depresses the disinhibitory inhibitory pathway at the PF-IN synapse, and potentiates direct excitatory inputs at the PF-Pkj synapse, contributing to the resulting monophasic, potentiated spike output. PF bursts potentiate both the feedforward inhibitory pathway at the PF-IN synapse as well as excitatory inputs at the PF-Pkj synapse leading to a biphasic spike response.

Such pattern-dependent reorganizations in feedforward inhibitory circuits could be useful for tuning the network to respond strongly to small changes in inputs encoding salient stimuli. When a particular input is activated with a pattern resembling baseline firing, the circuit relays activity reliably. However, if an input fires in a manner consistent with encoding sensory stimuli, the circuit reorganizes to increase the salience of the output, responding with a larger increase and a sharp decrease in spiking.

Cellular Mechanisms. Our observations can largely be accounted for by known forms of plasticity that have, until now, been studied in isolation. Two different mechanisms of LTP at the PF-Pkj synapse have been described by previous studies. One is induced with low rates of activity, can reverse LTD at this synapse, (24) and is resistant to postsynaptic chelation of calcium (16). The LTP we observed after LFS was likely due to this mechanism, in fact, the LFS protocol is identical to that used by Lev-Ram *et al.* (16). The other form of LTP is induced with higher rates of activity, depends on a presynaptic rise in cAMP concentration, and appears to be

mostly due to presynaptic mechanisms, because it is accompanied by a decrease in PPF and is insensitive to glutamate receptor blockade and high concentrations of postsynaptic calcium chelators (19, 20). This form of LTP was likely observed after PF burst stimulation.

Recently, Rancillac and Crepel have performed intracellular recordings to explore LTP and LTD at the PF-IN synapse (22). They found, and our results confirm, that LTP at this synapse depends on NMDA receptors and LTD depends on group II mGluRs. The later phase of inhibited spike output observed after PF bursts is likely due in large part to LTP at the PF-IN synapse for three reasons. First, we found in intracellular recordings from Pkjs that PF bursts potentiate stimulus-evoked inhibitory input. Second, intracellular recordings in INs showed that PF bursts induce an NMDA-receptor-dependent LTP of stimulus-evoked PF input onto those cells. Finally, extracellular experiments showed that the later inhibitory phase was blocked by a GABA_A antagonist. Another mechanism that may contribute to the late decrease in spiking is refractory effects due to the potentiated early spike response. However, if such mechanisms made a major contribution to the late decrease in firing in all experiments, then we would expect to see decreases in firing after any potentiated spike responses, and these decreases would not be blocked by PTX. After LFS, which also potentiates the early spike response, there is, in fact, an increase in firing during the same late time window despite the early potentiated increase in firing rate. Therefore, the contributions of refractory effects are likely smaller than those of PF-IN plasticity.

A depression of inhibitory input was observed after LFS in intracellular Pkj recordings. Additionally, after LFS conditioning, a group II mGluR-dependent increase in spike output during a late time window was also observed. These effects can be accounted for by LTD at the PF-IN synapse because intracellular recordings from INs showed that a group II mGluR-dependent LTD of PF input occurs with LFS.

Relevance to Models of Cerebellar Learning. Studies of Pkj firing during trace eyeblink conditioning paradigms have shown both simple monophasic and complex, multiphasic changes in spike output during conditioned stimuli (25, 26). These complex changes may be partially due to the mechanisms described in this paper.

The circuit phenomena outlined here may also shed light on mechanisms used to reset cerebellar circuits between bouts of motor learning. Granule cells have a low level of spontaneous activity in the absence of overt sensory stimuli (15). This activity is similar to LFS, which induces LTP at PF-Pkj synapses and LTD at PF-IN synapses. These processes would likely reverse LTD at PF-Pkj synapses (24) and counteract LTP at PF-IN synapses. Biphasic responses would be returned to simple excitatory responses, and the system would be returned to a baseline state.

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