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Long-Term Potentiation of the Responses to Parallel Fiber Stimulation in Mouse Cerebellar Cortex *in Vivo*

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Abstract

Long-term potentiation (LTP) of parallel fiber–Purkinje cell (PF–PC) synapses in the cerebellum has been suggested to underlie aspects of motor learning. Previous *in vitro* studies have primarily used low frequency PF stimulation conditioning paradigms to generate either presynaptic PF–PC LTP (4–8 Hz) or postsynaptic PF–PC LTP (1 Hz). Little is known about the conditions that evoke PF–PC LTP *in vivo*. High frequency stimulation *in vivo* increases PC responsiveness to peripheral stimuli; however, neither the site of action nor the signaling pathways involved have been examined. Using flavoprotein autofluorescence optical imaging in the FVB mouse *in vivo*, this report describes that a conditioning stimulation consisting of a high frequency burst of PF stimulation (100 Hz, 15 pulse trains every 3 s for 5 min) evokes a long-term increase in the response to PF stimulation. Following the conditioning stimulation, the response to PF stimulation increases over 20 min to ~130% above baseline and this potentiation persists for at least 2 h. Field potential recordings of the responses to PF stimulation show that the postsynaptic component is potentiated but the presynaptic, parallel fiber volley is not. Paired-pulse facilitation does not change after the conditioning stimulation, suggesting the potentiation occurs postsynaptically. Blocking non-NMDA (N-methyl-D-aspartic acid) ionotropic glutamate receptors with DNQX (6,7-dinitroquinoxaline-2,3-dione disodium salt, 50 μ M, bath application) during the conditioning stimulation has no effect on the long-term increase in fluorescence. However, blocking subtype I metabotropic glutamate receptors (mGluR₁) with LY367385 (200 μ M) during the conditioning stimulation abolishes the long-term increase in fluorescence. Blocking GABAergic neurotransmission is not required to evoke this long-term potentiation. Blocking GABA_A receptors reduces but does not eliminate the long-term potentiation. Therefore, this study demonstrates that high frequency PF stimulation generates long-term potentiation of PF–PC synapses *in vivo*. This novel form of LTP is generated primarily postsynaptically and is mediated by mGluR₁ receptors.

Keywords

synaptic plasticity; Purkinje cell; metabotropic glutamate receptors; flavoprotein imaging

Synaptic plasticity in the cerebellar cortex and cerebellar nuclei has been widely viewed as a mechanism for the long-term storage of information and has been implicated as the neural substrate underlying various forms of motor learning (Ito, 2006; De Zeeuw and Yeo, 2005). At various synapses in the cerebellar cortex and cerebellar nuclei, several forms of synaptic plasticity have been described, including long-term depression (LTD) and long-term potentiation (LTP) (Hansel et al., 2001; Ito, 2001; Jorntell and Hansel, 2006). Of particular interest are the long-term changes occurring at parallel fiber (PF)–Purkinje cell (PC) synapses.

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Conjunctive stimulation of climbing fibers (CFs) and PFs (PFs) results in LTD at the PF–PC synapses (Ito and Kano, 1982; Sakurai, 1987). Extensive studies *in vitro* have evaluated the properties and cellular and molecular mechanisms of PF–PC LTD. Expressed postsynaptically, the signaling cascade to evoke PF–PC LTD involves activation of metabotropic glutamate receptors subtype 1 (mGluR₁) (Conquet et al., 1994; Hartell, 1994; Ichise et al., 2000) and protein kinase C (PKC) (Crepel and Krupa, 1988; Linden and Connor, 1991). The activation of PKC results in clathrin-mediated internalization of postsynaptic AMPA receptors (Wang and Linden, 2000). Both postsynaptic α -calcium/calmodulin-dependent protein kinase II and nitric oxide-cyclic GMP-protein kinase G cascade play roles in LTD induction (Shibuki and Okada, 1991; Lev-Ram et al., 1997; Hansel et al., 2006). PF–PC LTD occurs when the intracellular Ca^{2+} levels are high, consistent with the requirements for conjunctive activation of both climbing fibers and PFs (Coesmans et al., 2004).

Several forms of LTP occur *in vitro* at PF–PC synapses. The first to be described is induced by 4–8 Hz PF stimulation in the absence of CF stimulation (Sakurai, 1987; Hirano, 1991; Kano et al., 1992; Hansel et al., 2001). Induced and expressed presynaptically, this form of LTP is triggered by presynaptic Ca^{2+} influx and is not altered by blocking postsynaptic glutamate receptors or by manipulating PC intracellular Ca^{2+} (Linden, 1998; Linden and Ahn, 1999; Salin et al., 1996). The resultant activation of Ca^{2+} -sensitive adenylyl cyclase in the presynaptic PFs gives rise to an increase in cAMP concentration with activation of protein kinase A (PKA) (Storm et al., 1998; Linden and Ahn, 1999; Salin et al., 1996). The activated PKA directly phosphorylates an active zone protein (RIM1 α) and leads to a long-lasting increase in neurotransmitter release (Lonart et al., 2003).

The second form of LTP at PF–PC synapses is induced by long duration (5 min), 1 Hz PF stimulation in the presence of bicuculline without CF activation (Lev-Ram et al., 2002). Expressed postsynaptically, induction of this LTP is dependent on postsynaptic Ca^{2+} and nitric oxide but not on cAMP or cGMP. Furthermore, paired-pulse facilitation (PPF, a measure of presynaptic transmitter release) does not change, consistent with the plasticity occurring postsynaptically. This form of PF–PC LTP involves activation of phosphatases (Belmeguenai and Hansel, 2005) and occurs when the increase in intracellular Ca^{2+} is smaller than that required to generate PF–PC LTD (Coesmans et al., 2004). One study in cerebellar slices showed that high frequency burst stimulation of PFs can evoke a short-term potentiation (at least 20 min) of PF–PC synapses (Smith and Otis, 2005).

The vast majority of both LTD and LTP studies described above have been *in vitro*. Linking any of these forms of synaptic plasticity to their functional consequences will require understanding the types and properties of synaptic plasticity that occur *in vivo*. However, our understanding of synaptic plasticity in the cerebellar cortex *in vivo* is rudimentary. PF–PC LTD has been shown to exist in decerebrate animals (Ito and Kano, 1982; Ekerot and Kano, 1985; Kano and Kato, 1987) and in the anesthetized, intact mouse (Gao et al., 2003). The latter study also demonstrated the spatial specificity of PF–PC LTD and confirmed its dependence on mGluR₁ and PKC activation. Even less is known about PF–PC LTP *in vivo*. The long-term expansion in PC receptive field size evoked by burst PF stimulation in the decerebrate cat appears to be a form of LTP (Jörntell and Ekerot, 2002; Ekerot and Jörntell, 2003). The underlying mechanisms were not evaluated, including whether this receptive field expansion is generated pre- or postsynaptically. Interestingly, the 4 Hz LTP induction protocol had no effect on PC receptive fields (Jörntell and Ekerot, 2002; Ekerot and Jörntell, 2003).

The goal of the present study was to determine if high frequency PF stimulation evokes LTP *in vivo* and, if so, to characterize the site of the plasticity and the signaling pathways involved. Also of interest, was the role of GABAergic neurotransmission in evoking LTP *in vivo*, as most previous studies blocked GABA_A receptors to generate PF–PC LTP and LTD. Using

flavoprotein optical imaging combined with electrophysiological recordings, this study documents that high frequency PF stimulation evokes a robust LTP in the mouse cerebellar cortex.

Experimental Procedures

Animal preparation and optical imaging

Male and FVB mice, ages 5–8 months (Charles River Laboratories, Wilmington, MA, USA) were used. All animal procedures were approved by and conducted in conformity with the Institutional Animal Care and Use Committee of the University of Minnesota and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize the number of animals and their suffering.

Experimental details on the animal preparation and optical imaging techniques are briefly described as the details have been provided in previous publications (Reinert et al., 2004; Gao et al., 2006). The mice were anesthetized by induction with acepromazine (2.0 mg/kg i.m.), followed by urethane (1.5 mg/kg i.p.). The electrocardiogram was monitored to assess the depth of anesthesia, supplementing anesthetics as needed. The mice were placed in a stereotaxic frame, mechanically ventilated and body temperature feedback-regulated. After the craniotomy a watertight chamber of dental acrylic was created around the exposed cerebellar cortex that included crus I and II. The chamber was filled and periodically rinsed with a gassed Ringer's solution. In the anesthetized mice drugs in normal Ringer's solution were applied to the cerebellar surface, including the AMPA receptor blocker DNQX (6,7-dinitroquinoxaline-2,3-dione disodium salt), the mGluR₁ antagonist LY367385 and the GABA_A receptor blocker SR95531. All drugs were purchased from Tocris (Ellisville, MO, USA).

Using a modified Nikon (Nikon Instruments, Inc., Melville, NY, USA) epifluorescence microscope fitted with a 4× objective, images were acquired with a Quantix cooled charge-coupled device camera with 12 bit digitization (Roper Scientific, Tucson, AZ, USA) at a final resolution of 256×256 pixels (~10×10 μm per pixel). A 100 W mercury-xenon lamp (Hamamatsu Photonics, Shizouka, Japan) with a d.c. controlled power supply (Opti Quip, Highland Mills, NY, USA) was used as the light source. Imaging flavoprotein autofluorescence used a band pass excitation filter (455±35 nm), an extended reflectance dichroic mirror (500 nm), and a >515 nm long pass emission filter (Reinert et al., 2004).

Stimulation of the PFs was delivered by a parylene-coated microelectrode to the surface of the cerebellar cortex. The first 35 min was used to establish the baseline response to the PF test stimulation (5 min intervals). Following this baseline period, the PF conditioning stimulation was applied ($t=0$ min). The “test” stimulation consisted of a train of 10 pulses at 100 Hz (175 μA, 150 μs duration). The “conditioning” stimulation followed the protocol of Jorntell and Ekerot (2002) and consisted of 15 pulses (175 μA, 150 μs duration) at 100 Hz every 3 s for 5 min. To evaluate the effect of the conditioning stimulation, the PF test stimulation was applied at 5 min intervals for 120 min. Variations on this basic design included the bath application of glutamate and GABAergic receptor antagonists after the baseline period and during the PF conditioning stimulation and control experiments with only PF test stimulation.

As detailed in the previous publications (Chen et al., 2005; Dunbar et al., 2004), for each PF test stimulation an image series consisting of 425 sequential frames was acquired (with an exposure time of 200 ms for each frame). The first 20 frames (control frames) provide a measure of the background fluorescence. The first step in the analysis was to generate a series of “difference” images by subtracting the average of the 20 controls from each control and experimental frame. These difference images were then divided by the control average on a

pixel-by-pixel basis, in which the intensity value of each pixel reflects the change in fluorescence intensity relative to the average of the control frames. This was converted to a percentage and is referred to as the $\Delta F/F$. To display the optical responses the images were statistically thresholded to highlight the activated pixels (pixels above/ below the mean ± 2.5 SD of the pixels in the difference images from the 20 control frames). These pixels were then displayed on an anatomical image of the background fluorescence of the folia (Gao et al., 2003).

To quantify the response to PF test stimulation, a region of interest (ROI) defined by the evoked beam was visually determined (Fig. 1B). The same ROI was used throughout an experiment to quantify any changes in the fluorescence response to the PF test stimulation. The response to the PF test stimulation consists of an initial period of increase in fluorescence followed by a longer duration decrease (Reinert et al., 2004,2007). The former is tightly coupled to the strength of the stimulation and is due to the oxidation of mitochondria flavoprotein in neurons activated by glutamate (Reinert et al., 2004,2007; Shibuki et al., 2003; Brennan et al., 2006). Therefore, the analysis was restricted to the light phase, averaging five frames centered on the peak amplitude of the light phase. The average $\Delta F/F$ for the frames throughout the ROI was determined.

The statistical analyses focused on comparing the responses in the baseline period with the responses following the conditioning stimulation. The latter were divided into early (0–60 min) and late (65–120 min) phases. The flavoprotein responses ($\Delta F/F$) at each 5 min interval were normalized to the average response during the baseline and defined as the “optical response.” Using an ANOVA (within-subject design with repeated measures), we tested for significant differences between the baseline period and the early and late phases ($\alpha=0.05$). In some instances a between-subjects design with repeated measures ANOVA was used to compare the optical responses between mice from different experiments.

Field potential recordings

Field potential recordings of the responses to PF stimuli in the molecular layer provided an electrophysiological assessment of any long-term changes and the effectiveness of the pharmacological agents. Field potentials were recorded using glass microelectrodes (2 M NaCl, 2–5 M Ω), digitized at 25 kHz and averaged (responses to 16 single PF stimuli at 1 Hz). The P_1/N_1 component was used as a measure of the presynaptic responses and the N_2 component as a measure of the postsynaptic response (Eccles et al., 1967; Reinert et al., 2004; Gao et al., 2003). Due to the long duration of the experiments, the N_2 was normalized to the P_1/N_1 to account for any variability in the PF volley. Normalization was also required because P_1/N_1 showed a gradual decline over the course of recordings (see Fig. 2).

PPF is a very short-term enhancement of synaptic efficacy that is usually attributed to residual presynaptic Ca^{2+} resulting in additional transmitter release (Salin et al., 1996; Lev-Ram et al., 2002). We tested PPF before and after the conditioning stimulation as a measure of synaptic transmitter release. Using the field potential recordings, two single pulses at a 50 ms interval were delivered to the molecular layer. The ratio of the N_2 amplitude evoked by the second pulse relative to the first pulse was determined before and after the conditioning stimulation. The statistical analysis of the field potential components and the PPF used are similar to that outlined above for the optical responses. All plots of field potentials, PPF or $\Delta F/F$ are mean $\pm$ standard deviation (SD).

Results

The main observation is that high frequency, burst conditioning stimulation induces an LTP-like response to the PF test stimulation. Previous studies using flavoprotein imaging have

shown that PF stimulation evokes a beam-like increase in fluorescence (Fig. 1A baseline) and that the vast majority of this signal arises from the postsynaptic targets of the PFs, including the PCs (Reinert et al., 2004; Gao et al., 2006). The responses to the PF test stimulations at 5 min intervals in the baseline period provide an estimate of the stability of the flavoprotein autofluorescence signal (Fig. 1B, C). Following the PF conditioning stimulation, the increase in the optical response is clearly evident at 25 min and persisted for at least 120 min (Fig. 1A, B). The optical images at the four selected time points (30, 60, 90 and 120 min) show the evoked beam increased (Fig. 1A). For this example experiment, the amplitudes of the response following the conditioning stimulation were $133.8\% \pm 20.5\%$ (early phase) and $136.2\% \pm 7.1\%$ (late phase) above the baseline period.

The population results from seven animals are consistent with the example data (Fig. 1C). The conditioning stimulation evoked a long-term increase in the response to PF test stimulation that lasted at least 120 min. The amplitude of the response was $130.8\% \pm 14.7\%$ above the baseline response for the early phase ($F(1,6)=245.1$, $P<0.0001$) and $129.4\% \pm 7.9\%$ for the late phase ($F(1,6)=160.8$, $P<0.0001$). The population data showed a gradual increase in the 20 min following the conditioning stimulation. Therefore, the conditioning stimulation evokes a significant, long-term increase in response to PF test stimulation as assayed by flavoprotein imaging.

Over the 120 min period following the conditioning stimulation, there is a gradual decrease in the optical response. To determine the source of this decrease, a control experiment was done in which PF test stimulations were performed over the same time period without the conditioning stimulation (Fig 1C, blue line). This control experiment revealed a gradual decrease over the duration of the experiment. The response amplitude was not significantly different during the early phase ($95.8\% \pm 9.4\%$, $F(1,9)=0.7$, $P=0.45$). However, the amplitude of the responses in the late phase decreased significantly compared to the baseline ($78.9\% \pm 10.0\%$, $F(1,3)=24.2$, $P=0.016$). This gradual decrease in response amplitude is not unexpected given the long duration of each experiment (~ 8 h) and the difficulty of maintaining completely constant conditions in the cerebellar cortex over that time period. The LTP induced by the high frequency conditioning stimulation is maintained in spite of this reduction in the response to PF stimulation over time ($F(1,9)=129.6$, $P<0.0001$ for the comparison of late phase with and without conditioning, Fig. 1C).

The degree of potentiation depends on the duration (i.e. number of high frequency bursts) in the conditioning stimulation. Conditioning stimulation of 2.5 min duration (50 bursts) resulted in a potentiation of $120.8\% \pm 7.4\%$ in the early phase ($F(1,3)=1613$, $P<0.0001$). However, the potentiation did not persist into the late phase ($106.1\% \pm 9.1\%$, $F(1,3)=4.0$, $P=0.14$). Conditioning stimulation of 1.0 min duration (20 bursts) did not produce a significant potentiation.

In another group of mice ($n=4$), field potential recordings were combined with the optical imaging to assess the electrophysiology correlates of the LTP. In these experiments, the optical response evoked by the PF test stimulation is used to guide the placement of the recording electrode to the center of the optical beam (Fig. 2A). The amplitude of the normalized N_2 component (Fig. 2B), a measure of the postsynaptic response, increased following the conditioning burst stimulation (Fig 2D, E) and the increase was maintained for the entire 120 min period (early phase: $123.9\% \pm 15.7\%$, $F(1,3)=9.2$, $P=0.056$ and late phase: $129.4\% \pm 13.4\%$, $F(1,3)=17.9$, $P=0.02$). Therefore, the long-term increase in the optical response is mirrored by a long-term increase in the postsynaptic response.

In contrast, P_1/N_1 was not potentiated but instead tended to decrease over the course of the experiment (Fig. 2D, red line). The change was not significant in the early phase ($93.9\% \pm 8.2\%$

of baseline, $F(1,3)=5.40$, $P=0.10$) but did reach significance in the late phase ($84.3\pm7.3\%$ of baseline, $F(1,3)=24.7$, $P=0.016$). This decrease in the PF volley is consistent with the decrease in the optical response over time without the conditioning stimulation as shown in Fig. 1C (blue line). However, the magnitude of the decrease and the lack of a significant decrease in the early period demonstrate that the larger and consistent significant increase in the normalized N_2 can be dissociated from the changes in the PF volley.

To assess whether the potentiation in the field potentials is due to changes in the presynaptic or postsynaptic elements, the degree of PPF at 50 ms intervals was also determined (Fig. 2C–E). During the baseline period, the average PPF was $144.2\pm17.2\%$. The PPF was unchanged following the conditioning stimulation, suggesting that the long-term changes in the PF–PC responses occur postsynaptically (early phase: $136.5\pm13.2\%$, $F(1,3)=2.8$, $P=0.19$ and late phase: $133.1\pm14.2\%$, $F(1,3)=3.6$, $P=0.16$).

To investigate the signaling pathway(s) involved in the LTP of the PF–PC response we examined the role of glutamate receptors. Because both AMPA and mGluR₁ receptors are major contributors to the postsynaptic responses generated by both PCs and cerebellar interneurons (Finch and Augustine, 1998; Konnerth et al., 1990; Knopfel and Grandes, 2002; Karakossian and Otis, 2004), the next series of experiments blocked these two classes of receptors during LTP induction. Both the AMPA receptor antagonist, DNQX (50 μ M), and the GluR₁ antagonist, LY367385 (200 μ M), were added to the bath 20 min before the onset of the conditioning stimulation and washed out immediately following. As shown for both the example (Fig. 3A) and the population data (Fig. 3B, $n=5$), the two antagonists resulted in a $84.4\pm4.1\%$ decrease in the optical response, similar to that previously observed *in vivo* (Dunbar et al., 2001). Upon washout of the drugs, the optical response recovered over 60 min to approximately the baseline levels. There was no evidence for potentiation of the optical response. The amplitude of the optical response in the late phase was $94.2\pm8.4\%$ and did not differ significantly from the baseline response ($F(1,4)=2.65$, $P=0.18$). We did not consider the early phase due to the obvious reduction in the responses as the antagonists were washed out. Therefore, blocking AMPA and mGluR₁ receptors prevents the induction of the LTP.

The individual contribution of these receptors was evaluated by selectivity, blocking AMPA and mGluR₁ receptors using the same protocol (Figs. 4 and 5). The application of DNQX (50 μ M) decreased the amplitude of the optical response to the PF test stimulation by $50.3\pm3.5\%$. This is consistent with previous results showing that 50%–60% of the optical response is due to activation of AMPA receptors and another 20%–25% (see Fig. 5) is due to activation of mGluR₁ receptors (Dunbar et al., 2001; Reinert et al., 2004). Although unresolved, the remaining 10%–15% is possibly presynaptic in origin.

Upon washout of the drug, the optical response recovered over 30 min to approximately the baseline level. At 40 min a LTP of the response was evident and in the late phase was $130.4\pm17.1\%$ of the baseline response ($F(1,3)=12.2$, $P=0.04$). The increase in this phase was not significantly different ($F(1,9)=2.7$, $P=0.13$) from the potentiation obtained with normal Ringer's (Fig. 4B, red vs. black lines).

An additional control experiment in four animals tested whether the potentiation was due to a rebound on removal of the DNQX after the conditioning stimulation (Fig. 4C). Following the same protocol, the DNQX was applied without the conditioning stimulation. Upon removal of the DNQX, the amplitude of the optical response returned to that of the baseline period over 40 min (Fig. 4C, red line). The response amplitude in the late phase was $85\pm14.5\%$. The amplitude and time course of the optical response are essentially identical to the results obtained in normal Ringer's without conditioning stimulation ($F(1,6)=1.0$, $P=0.36$, Fig. 4C, blue line). As shown previously (Reinert et al., 2004; Chen et al., 2001), DNQX completely blocked the

postsynaptic (N_2) component of the evoked field potential (at 1 Hz) and did not affect the PF volley (P_1/N_1) (Fig. 4C). Therefore, AMPA receptors appear to play little role in the LTP evoked by high frequency burst stimulation.

Blocking mGluR₁s (LY367385, 200 μ M) during the conditioning stimulation prevented the induction of LTP (Fig. 5). LY367385 reduced the optical response $22.2\% \pm 4.3\%$ from the baseline period and was similar to the decrease observed previously *in vivo* (Dunbar et al., 2001). Following the conditioning stimulation and washout of the LY367385, the response amplitude returned in 20 min to that observed in the baseline period without any evidence of potentiation. After conditioning stimulation, the response amplitude was $105.8\% \pm 11.8\%$ in the early phase ($F(1,3)=6.1$, $P=0.09$) and $95.8\% \pm 14\%$ in the late phase ($F(1,3)=0.4$, $P=0.57$), and neither was significantly different from the baseline response. An additional control experiment ($n=4$ animals) applied LY367385 without the conditioning stimulation and resulted in complete recovery of the optical response after removal of the antagonist for both the early phase ($97.0\% \pm 8.0\%$, $F(1,3)=1.1$, $P=0.36$) and the late phase ($93.7\% \pm 7.2\%$, $F(1,3)=6.0$, $P=0.09$) (Fig. 5C). This experiment rules out the possibility that the LY367385 masked the expression of LTP by simply suppressing the optical responses. Therefore, the induction of LTP requires activation of mGluR₁ receptors.

The last experiment examined the role of GABAergic inhibitory transmission in the induction and maintenance of the LTP. The first step was to establish a dose-response curve for the GABA_A receptor antagonist, SR95531, using 1, 2.5, 5.0 and 10.0 μ M. While each concentration resulted in increases in the optical response to PF test stimulation (Fig. 6A), 10 μ M SR95531 produced widespread changes in the fluorescence not confined to the evoked beam as well as movements in the animals (presumably due to brain stem effects). Therefore, the 5 μ M concentration was used. When SR95531 was added to the bath before the onset of the PF conditioning stimulation the beam-like response to the PF test stimulation increased ($142.1\% \pm 13.3\%$). Following removal of the SR95531 at the end of the conditioning stimulation, the response amplitude remained elevated in the early phase ($118\% \pm 10.4\%$, $F(1,4)=117.2$, $P=0.0004$) but not in the late phase ($94.2\% \pm 13.4\%$, $F(1,4)=1.8$, $P=0.24$). To assess whether there was any potentiation, an additional control experiment was performed using the PF test stimulation with the application of the SR95531 but without the conditioning stimulation (Fig. 6B, blue line). After removal of SR95531, the response amplitude returned to the baseline level in the early phase ($91.3\% \pm 13.7\%$, $F(1,4)=3.04$, $P=0.17$) and decreased to below the baseline level in the late phase ($74.1\% \pm 16.4\%$, $F(1,4)=11.8$, $P=0.027$). A comparison of the data with (red) and without (blue) the conditioning stimulation reveals a significant potentiation in the early ($F(1,8)=11.6$, $P=0.009$) but not the late phase ($F(1,8)=4.9$, $P=0.06$). Therefore, blocking GABA_A receptors alters but does not prevent the induction of LTP. Specifically, the duration of the LTP decreased.

Finally, as for the application of other drugs without the conditioning stimulation (Figs. 3 and 4), there was a gradual decrease in the amplitude of the response over the 120 min period after removing the SR95531 (Fig. 6B, blue line). In fact, the amplitude for these control responses to application and washout of the SR95531 did not differ from the control curves (Fig. 1C, blue line) obtained in Ringer's and without conditioning stimulation (early phase; $F(1,7)=0.40$, $P=0.55$ and late phase; $F(1,7)=0.28$, $P=0.62$). This demonstrates that the GABA_A antagonist did not have a persistent effect on the response to the PF test stimulation after washout and therefore, did not interfere with the assessment of the effects of the conditioning stimulation.

Discussion

The main finding of this study is that high frequency burst stimulation of the PFs produces a robust LTP of the response to PF stimulation in the cerebellar cortex *in vivo*. The potentiation

occurs along the entire length of the stimulated PFs and persists for at least 2 h. Field potential recordings confirm that the postsynaptic response was potentiated. This form of LTP appears to be fundamentally different from the two forms of PF–PC LTP described *in vitro*, either the presynaptic form evoked by 4–8 Hz stimulation (Sakurai, 1987; Kano et al., 1992; Linden, 1998) or the postsynaptic form evoked by 1 Hz stimulation (Lev-Ram et al., 2002; Belmeguenai and Hansel, 2005). Instead, the LTP described here is most likely to underlie the potentiation of PC receptive fields described by Jorntell and Ekerot that used the same conditioning paradigm (Jorntell and Ekerot, 2002; Ekerot and Jorntell, 2003). Furthermore, this implies that the LTP evoked by high frequency burst stimulation is operative in the intact cerebellar cortex.

The LTP evoked by high frequency stimulation is postsynaptic in origin. The assessment of PPF showed no change and glutamate receptor antagonists prevented the induction of the LTP. Several preliminary experiments removing extracellular Ca^{2+} also blocked the potentiation (data not shown). The mGluR₁ receptors were critical to the induction and AMPA receptors were not. As mGluR₁ receptors are located predominantly on PCs and molecular layer interneurons (Grandes et al., 1994; Mateos et al., 2000), this provides the strongest evidence that the LTP induced by high frequency stimulation of the PFs is primarily postsynaptic. The dependence on mGluR₁ receptors demonstrates another fundamental difference from PF–PC LTP induced by 1 Hz in the cerebellar slice, as the latter is not dependent on mGluR₁ receptors (Belmeguenai et al., 2008).

The PPF results also suggest that the LTP evoked by high frequency burst PF stimulation is not presynaptic in origin. However, it is not possible to completely rule out a presynaptic contribution. A presynaptic locus for the LTP would produce a reduction in PPF (Salin et al., 1996; Jacoby et al., 2001). Although there was no statistical change in the PPF after the conditioning stimulation, there was a consistent, small decrease (~92% of baseline) in the PPF (Fig. 2D). This is similar to the findings in the slice preparation in which PF burst stimulation results in a small (~82% of baseline), non-significant decrease in PPF based on recordings from molecular layer interneurons (Smith and Otis, 2005). Further examination is needed to sort out if there is a presynaptic component to the LTP observed *in vivo*. However, there is no evidence that the PF volley increased, instead the P₁/N₁ amplitude gradually decreased throughout the course of the experiment. This is not unexpected given the long duration of these recordings. The decrease in the PF volley (~84% of baseline in the late phase) can account for much of the gradual decrease in the optical response in both experiments with and without the conditioning stimulation (~79% of the baseline in the late phase). Finally, the analysis of the PF volley did not provide any evidence that the conditioning stimulation resulted in the increased excitability or recruitment of additional PFs.

The induction by high frequency PF stimulation is consistent with both the properties of cerebellar granule cells and the mGluR₁ receptors on PCs and molecular layer interneurons. Granule cells respond with high frequency bursts of activity to mossy fiber inputs (Chadderton et al., 2004), suggesting that bursts of PF activity are inherently physiological. Furthermore, activation of ionic currents via mGluR₁ receptors on PCs or inhibitory interneurons requires high frequency PF activation (typically 50–100 Hz) (Batchelor and Garthwaite, 1997; Takechi et al., 1998; Finch and Augustine, 1998; Karakossian and Otis, 2004). Therefore, one can speculate that the granule cell–PF–PC circuit is specialized for high frequency activation and that synaptic plasticity in this circuit utilizes these specializations.

The requirement for activation of mGluR₁ and not AMPA receptors suggests that this form of LTP involves the same signaling pathway as PF–PC LTD (Conquet et al., 1994; Hartell, 1994). However, a fundamental difference is that the LTP described in this report relies on high frequency activation of the PFs and PF–PC LTD relies on low frequency conjunction of PF and CF inputs (Ito and Kano, 1982; Sakurai, 1987). This difference in induction frequencies

implies that these two forms of synaptic plasticity are generated by the cerebellar cortical circuitry under fundamentally different conditions.

Another important issue is the role of GABA_A receptors and the molecular layer inhibitory interneurons. As noted, most previous studies of PF–PC LTP or LTD invariably used GABA_A antagonists (however, see; Chen and Thompson, 1995; Schreurs and Alkon, 1993). In fact, blocking GABA_A receptors is generally considered a requirement for the generation of both forms of plasticity *in vitro*. The LTP observed in this report did not require the use of GABA receptors blockers. This avoids the criticism that use of GABA receptor antagonists is not physiological.

However, the role of GABA_A receptors and the inhibitory neurons needs additional clarification. The reduction in the duration of the LTP when SR95531 was applied during conditioning may reflect the overall increase in excitability in the cerebellar cortical circuitry. As shown, the amplitude of the response to PF stimulation increased to 140% of baseline, a large change in the excitability. Another possibility is that the high frequency conditioning stimulation produces LTD at PF–interneuron synapses, which in turn would increase PC excitability by disinhibition. We cannot completely rule out this possibility, as mGluR₁ receptors are found on both PCs and molecular layer interneurons (Grandes et al., 1994; Mateos et al., 2000). However, blocking GABA_A receptors reduced but did not prevent the LTP, implying that plasticity at the PF–molecular layer interneurons cannot be the only mechanism for the LTP of the response to PF stimulation.

The bi-directional plasticity of the PF–PC synapse has been hypothesized to be governed by a calcium threshold rule (Coesmans et al., 2004; Jorntell and Hansel, 2006). It has been proposed that PF–PC LTD requires higher calcium levels than PF–PC LTP (Coesmans et al., 2004), and this rule is the inverse of what has been typically observed at other glutamatergic synapses (Bear et al., 1987; Bienenstock et al., 1982; Lisman, 1989). The inverse rule helps explain the observations that LTD requires the co-activation of climbing fibers and PFs with the concomitant increase in intracellular Ca²⁺ (Eilers et al., 1995; Sakurai, 1987; Wang et al., 2000) and that the postsynaptic form of LTP is evoked by lower frequency stimulation (Lev-Ram et al., 2002; Belmeguenai and Hansel, 2005). The present finding of LTP by high frequency PF stimulation will undoubtedly result in large increases in intracellular Ca²⁺ due to activation of mGluR₁ receptors (Finch and Augustine, 1998; Takechi et al., 1998; Wang et al., 2000). Furthermore, the high frequency conditioning stimulation is similar to the induction paradigms observed at other glutamatergic synapses (Bear et al., 1987). Conversely, the intact GABAergic inhibitory activity is likely to reduce the Ca²⁺ influx. Whether the Ca²⁺ changes for the form of LTP reported in this study are consistent with the inverse calcium rule found for PF–PC synapses *in vitro* remains to be determined (Coesmans et al., 2004).

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References

- Batchelor AM, Garthwaite J. Frequency detection and temporally dispersed synaptic signal association through a metabotropic glutamate receptor pathway. *Nature* 1997;385:74–77. [PubMed: 8985249]
- Bear MF, Cooper LN, Ebner FF. A physiological basis for a theory of synapse modification. *Science* 1987;237:42–48. [PubMed: 3037696]

- Belmeguenai A, Botta P, Weber JT, Carta M, De Ruiter M, De Zeeuw CI, Valenzuela CF, Hansel C. Alcohol impairs long-term depression at the cerebellar parallel fiber-Purkinje cell synapse. *J Neurophysiol* 2008;100:3167–3174. [PubMed: 18922952]
- Belmeguenai A, Hansel C. A role for protein phosphatases 1, 2A, and 2B in cerebellar long-term potentiation. *J Neurosci* 2005;25:10768–10772. [PubMed: 16291950]
- Bienenstock EL, Cooper LN, Munro PW. Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. *J Neurosci* 1982;2:32–48. [PubMed: 7054394]
- Brennan AM, Connor JA, Shuttleworth CW. NAD(P)H fluorescence transients after synaptic activity in brain slices: predominant role of mitochondrial function. *J Cereb Blood Flow Metab* 2006;26:1389–1406. [PubMed: 16538234]
- Chadderton P, Margrie TW, Hausser M. Integration of quanta in cerebellar granule cells during sensory processing. *Nature* 2004;428:856–860. [PubMed: 15103377]
- Chen C, Thompson RF. Temporal specificity of long-term depression in parallel fiber-Purkinje synapses in rat cerebellar slice. *Learn Mem* 1995;2:185–198. [PubMed: 10467575]
- Chen G, Dunbar RL, Gao W, Ebner TJ. Role of calcium, glutamate neurotransmission, and nitric oxide in spreading acidification and depression in the cerebellar cortex. *J Neurosci* 2001;21:9877–9887. [PubMed: 11739595]
- Chen G, Gao W, Reinert KC, Popa LS, Hendrix CM, Ross ME, Ebner TJ. Involvement of Kv1 potassium channels in spreading acidification and depression in the cerebellar cortex. *J Neurophysiol* 2005;94:1287–1298. [PubMed: 15843481]
- Coesmans M, Weber JT, De Zeeuw CI, Hansel C. Bidirectional parallel fiber plasticity in the cerebellum under climbing fiber control. *Neuron* 2004;44:691–700. [PubMed: 15541316]
- Conquet F, Bashir ZI, Davies CH, Daniel H, Ferraguti F, Bordi F, Franz-Bacon K, Reggiani A, Matarese V, Conde F, Collingridge GL, Crepel F. Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1. *Nature* 1994;372:237–243. [PubMed: 7969468]
- Crepel F, Krupa M. Activation of protein kinase C induces a long-term depression of glutamate sensitivity of cerebellar Purkinje cells. An *in vitro* study. *Brain Res* 1988;458:397–401. [PubMed: 2905201]
- De Zeeuw CI, Yeo CH. Time and tide in cerebellar memory formation. *Curr Opin Neurobiol* 2005;15:667–674. [PubMed: 16271462]
- Dunbar RL, Chen G, Gao W, Reinert KC, Feddersen R, Ebner TJ. Imaging parallel fiber and climbing fiber responses and their short-term interactions in the mouse cerebellar cortex *in vivo*. *Neuroscience* 2004;126:213–227. [PubMed: 15145087]
- Dunbar RL, Gao WC, Chen G, Ebner TJ. Characterization of short-term parallel fiber and climbing fiber interaction in the mouse cerebellar cortex using optical imaging *in vivo*. *Soc Neurosci Abstr* 2001;27:828–829.
- Eccles, JC.; Ito, M.; Szentagothai, J. The cerebellum as a neuronal machine. Berlin: Springer-Verlag; 1967.
- Eilers J, Augustine GJ, Konnerth A. Subthreshold synaptic Ca^{2+} signalling in fine dendrites and spines of cerebellar Purkinje neurons. *Nature* 1995;373:155–158. [PubMed: 7816097]
- Ekerot CF, Jorntell H. Parallel fiber receptive fields: a key to understanding cerebellar operation and learning. *Cerebellum* 2003;2:101–109. [PubMed: 12880177]
- Ekerot CF, Kano M. Long-term depression of parallel fibre synapses following stimulation of climbing fibers. *Brain Res* 1985;342:357–360. [PubMed: 4041837]
- Finch EA, Augustine GJ. Local calcium signaling by inositol-1,4,5-trisphosphate in Purkinje cell dendrites. *Nature* 1998;396:753–756. [PubMed: 9874372]
- Gao W, Chen G, Reinert KC, Ebner TJ. Cerebellar cortical molecular layer inhibition is organized in parasagittal zones. *J Neurosci* 2006;26:8377–8387. [PubMed: 16899733]
- Gao W, Dunbar RL, Chen G, Reinert KC, Oberdick J, Ebner TJ. Optical imaging of long-term depression in the mouse cerebellar cortex *in vivo*. *J Neurosci* 2003;23:1859–1866. [PubMed: 12629190]
- Grandes P, Mateos JM, Ruegg D, Kuhn R, Knopfel T. Differential cellular localization of three splice variants of the mGluR1 metabotropic glutamate receptor in rat cerebellum. *Neuroreport* 1994;5:2249–2252. [PubMed: 7881038]

- Hansel C, de Jeu M, Belmeguenai A, Houtman SH, Buitendijk GH, Andreev D, De Zeeuw CI, Elgersma Y. α CaMKII Is essential for cerebellar LTD and motor learning. *Neuron* 2006;51:835–843. [PubMed: 16982427]
- Hansel C, Linden DJ, D'Angelo E. Beyond parallel fiber LTD: the diversity of synaptic and nonsynaptic plasticity in the cerebellum. *Nat Neurosci* 2001;4:467–475. [PubMed: 11319554]
- Hartell NA. Induction of cerebellar long-term depression requires activation of glutamate metabotropic receptors. *Neuroreport* 1994;5:913–916. [PubMed: 8061295]
- Hirano T. Differential pre- and postsynaptic mechanisms for synaptic potentiation and depression between a granule cell and a Purkinje cell in rat cerebellar culture. *Synapse* 1991;7:321–323. [PubMed: 1645891]
- Ichise T, Kano M, Hashimoto K, Yanagihara D, Nakao K, Shigemoto R, Katsuki M, Aiba A. mGluR1 in cerebellar Purkinje cells essential for long-term depression, synapse elimination, and motor coordination. *Science* 2000;288:1832–1835. [PubMed: 10846166]
- Ito M. Cerebellar long-term depression: characterization, signal transduction, and functional roles. *Physiol Rev* 2001;81:1143–1195. [PubMed: 11427694]
- Ito M. Cerebellar circuitry as a neuronal machine. *Prog Neurobiol* 2006;78:272–303. [PubMed: 16759785]
- Ito M, Kano M. Long-lasting depression of parallel fiber-Purkinje cell transmission induced by conjunctive stimulation of parallel fibers and climbing fibers in the cerebellar cortex. *Neurosci Lett* 1982;33:253–258. [PubMed: 6298664]
- Jacoby S, Sims RE, Hartell NA. Nitric oxide is required for the induction and heterosynaptic spread of long-term potentiation in rat cerebellar slices. *J Physiol* 2001;535:825–839. [PubMed: 11559778]
- Jörntell H, Ekerot CF. Reciprocal bidirectional plasticity of parallel fiber receptive fields in cerebellar Purkinje cells and their afferent interneurons. *Neuron* 2002;34:797–806. [PubMed: 12062025]
- Jörntell H, Hansel C. Synaptic memories upside down: bidirectional plasticity at cerebellar parallel fiber-Purkinje cell synapses. *Neuron* 2006;52:227–238. [PubMed: 17046686]
- Kano M, Kato M. Quisqualate receptors are specifically involved in cerebellar synaptic plasticity. *Nature* 1987;325:276–279. [PubMed: 2880297]
- Kano M, Rexhausen U, Dreessen J, Konnerth A. Synaptic excitation produces a long-lasting rebound potentiation of inhibitory synaptic signals in cerebellar Purkinje cells. *Nature* 1992;356:601–604. [PubMed: 1313949]
- Karakossian MH, Otis TS. Excitation of cerebellar interneurons by group I metabotropic glutamate receptors. *J Neurophysiol* 2004;92:1558–1565. [PubMed: 15152021]
- Knopfel T, Grandes P. Metabotropic glutamate receptors in the cerebellum with a focus on their function in Purkinje cells. *Cerebellum* 2002;1:19–26. [PubMed: 12879970]
- Konnerth A, Llano I, Armstrong CM. Synaptic currents in cerebellar Purkinje cells. *Proc Natl Acad Sci U S A* 1990;87:2662–2665. [PubMed: 1969639]
- Lev-Ram V, Jiang T, Wood J, Lawrence DS, Tsien RY. Synergies and coincidence requirements between NO, cGMP, and Ca^{2+} in the induction of cerebellar long-term depression. *Neuron* 1997;18:1025–1038. [PubMed: 9208868]
- Lev-Ram V, Wong ST, Storm DR, Tsien RY. A new form of cerebellar long-term potentiation is postsynaptic and depends on nitric oxide but not cAMP. *Proc Natl Acad Sci U S A* 2002;99:8389–8393. [PubMed: 12048250]
- Linden DJ. Synaptically evoked glutamate transport currents may be used to detect the expression of long-term potentiation in cerebellar culture. *J Neurophysiol* 1998;79:3151–3156. [PubMed: 9636115]
- Linden DJ, Ahn S. Activation of presynaptic cAMP-dependent protein kinase is required for induction of cerebellar long-term potentiation. *J Neurosci* 1999;19:10221–10227. [PubMed: 10575019]
- Linden DJ, Connor JA. Participation of postsynaptic PKC in cerebellar long-term depression in culture. *Science* 1991;254:1656–1659. [PubMed: 1721243]
- Lisman J. A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. *Proc Natl Acad Sci U S A* 1989;86:9574–9578. [PubMed: 2556718]

- Lonart G, Schoch S, Kaeser PS, Larkin CJ, Sudhof TC, Linden DJ. Phosphorylation of RIM1 α by PKA triggers presynaptic long-term potentiation at cerebellar parallel fiber synapses. *Cell* 2003;115:49–60. [PubMed: 14532002]
- Mateos JM, Benitez R, Elezgarai I, Azkue JJ, Lazaro E, Osorio A, Bilbao A, Donate F, Sarria R, Conquet F, Ferraguti F, Kuhn R, Knopfel T, Grandes P. Immunolocalization of the mGluR1b splice variant of the metabotropic glutamate receptor 1 at parallel fiber-Purkinje cell synapses in the rat cerebellar cortex. *J Neurochem* 2000;74:1301–1309. [PubMed: 10693964]
- Reinert KC, Dunbar RL, Gao W, Chen G, Ebner TJ. Flavoprotein autofluorescence imaging of neuronal activation in the cerebellar cortex *in vivo*. *J Neurophysiol* 2004;92:199–211. [PubMed: 14985415]
- Reinert KC, Gao W, Chen G, Ebner TJ. Flavoprotein autofluorescence imaging in the cerebellar cortex *in vivo*. *J Neurosci Res* 2007;85:3221–3232. [PubMed: 17520745]
- Sakurai M. Synaptic modification of parallel fibre-Purkinje cell transmission in *in vitro* guinea-pig cerebellar slices. *J Physiol* 1987;394:463–480. [PubMed: 2832595]
- Salin PA, Malenka RC, Nicoll RA. Cyclic AMP mediates a presynaptic form of LTP at cerebellar parallel fiber synapses. *Neuron* 1996;16:797–803. [PubMed: 8607997]
- Schreurs BG, Alkon DL. Rabbit cerebellar slice analysis of long-term depression and its role in classical conditioning. *Brain Res* 1993;631:235–240. [PubMed: 8131052]
- Shibuki K, Hishida R, Murakami H, Kudoh M, Kawaguchi T, Watanabe M, Watanabe S, Kouuchi T, Tanaka R. Dynamic imaging of somatosensory cortical activity in the rat visualized by flavoprotein autofluorescence. *J Physiol* 2003;549:919–927. [PubMed: 12730344]
- Shibuki K, Okada D. Endogenous nitric oxide release required for long-term synaptic depression in the cerebellum. *Nature* 1991;349:326–328. [PubMed: 1702879]
- Smith SL, Otis TS. Pattern-dependent, simultaneous plasticity differentially transforms the input-output relationship of a feedforward circuit. *Proc Natl Acad Sci U S A* 2005;102:14901–14906. [PubMed: 16199519]
- Storm DR, Hansel C, Hacker B, Parent A, Linden DJ. Impaired cerebellar long-term potentiation in type I adenylyl cyclase mutant mice. *Neuron* 1998;20:1199–1210. [PubMed: 9655507]
- Takechi H, Eilers J, Konnerth A. A new class of synaptic response involving calcium release in dendritic spines. *Nature* 1998;396:757–760. [PubMed: 9874373]
- Wang SS, Denk W, Hausser M. Coincidence detection in single dendritic spines mediated by calcium release. *Nat Neurosci* 2000;3:1266–1273. [PubMed: 11100147]
- Wang YT, Linden DJ. Expression of cerebellar long-term depression requires postsynaptic clathrin-mediated endocytosis. *Neuron* 2000;25:635–647. [PubMed: 10774731]

Abbreviations

CF	climbing fiber
DNQX	6,7-dinitroquinoxaline-2,3-dione disodium salt
LTD	long-term depression
LTP	long-term potentiation
mGluR₁	metabotropic glutamate receptor subtype 1
PC	Purkinje cell
PF	

	parallel fiber
PKA	protein kinase A
PKC	protein kinase C
PPF	paired-pulse facilitation
ROI	region of interest

Fig. 1.

High frequency, burst PF stimulations evoke LTP of the response to test PF stimulation. (A) Sequential images of the pseudocolored threshold optical responses evoked by PF test stimulation (10 pulses of $150\ \mu\text{s}$, $175\ \mu\text{A}$ at 100 Hz) from an example experiment. The response to the test stimulation was obtained prior to and following the conditioning stimulation (10 pulses at $175\ \mu\text{A}$, $150\ \mu\text{s}$ at 100 Hz, every 3 s for 5 min, denoted by the gray vertical bar). Pseudocolored scale bar is shown at the bottom. The striped region in the center of the bar represents the pixels falling within the mean ± 2.5 SD statistical threshold and these pixels are not displayed. Time relative to the onset of the conditioning stimulation indicated above each image. (B) Amplitude of the optical responses to PF test stimulation from the same experiment in A. The optical responses were from the ROI shown in the inset, and normalized to the average responses during the baseline period. Black arrows denote the times at which the images in A were acquired. (C) Average optical responses (mean $\pm$ SD) in animals with ($n=7$, black) and without ($n=4$, blue) PF conditioning stimulation. For subsequent figures, the stimulation and recording parameters, protocols and labeling conventions are the same, unless noted otherwise.

Fig. 2.

PF conditioning stimulation potentiates the postsynaptic response with no change in PPF. (A) Experimental layout for the recording of both electrical and optical responses in crus II. Field potentials (FP Rec. Electrode) were recorded in the center of the evoked optical beam. (B) Extracellular field potential showing the presynaptic P₁/N₁/P₂ waveform and the postsynaptic N₂ component (PF stimulation parameters: 100 μ A, 100 μ s pulses at 1 Hz, average of 16 trials). (C) Example of PPF evoked at an interval of 50 ms using the same stimulation parameters as in B. Note the increase in the second postsynaptic response (N₂, 2nd) relative to the initial N₂ component (N₂, 1st). (D) Optical response ($\Delta F/F$, black), postsynaptic response (normalized N₂, green), PF volley (P₁/N₁, red), and PPF (orange) before and after the conditioning stimulation ($n=4$). (E) Average N₂ and PPF in the baseline and the early and late phases following conditioning stimulation.

Fig. 3.

Induction of LTP depends on the activation of glutamate receptors. (A) Example optical responses evoked by PF test stimulation at different times relative to the PF conditioning stimulation. DNQX (50 μ M) and LY367385 (200 μ M) were applied after the baseline period prior to the conditioning stimulation and then washed out after the conditioning stimulation. (B) Average optical responses to PF test stimulation with the application and removal of the glutamate receptor antagonists ($n=5$). Brown up-down arrows on the top show the timing of the application and removal of the antagonists.

Fig. 4.

Induction of LTP is not dependent on AMPA receptors. (A) Sequential images of the optical responses evoked by PF test stimulation. The AMPA receptor antagonist, DNQX (50 μ M), was applied prior to the conditioning stimulation and washed out after conditioning stimulation. (B) Average optical responses with the application and removal of DNQX ($n=4$, red), and optical responses without the DNQX application ($n=7$, black, same data shown in Fig. 1C). (C) Average optical responses with the application and removal of DNQX but without the PF conditioning stimulation ($n=4$, red). Also shown are the optical responses without DNQX and without the conditioning stimulation ($n=4$, blue, same data shown in Fig. 1C). Insets are examples of the field potential recordings before, during and after the application of DNQX. Calibration bars for the field potentials are included.

Fig. 5.

MGLuR₁s are required for LTP. (A) Sequential images of the optical responses evoked by PF test stimulation at different times relative to conditioning stimulation. The mGluR₁ antagonist, LY367385 (200 μ M), was applied prior to and during the conditioning stimulation. (B) Optical responses obtained with the application and removal of LY367385 ($n=4$, red). (C) Optical responses with application of LY367385 but without the conditioning stimulation ($n=4$, blue).

Fig. 6. Role of GABA_A receptors in LTP. (A) Dose-response curve for the GABA_A receptor antagonist SR95531. (B) Optical responses to PF test stimulation with SR95531 applied prior to, during and after the conditioning ($n=5$).