Neuron, Vol. 42, 973–982, June 24, 2004, Copyright ©2004 by Cell Press

Long-Term Synaptic Changes Induced in the Cerebellar Cortex by Fear Conditioning

Benedetto Sacchetti,[1] Bibiana Scelfo,[1] Filippo Tempia,[2] and Piergiorgio Strata[1,3,*]
[1]Rita Levi-Montalcini Center for Brain Repair
Department of Neuroscience
University of Turin
Corso Raffaello 30
I-10125 Turin
Italy
[2]Department of Internal Medicine
Section of Human Physiology
University of Perugia
Via del Giochetto
I-06126 Perugia
Italy
[3]Rehabilitation Hospital and Research Institute
Santa Lucia Foundation
Via Ardeatina 306
I-00179 Rome
Italy

Summary

To better understand learning mechanisms, one needs to study synaptic plasticity induced by behavioral training. Recently, it has been demonstrated that the cerebellum is involved in the consolidation of fear memory. Nevertheless, how the cerebellum contributes to emotional behavior is far from known. In cerebellar slices at 10 min and 24 hr following fear conditioning, we found a long-lasting potentiation of the synapse between parallel fibers and Purkinje cells in vermal lobules V-VI, but not in the climbing fiber synapses. The mechanism is postsynaptic, due to an increased AMPA response. In addition, in *hotfoot* mice with a primary deficiency of the parallel fiber to Purkinje cell synapse, cued (but not contextual) fear conditioning is affected. We propose that this synapse plays an important role in the learned fear and that its long-term potentiation may represent a contribution to the neural substrate of fear memory.

Introduction

Fear is a basic evolutionarily conserved emotion that is essential for survival because it triggers a set of defensive reactions aimed at counteracting the threatening events. The memory of learned fear can be assessed using a Pavlovian fear conditioning paradigm (Kim and Fanselow, 1992; Rogan et al., 1997; Fendt and Fanselow, 1999; Sacchetti et al., 1999; LeDoux, 2000; Walker and Davis, 2002; Sanders et al., 2003). Here, a neutral stimulus, usually a tone acting as a conditioned stimulus (CS), is repeatedly paired with an aversive foot shock, acting as an unconditioned stimulus (US). As a result of this pairing, the CS comes to elicit a set of defensive behavioral responses that include freezing, increased heart rate, and startle (Kim and Fanselow, 1992; Rogan et al., 1997; Fendt and Fanselow, 1999; Sacchetti et al., 1999; LeDoux, 2000; Walker and Davis, 2002; Sanders et al., 2003). Learned fear is commonly employed to study the neural circuits and the cellular mechanisms underlying associative memory processes related to emotional behavior.

A growing body of data suggests that the cerebellum is involved in fear behavior both in experimental animals and humans (Snider and Maiti, 1976; Heath et al., 1981; Supple et al., 1987, 1988; Supple and Leaton, 1990; Sebastiani et al., 1992; Supple and Kapp, 1993; Schmahmann and Sherman, 1998; Parvizi et al., 2001; Maschke et al., 2002; Sacchetti et al., 2002b). Stimulation of cerebellar vermis induces a complex behavioral pattern indicative of emotional arousal in animals (Snider and Maiti, 1976) and elicits psychotic symptoms in humans (Heath et al., 1981). Cerebellar pathologies have also been correlated with various emotional disorders such as depression and autism (Snider and Maiti, 1976; Heath et al., 1981; Schmahmann and Sherman, 1998; Parvizi et al., 2001). In addition, cerebellar dysfunction affects autonomic and behavioral conditioned fear responses (Supple et al., 1987, 1988; Supple and Leaton, 1990; Sebastiani et al., 1992; Supple and Kapp, 1993; Schmahmann and Sherman, 1998; Maschke et al., 2002; Sacchetti et al., 2002b). Nevertheless, how the cerebellum contributes to emotional spontaneous and learned behavior is not known. A recent report showed that the reversible inactivation of the cerebellar cortex abolishes the retention of fear conditioning (Sacchetti et al., 2002b). This effect was obtained by injecting tetrodotoxin after the acquisition session and performing the retention session when the tetrodotoxin reversible blockade was over. This procedure ensured that there was no interference with sensory or motor response so that any amnesic effect was due only to the disruption of the fear memory trace. Nevertheless, there are no data on synaptic changes inside the cerebellum after behavioral training. Long-term depression (LTD) and long-term potentiation (LTP) in the parallel fiber (PF) to Purkinje cell (PC) synapses were obtained by electrical stimulation in vitro in the cerebellar cortex, and they have been assumed to play a role in cerebellar motor learning (Marr, 1969; Albus, 1971; Ito, 1984, 2001; Kim and Thompson, 1997; Medina et al., 2000, 2002; Attwell et al., 2002; Koekkoek et al., 2003). While for the vestibulo-ocular reflex and the eye blink conditioning there is a satisfactory model of operation, nothing is known about fear conditioning. Therefore, the aim of the present work is to identify possible long-term synaptic plasticity in the cerebellar cortex associated with emotional memory.

Results

Fear Conditioning Elicits an LTP of PF-PC Synapses

Three groups, each of 12 rats, were studied. The three categories were (1) naive animals that received no train-

*Correspondence: piergiorgio.strata@unito.it

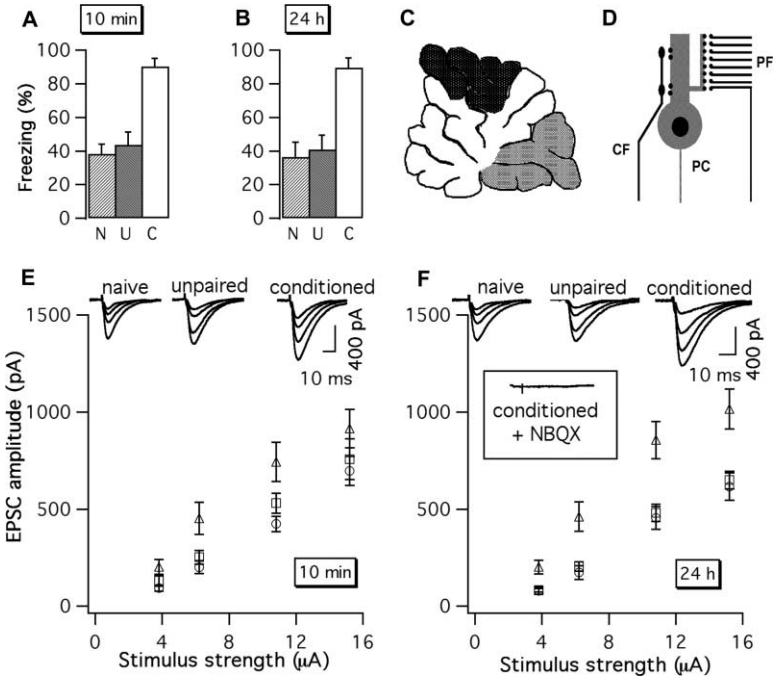

Figure 1. Fear Conditioning Induces a Long-Lasting Increase in the PF-PC Synaptic Strength

(A) Freezing response was measured as percentage of immobility during retention session performed 10 min after acquisition paradigm in naive (N, n = 12), unpaired (U, n = 12), and conditioned (C, n = 12) groups. Mean ± SEM freezing as percentage of immobility.

(B) Fear retention measured 24 hr after acquisition session.

(C) Lobules V and VI (dark gray) and lobules IX and X (light gray) of cerebellar vermis.

(D) PC with the two excitatory inputs, PF and CF.

(E) Input-output data for PF-PC EPSCs from naive (circle, n = 17), unpaired (square, n = 16), and conditioned (triangle, n = 22) groups in slices prepared 10 min after acquisition session.

(F) Input-output measured 24 hr after acquisition session. Reported values are mean ± SEM.

ing, (2) conditioned animals that received a pairing of a tone with an electric shock, and (3) unpaired control animals that received tone and electrical stimulation in an unpaired pattern. Fear retention was evaluated by measuring freezing, the expression of fear behavior (Kim and Fanselow, 1992; Rogan et al., 1997; Fendt and Fanselow, 1999; Sacchetti et al., 1999; LeDoux, 2000), during the administration of the CS alone at 10 min and 24 hr after the acquisition session. At both time intervals, in the fear-conditioned rats, the periods of immobility, expressed as percentage of the total time during the retrieval phase, were increased relative to the unpaired and naive groups ($p < 0.005$ in all instances; one-way ANOVA test and Newman Keuls posttest) [10 min: naive, 38.18 ± 5.99; unpaired, 43.59 ± 7.81; conditioned, 90.378 ± 4.78; $F(2,35) = 20.32$; Figure 1A; 24 hr: naive, 36.32 ± 8.93; unpaired, 40.86 ± 8.55; conditioned, 89.62 ± 5.76; $F(2,35) = 13.23$; Figure 1B]. There was no significant difference between naive and unpaired groups ($p > 0.05$). Therefore, the increased freezing response, observed in fear-conditioned rats, was specifically due to the associative processes underlying CS-US association (Rogan et al., 1997; McKernan and Shinnick-Gallagher, 1997). From other naive, unpaired, and conditioned groups of rats, we prepared parasagittal slices near the midline from the dorsal vermis of the cerebellum at 10 min and 24 hr (Figure 1C). The procedure was directed at revealing possible long-term synaptic changes related to memory consolidation (McGaugh, 2000; Sacchetti et al., 2001, 2002a). We recorded from P14–P16 animals in which the cerebellar circuitry has already achieved its final architecture at qualitative but not quantitative levels (Ito, 1984). Indeed, with the further development of dendrites, voltage clamp recordings become less reliable because of dendritic filtering and space clamp errors (see Experimental Procedures). Fear conditioning, however, is already present at this age (our data; Moye and Rudy, 1987). We recorded from cerebellar lobules V and VI (Figure 1C), an area that represents the site of dominant convergence of acoustic and nociceptive stimuli (Snider and Stowell, 1944; Huang et al., 1982) and is related to the expression of emotional behavior (Supple and Kapp, 1993; Sebastiani et al., 1992).

Figure 1E illustrates the amplitude of the current evoked in the PC by stimulating the PF at increasing strength. To provide a quantitative evaluation of the whole response in the PC, we calculated the slope of the curves. Input-output relations measuring excitatory postsynaptic current (EPSC) amplitude (pA, output) as a function of PF stimulus intensity (μA, input) for each neuron were compared in the three groups. One-way ANOVA test was performed on the slope values of the linear fits obtained in each cell for the first three points of the stimulus-response curve. In the slices prepared at 10 min, the average slope value for the conditioned group (91.12 ± 16.98 pA/μA, n = 17) was significantly higher relative to the unpaired (57.88 ± 5.77 pA/μA, n = 16) and naive (45.36 ± 4.02 pA/μA, n = 22) groups [$F(2,52) = 5.731$; $p < 0.005$ naive-conditioned, $p < 0.05$ unpaired-conditioned; Newman Keuls posttest; Figure 1E]. There was no significant difference between naive and unpaired groups ($p > 0.05$). Similar results were obtained in the slices prepared at 24 hr [naive, 54.74 ± 6.99 pA/μA, n = 17; unpaired, 48.33 ± 4.94 pA/μA, n = 23; conditioned, 111.4 ± 18.53 pA/μA, n = 17; $F(2,54) = 9.925$; $p < 0.001$ for naive-conditioned and unpaired-conditioned; $p > 0.05$ naive-unpaired; Figure 1F]. We conclude that the potentiation observed in the fear-conditioned animals is specifically related to the associative processes that underlie emotional memories.

The fast PF-PC EPSCs are due to ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazalproprionate (AMPA) receptors (Konnerth et al., 1990; Perkel et al., 1990). To

confirm that the increase of the recorded current was due to a potentiation of the AMPA-mediated responses and not to the recruitment of a non-AMPA current, we performed similar recordings in the presence of NBQX, a specific AMPA antagonist (10 μM). In all instances, we observed no response (Figure 1F). Our experiments provide a demonstration that a potentiation of a synaptic response is associated in the cerebellum with the formation of new memories.

Pre- versus Postsynaptic Localization of LTP

In order to assess whether the observed potentiation was due to a change in the axonal excitability of the PFs axons, we measured the amplitude of the PF volley in relation to the strength of stimulation. PF stimulation produced a well-characterized extracellular triphasic potential (p1-n1-p2) that corresponds to the current generated by propagating action potentials along the PFs (Figure 2A; Ito, 1984; Salin et al., 1996; Levenes et al., 1998). These experiments were performed in the presence of kynurenic acid (1 mM) in order to prevent contamination by postsynaptic signals (Levenes et al., 1998). One-way ANOVA test was performed on the slope values of the linear fits obtained for the first three points of the stimulus-response curve and on the presumed volley threshold obtained by extrapolating the intercept to the *x* axis. There was no significant difference for both the slope (naive, 72.17 ± 6.6 mV/μA, n = 25; unpaired, 69.24 ± 8.7 mV/μA, n = 24; conditioned, 72.45 ± 9.9 mV/μA, n = 25; $p > 0.05$) and the threshold (naive, 9 ± 1.4 μA, n = 25; unpaired, 8.6 ± 1.7 μA, n = 24; conditioned, 9.8 ± 1 μA, n = 25; $p > 0.05$; Figure 2B) values.

The next step was to test whether our synaptic potentiation occurred at pre- and/or postsynaptic levels. For this, we first analyzed the paired-pulse facilitation (PPF) of PF-PC responses (Figures 2C and 2D). Such facilitation is an index of a short-term enhancement in synaptic efficacy attributed to residual calcium that facilitates transmitter release (Salin et al., 1996; McKernan and Shinnick-Gallagher, 1997; Jacoby et al., 2001; Lev-Ram et al., 2002). Changes of PPF during long-term plasticity suggest the involvement of presynaptic component, whereas unchanged PPF shows that the long-term plasticity is postsynaptic (Salin et al., 1996; McKernan and Shinnick-Gallagher, 1997; Jacoby et al., 2001; Lev-Ram et al., 2002). One-way ANOVA test at each of the four time intervals tested (50, 100, 150, 200 ms) showed no difference ($p > 0.05$) in the PPF of the three behavioral groups (Figures 2C and 2D). These data suggest that the increase of synaptic efficacy has no significant presynaptic component and is completely postsynaptically expressed.

Additional support for this interpretation was provided by analysis of single evoked quantal events. An amplitude increase of the postsynaptic response to a single quantal release is an index of a postsynaptic potentiation, while a change in frequency is an index of a presynaptic modification (Oliet et al., 1996; Levenes et al., 1998). Quantal events were obtained by substituting Ca^{2+} with Sr^{2+} in the bathing medium (Oliet et al., 1996; Levenes et al., 1998). In this condition, the PF stimulation-evoked synchronous release of transmitter is reduced, but asynchronous release of quanta is markedly

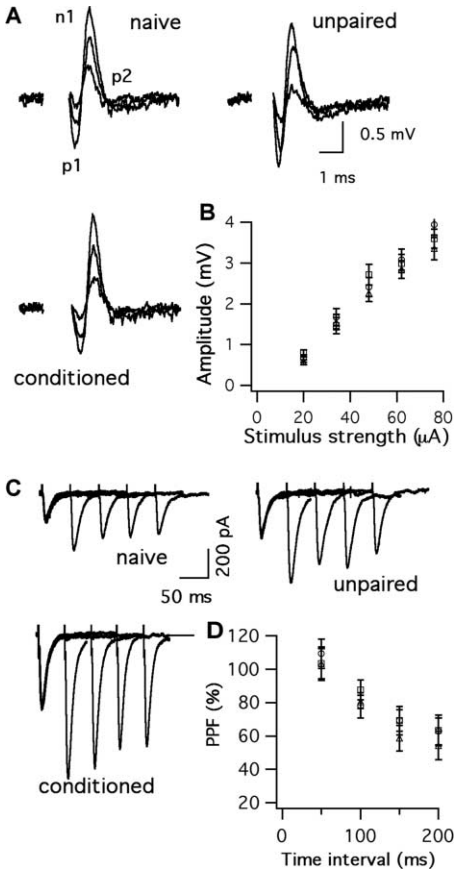

Figure 2. Fear Learning Does Not Change PF Axon Volley or PF Synaptic Facilitation

(A) Superimposed traces obtained by stimulating PF axons at three stimulus strengths. The triphasic potential (p1-n1-p2) corresponds to the field generated by evoked action potentials propagating along PFs.

(B) Input-output data for PF volley from naive (circle, n = 25), unpaired (square, n = 24), and conditioned (triangle, n = 25) groups in slices prepared 24 hr after acquisition session. Reported values are mean ± SEM.

(C) Superimposed traces of EPSCs obtained by paired PF stimuli with four different stimulus intervals. The synaptic current elicited by a second stimulus is similarly facilitated in cells from naive, unpaired, and conditioned groups at the four different time intervals (50, 100, 150, 200 ms) between the first and the second responses.

(D) PPF ratio in naive (circle, n = 15), unpaired (square, n = 14), and conditioned (triangle, n = 12) groups obtained in slices prepared 24 hr after acquisition session. Reported values are mean ± SEM.

and selectively enhanced, allowing detailed analysis of evoked quantal events (Figure 3A; Oliet et al., 1996; Levenes et al., 1998). Asynchronous events arising from the stimulated PF-PC synapses were accumulated into amplitude and frequency histograms. The Kolmogorov-Smirnov statistics revealed that the amplitude of Sr^{2+}-induced asynchronous events was significantly greater ($p < 0.001$) in the conditioned (27.49 ± 12.30 pA; n = 118 events in 10 cells) relative to the naive (20.10 ± 6.07 pA; n = 106 events in 9 cells) and unpaired (21.3 ± 7.73 pA; n = 86 events in 9 cells) animals (Figure 3B). In contrast, for the frequency there was no significant difference (respectively, 1.32 ± 1.14 Hz, 1.18 ± 1.14 Hz, and 0.96 ± 0.93 Hz; $p > 0.05$; Figure 3C).

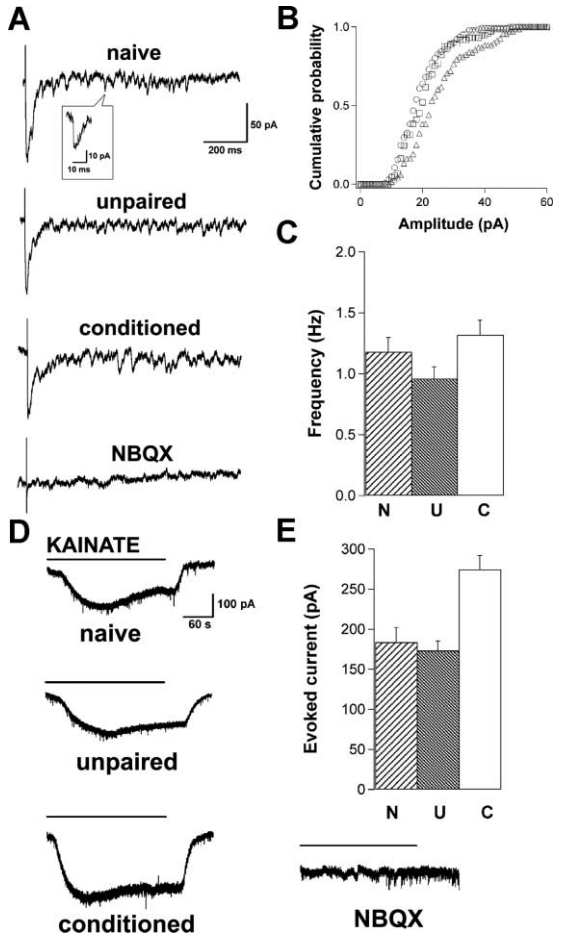

Figure 3. Asynchronous Evoked Events Analysis and Kainate-Evoked Currents Indicate a Postsynaptic Localization of the LTP Induced by Fear Learning

(A) Examples of evoked asynchronous events recorded in presence of Sr^{2+} in naive, unpaired, and conditioned groups. The AMPA receptor antagonist NBQX blocks these responses.

(B) Cumulative mean amplitude distributions of the asynchronous events in naive (circle), unpaired (square), and conditioned (triangle) groups.

(C) Mean detection rates (+SEM) in naive (N), unpaired (U), and conditioned (C) groups.

(D) Kainate-evoked currents (1 μM) in cells from naive, unpaired, and conditioned groups. The response is completely blocked in the conditioned group by NBQX.

(E) Mean amplitude (+SEM) of kainate-evoked currents in naive (N, $n = 6$), unpaired (U, $n = 8$), and conditioned (C, $n = 11$) groups in slices prepared at 24 hr after acquisition session.

To further confirm the postsynaptic nature of the potentiation, we perfused the slices with kainate (1 μM) in the presence of tetrodotoxin (1 μM) to activate only the postsynaptic AMPA receptors of the recorded Purkinje cells, thus avoiding indirect effects through other neurons (Hollmann and Heinemann, 1994; Tempia et al., 1996). Unlike other AMPA receptor agonists (glutamate and AMPA), kainate does not desensitize AMPA receptors (Hollmann and Heinemann, 1994; Tempia et al., 1996). Moreover, kainate evokes currents, which are almost exclusively mediated by AMPA receptors, since the responses of high-affinity kainate receptors are quickly desensitized (Hollmann and Heinemann, 1994). One-way ANOVA test showed a significant increase of kainate-evoked currents in the fear-conditioned group (274.67 ± 7.22 pA, $n = 11$) relative to unpaired (173.3 ± 12.12 pA, $n = 8$) and naive (183.59 ± 18.56 pA, $n = 6$) groups [$F(2,24) = 12.53$; $p < 0.001$; Figures 3D and 3E]. No difference was present between naive and unpaired groups ($p > 0.05$).

When both quantal recordings and kainate perfusion were performed in the presence of NBQX, no responses occurred (Figures 3A and 3D), thus demonstrating the AMPA nature of the response. All these data support the conclusion that fear learning induces a long-lasting increase of PF-PC synaptic efficacy and that this effect is mediated by the postsynaptic AMPA receptors.

Fear Conditioning Does Not Modify CF Synapse onto PC

In addition to the PF input, the PC receives a single climbing fiber (CF) that is the terminal arborization of an inferior olivary neuron (Figure 1D; Ito, 1984). The postsynaptic current evoked by CF stimulation is characterized by an all-or-none response (Konnerth et al., 1990; Perkel et al., 1990). Like PF-PC synapses, fast EPSCs in the CF-PC synapses are mediated by AMPA receptors (Konnerth et al., 1990; Perkel et al., 1990). In addition, this input is characterized by a high degree of morphological (Strata and Rossi, 1998; Bravin et al., 1999; Strata, 2002; Cesa et al., 2003) and electrophysiological (Hansel and Linden, 2000) plasticity. To test whether fear conditioning induces a long-term change in the CF-PC transmission, we recorded the responses to CF stimulation from the same cells in which we previously measured PF-PC EPSCs. One-way ANOVA test showed no difference between the three behavioral groups both at 10 min [naive, 1390 ± 107.50 pA, $n = 11$; unpaired, 1404 ± 135.12 pA, $n = 7$; conditioned, 1428 ± 117.09 pA, $n = 13$; $F(2,28) = 0.0305$, $p > 0.05$; Figures 4A and 4C] and at 24 hr [naive, 1409 ± 125.55 pA, $n = 9$; unpaired, 1516 ± 198.59 pA, $n = 5$; conditioned 1458 ± 157.70 pA, $n = 13$; $F(2,20) = 0.0291$, $p > 0.05$; Figures 4B and 4D] time intervals. Paired-pulse stimulation of the CF is characterized by a depression of the second EPSC (paired-pulse depression, PPD) (Konnerth et al., 1990; Perkel et al., 1990). One-way ANOVA test showed no difference between all groups both at 10 min and 24 hr ($p > 0.05$ in all the instances; Figures 4E and 4F). Our experiments thus show that this form of emotional learning does not induce long-term changes in CF-PC synapses. However, since the recordings were done after the acquisition session, an involvement of the CF synapses during the acquisition phase of fear learning cannot be excluded.

PF-PC LTP Is Present in Cerebellar Lobules V and VI, and not in Lobules IX and X

Lobules V and VI were selected in our study because, in addition to receiving nociceptive input, they have a dominant acoustic projection (Snider and Stowell, 1944; Huang et al., 1982; Saab and Willis, 2003). The positive effects obtained in this area prompted us to investigate an area that is known not to be involved in aversive behavior, like the lobules IX and X (Figure 1C; Supple

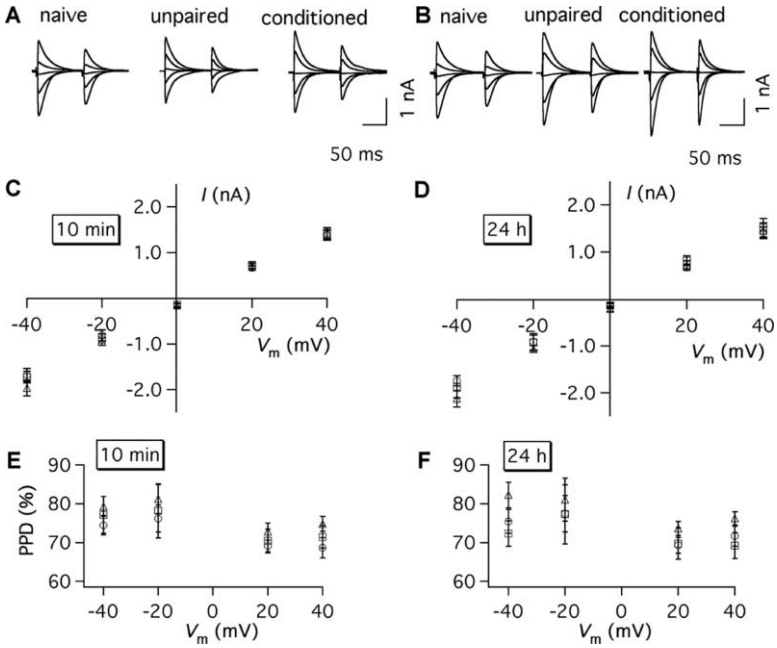

Figure 4. CF-PC Responses Do Not Change after Fear Learning

(A) CF-PC EPSCs at different holding potentials in slices prepared 10 min after the behavioral procedures.

(B) CF-PC EPSCs in slices prepared 24 hr after acquisition session.

(C) I-V curves of naive (circle, n = 11), unpaired (square, n = 7), and conditioned (triangle, n = 13) groups in slices prepared 10 min after the behavioral procedures.

(D) I-V curves of naive (circle, n = 9), unpaired (square, n = 5), and conditioned (triangle, n = 9) groups in slices prepared at 24 hr.

(E) PPD (100 ms) at the different holding potentials 10 min after acquisition.

(F) PPD obtained 24 hr after acquisition session. Reported values are mean ± SEM.

and Kapp, 1993; Sebastiani et al., 1992). One-way ANOVA test showed no difference in the slope of the input-output curves of all groups [naive, 72.72 ± 8.60 pA/μA, n = 12; unpaired, 85.5 ± 9.98 pA/μA, n = 7; conditioned, 81.98 ± 17.51 pA/μA, n = 13; $F(2,33) = 0.2878$, $p > 0.05$; Figures 5A and 5B] and in the PPF ($p > 0.05$ in all the instances; Figures 5C and 5D). Thus, the long-lasting changes seem specifically localized to the areas involved in fear conditioning.

Fear Memories in *hotfoot* Mutant Mice

To add further support to the cerebellar contribution to fear memory, we have used *hotfoot 4J* mice, which are characterized by a primary deficiency of the PF-PC synapses. These mice lack the ionotropic glutamate receptor delta2 subunit (GluRδ2), which is normally targeted selectively to the postsynaptic site of the PF-PC synapse (Yuzaki, 2003). In these mice, like GluRδ2$^{-/-}$ mice, nearly one-half of the branchlet spines are free of innervation, although the total spine density is normal.

In these mice, we tested the acquisition and retention of fear-conditioned responses. To test the acquisition, we compared *hotfoot* and control mice freezing response during (1) the two minutes before CS-US presentation ("pre-acquisition" session), (2) the eight 30 s intrashock periods ("acquisition"), and (3) the one minute immediately after the acquisition. Student's t test showed no difference in the freezing response between control and mutant mice during the pre-acquisition period and during the immediate postacquisition interval ($p > 0.05$; Figure 6A). Mixed ANOVA test (2×8) (2 treatments, control and mutant mice, as between subjects variable; 8 time intervals, as within subjects variable) showed no difference between the control and mutant mice in the freezing levels during the acquisition session ($p > 0.05$; Figure 6A). These data show that *hotfoot* mutant mice do not have sensory or motor deficits that could occlude fear memory acquisition. We then checked the retention

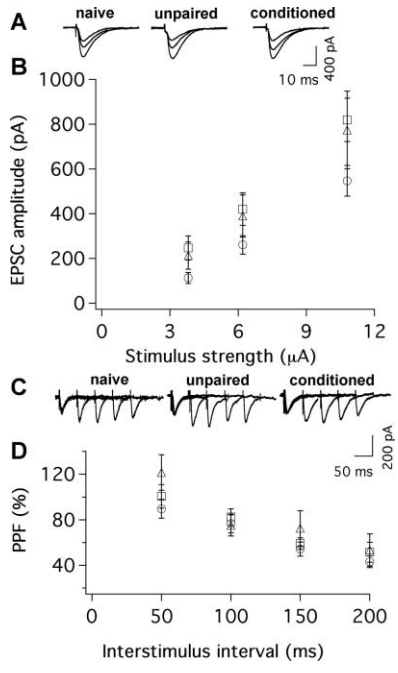

Figure 5. PF-PC Responses in the Lobules IX and X Do Not Change after Fear Learning

(A) Synaptic currents of cells from naive, unpaired, and conditioned groups 24 hr after acquisition session.

(B) Input-output data on EPSC PF-PC synapses in the naive (circle, n = 12), unpaired (square, n = 11), and conditioned (triangle, n = 11) groups in the slices prepared 24 hr after acquisition session.

(C) PPF obtained in the same cells at four different time intervals (50, 100, 150, 200 ms).

(D) PPF ratio of naive, unpaired, and conditioned groups. Reported values are mean ± SEM.

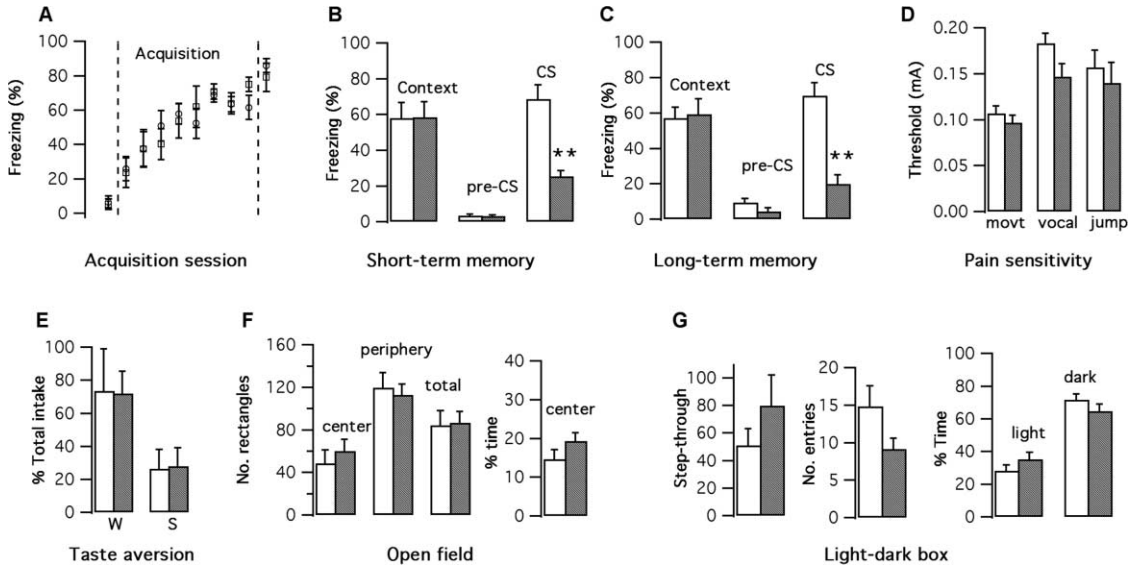

Figure 6. Innate and Conditioned Fear Behavior in the *hotfoot* Mice

(A) Freezing response was measured as percentage of immobility during (1) the 2 min before the acquisition session, (2) the eight 30 s intrashock periods, and (3) the 1 min immediately after the acquisition in the control (circle, n = 6) and *hotfoot* mutant (square, n = 6) mice.

(B) Short-term memory was tested by measuring context and cued (CS) freezing response 10 min after acquisition session in the control (empty columns) and in *hotfoot* (hatched columns) mice. Freezing before CS retention is also shown (pre-CS). Mean ± SEM freezing as percentage of immobility.

(C) Long-term memory 24 hr after the acquisition session.

(D) Pain sensitivity assessed as lowest intensity of shocks required to elicit movement (movt), vocalization (vocal), and jump.

(E) Conditioned taste aversion (CTA) learning was assessed by measuring the percentage of water (W) and saccharine (S) consumption on the total fluid intake during retrieval test.

(F) Open field test: number of rectangles crossed in the center and in the periphery, percentage of time spent in the center. No difference was found between control (n = 6) and mutant (n = 6) mice.

(G) Anxiety-related behavior in the light-dark box: first step-through latency to enter the dark compartment, number of entries into the dark compartment, and percentage of time spent in the lit and in the dark compartments. In all panels, reported values are mean ± SEM.

of both the short- and long-term memory by testing context and cued fear responses, respectively, 10 min and 24 hr after the acquisition phase. Mixed ANOVA test (2×2×2) (2 treatments, control and mutant mice, as between subjects variable; 2 retention intervals, 10 min and 24 hr, as within subjects variable; 2 fear responses, context and CS freezing, as within subjects variable) showed differences between *hotfoot* and control mice related to the cued fear responses both at 10 min and 24 hr time intervals. Thus, in the *hotfoot* mice, the periods of immobility, expressed as percentage of the total time, were decreased relative to the control mice (10 min: control, 68.61 ± 7.99; *hotfoot*, 25.26 ± 3.2; Figure 6B; 24 hr: control, 69.68 ± 7.44; *hotfoot*, 19.85 ± 5.35; Figure 6C; $p < 0.001$ in all the instances). No difference was found for context fear retention ($p > 0.05$ in all the instances; Figures 6B and 6C). These data clearly show that the PF-PC synapse alteration strongly affects both short- and long-term cued fear memories. The normal context freezing response in the *hotfoot* mice shows that the cerebellar dysfunction does not alter this motor response and that the amnesic CS response is not due to a decreased sensitivity to the shock. To further verify this point, we performed a control experiment in which we administered electric shock of increasing intensity while recording the behavioral response exhibited by the mice. There was no difference between the two groups in the intensity of shocks required to elicit movement, vocalization, or jump (Student's t test; $p > 0.05$ in all the instances; Figure 6D), indicating no difference in pain sensitivity.

In order to verify that the amnesic effects found in the *hotfoot* mice is specifically related to the cerebellar dysfunction and is not due to some secondary alterations in other structures such as the amygdala, known to be crucial for fear memory (Rogan et al., 1997; McKernan and Shinnick-Gallagher, 1997; Fendt and Fanselow, 1999; Sacchetti et al., 1999; LeDoux, 2000; Walker and Davis, 2002), these mice were submitted to the conditioned taste aversion (CTA) learning. In this aversive task, the amygdala and many other systems (serotoninergic and noradrenergic systems) are required (Bures et al., 1998), but not the cerebellum (Supple et al., 1987; Bures et al., 1998; Mediavilla et al., 1999). In the CTA learning, the subjects learn to associate a gustatory stimulus (most often saccharine as the CS) with an aversive stimulus (most often the distress induced by intraperitoneal administration of LiCl as the US), so that the behavior toward saccharine becomes aversive instead of appetitive (Bures et al., 1998). One-way ANOVA test showed no difference between the two groups for the saccharine and water consumption during the retrieval

session ($p > 0.05$; Figure 6E), thus confirming that the amnesic effect on fear memory is related specifically to cerebellar dysfunction.

hotfoot Mice and Anxiety-Related Behavior

To explore the tendency for innate fear in *hotfoot* mice and their exploratory behavior, the animals were tested in two well-established models of anxiety, namely the open field test and the light-dark test. In both paradigms, the mice face a conflict between an innate aversion to the open space and the motivation to explore (Supple et al., 1987; Johansson et al., 2001). A greater amount of time spent in the brightly lit space is an index of decreased anxiety-like behavior. As shown in Figures 6F and 6G, the time spent in the center of the open field and the number of rectangle crossings by the *hotfoot* mice did not differ from the control group (Student's t test, $p > 0.05$). Similar results were found in the light-dark box test (Student's t test, $p > 0.05$). It should be noted that a complete vermal lesion performed in adult animals led to a significant decrease in the fear-related innate behavior (Supple et al., 1987, 1988). Such a difference might be due to the fact that in the *hotfoot* mice, the cerebellar deficiency is primarily limited to the PF-PC synapses and that the deficiency is present during development, when compensatory mechanisms might take place. These data support the view that the fear-conditioned amnesia observed in the *hotfoot* mice is not due to a change in the spontaneous fear state.

Discussion

In this paper, we show that following fear conditioning, obtained by associating a sound with an electric shock, there is a long-term change in the PF-PC synapses and that a cerebellar dysfunction due to a primary impairment of these synapses does not prevent the acquisition, but impairs short- and long-term retention, of this learning paradigm.

Evidence that the cerebellum participates in the neural circuitry subserving emotional processes has been widely provided (Snider and Maiti, 1976; Heath et al., 1981; Supple et al., 1987, 1988; Supple and Leaton, 1990; Sebastiani et al., 1992; Supple and Kapp, 1993; Schmahmann and Sherman, 1998; Parvizi et al., 2001; Maschke et al., 2002; Sacchetti et al., 2002b). Among others, the cerebellum is connected with the amygdala, the limbic system, and the paralimbic and neocortical association areas (Snider and Maiti, 1976; Schmahmann and Sherman, 1998). Human cerebellar dysfunctions also lead to emotional abnormalities (Snider and Maiti, 1976; Heath et al., 1981; Schmahmann and Sherman, 1998; Parvizi et al., 2001; Maschke et al., 2002). Moreover, vermal stimulation affects the activity of the amygdala, the septum, and the hippocampus (Snider and Maiti, 1976), while lesions of the vermis or its reversible blockade affect autonomic and behavioral fear-conditioned responses (Supple et al., 1987, 1988; Supple and Leaton, 1990; Sebastiani et al., 1992; Supple and Kapp, 1993; Sacchetti et al., 2002b). Therefore, it is likely that the cerebellum is linked to these extracerebellar circuits.

In the *hotfoot* mice, we found no statistically significant difference in the spontaneous anxiety-related behavior. Therefore, the amnesic effects observed in these mice are likely not due to the interference with anxiety state. In addition, our data show that the cerebellum does not simply control the motor responses related to emotions. In fact, in the *hotfoot* mice, the freezing response is still present in the acquisition phase and in the context retention, but it is selectively abolished in the cued retention. Therefore, our experiments suggest that the cerebellum is a link between sensory stimulus (a sound), its emotional significance, and the correct motor behavior. This view is supported by our demonstration that in normal animals, persistent modifications of the PF-PC synapses occur in those cerebellar lobuli where there is a convergence of acoustic and nociceptive stimuli.

The presence of a context freezing response in the *hotfoot* mice is in line with a previous experiment on irreversible pre-acquisition vermal lesion (Supple et al., 1987). However, it is at variance with another report where tetrodotoxin application to the cerebellar vermis, after the acquisition phase, impaired both context and cued fear-conditioned responses (Sacchetti et al., 2002b). Since hippocampus is known to mediate context, but not cued, fear memory (Kim and Fanselow, 1992; Fendt and Fanselow, 1999; Sacchetti et al., 1999; Sanders et al., 2003), the latter experiment indicates that the functional integrity of the cerebellum is necessary for the hippocampus to mediate the context response. Therefore, it is likely that in the *hotfoot* mice, where the cerebellum is impaired from birth, as well as in long-term lesioned animals, the hippocampus has become relatively independent from the interaction with the cerebellum.

In line with the demonstration that a primary selective dysfunction of the PF-PC synapse affects fear memory, our results provide evidence of a synaptic plasticity induced by behavioral learning in the cerebellum. These changes are long lasting, since they persist for at least 24 hr after acquisition. Changes in synaptic strength following electrical stimulation have been assumed to underlie behavioral learning (Carey and Lisberger, 2002). In addition, behavior-induced synaptic plasticity has been described in several brain regions (Moyer et al., 1996; Rogan et al., 1997; McKernan and Shinnick-Gallagher, 1997; Rioult-Pedotti et al., 2000; Sacchetti et al., 2001, 2002a). Although this plasticity may represent metaplastic changes that facilitate the storage of memory trace by other mechanisms or at another location, they are often interpreted to represent a memory trace. In line with this assumption, we interpret our demonstration of an increased synaptic strength in the PF-PC synapses as suggesting that this synaptic change might be part of the neural substrate of fear memory. This conclusion is in line with two recent studies, performed by reversible inactivation techniques, strongly suggesting that cerebellar cortex may contain a memory trace (Attwell et al., 2002; Sacchetti et al., 2002b).

Previous lesion studies have already shown the critical role of the cerebellum in learning motor tasks, such as eye blink conditioning and adaptation of the vestibulo-ocular reflex (Ito, 1984, 2001; Kim and Thompson, 1997; Medina et al., 2000, 2002; Attwell et al., 2002;

Koekkoek et al., 2003). Damage to the cerebellar cortex, cerebellar nuclei, or inferior olive impairs both of these tasks (Ito, 1984, 2001; Kim and Thompson, 1997; Medina et al., 2000, 2002; Attwell et al., 2002; Koekkoek et al., 2003). In addition, eye blink conditioning and adaptation of the vestibulo-ocular reflex have been studied also by recording neuronal activity during behavioral training (Ito, 1984, 2001; Kim and Thompson, 1997; Schreurs et al., 1997, 1998; Medina et al., 2000). These results helped to identify the structures and online changes that occur during learning, but they did not provide direct evidence for long-term traces in the cerebellum.

The plastic changes we observed consist of a long-lasting potentiation of the synaptic transmission between PF and PC. This phenomenon has never been described in the cerebellum in vivo, although it has been described in the hippocampus (Sacchetti et al., 2001, 2002a) and amygdala (Rogan et al., 1997; McKernan and Shinnick-Gallagher, 1997). In vitro experiments have shown that a postsynaptic LTP is induced in the cerebellum by a repetitive activation of the PF (Lev-Ram et al., 2002, 2003). Therefore, it is possible that our LTP is the result of a conjunctive activation of two separate PF channels under CS and US stimuli. In this respect, it has to be underlined that PFs are known to carry, together with acoustic inputs, nociceptive information (Saab and Willis, 2003). In order to check whether our potentiation shares common cellular mechanisms with this artificially induced LTP, a further issue will be to test if fear conditioning experience occludes subsequent LTP and whether it interferes with LTD in the PF-PC synapses. Since LTP and LTD can both be expressed postsynaptically, this synaptic site is important as a place capable of use-dependent bidirectional modification. Interestingly, both our LTP and the previously studied LTD (Ito, 2001) are mediated by AMPA receptors. An increasing number of investigations show that this family of glutamate receptors plays an important role in long-term plasticity in vitro (Bredt and Nicoll, 2003). Moreover, administration of agonists and antagonists of these receptors in the amygdala modifies fear retention (Walker and Davis, 2002). Finally, it should be noted that recent reports showed that following eye blink conditioning, there is an increase of PC excitability (Schreurs et al., 1997, 1998) and that motor learning increases the number of excitatory synapses onto the Purkinje cell (Kleim et al., 1997), results that are congruent with our data on PF-PC potentiation.

In conclusion, our data provide evidence of an involvement of the PF-PC synapse in fear memories. In addition to better characterizing the role of the cerebellum in emotional behavior, our results identify a synaptic location and mechanism of this process. It thus seems likely that the cerebellum is part of a complex system controlling emotional behavior.

Experimental Procedures

Behavioral Procedures

Fear Conditioning

Young Wistar rats (14- to 16-day-old) were randomly divided into three behavioral groups. The first group (fear conditioned) was trained in a basic Skinner box module (Coulbourn Instruments, Rat Test Cage). Once placed inside the conditioning apparatus, the rats were left undisturbed for 2 min. After this time, the CS (a 1000 Hz tone amplified to 70 dB lasting 6 s) was administered 8 times at 25 s intervals. The last 1 s of each CS was paired with the US, consisting of an electric foot shock (1 mA). The second group of rats (unpaired) received the same number of conditioned stimuli administered at 1 s intervals. Immediately afterward, the rats were placed back in the home cage. After 45 min, the animals were placed back in the apparatus, where they immediately received the same number of unconditioned stimuli at 1 s intervals. These procedures were designed to make it difficult for the animals to associate the US with the CS and the surrounding. The third group of rats never left the home cage (naive). Fear retention was tested 10 min and 24 hr after the training session or after a similar time lapse in the home cage for the naive group. The subjects were placed inside the conditioning apparatus and were left there for 2 min. During a subsequent 25 s period, a series of eight acoustic stimuli (CS) were administered, identical to those used during the acquisition session.

hotfoot and control mice (females, 2 months old) underwent a similar procedure for fear memory acquisition: after 2 min of free exploration in the conditioning chamber, a series of conditional stimuli (3500 Hz tones lasting 6 s) was administered 8 times at 30 s intervals. The last 2 s of each CS were paired with the US consisting of an electric foot shock (0.75 mA). The mice were left there for an additional 1 min, and afterward they were returned to their home cages. 10 min after this acquisition session, we tested short-term memory fear retention by representing to the mice the same context in which they were trained (contextual fear conditioning). 2 min after this, the subjects were placed in a novel environment ("pre-CS") for an additional 2 min, and afterward a series of eight acoustic stimuli (CS) were administered, identical to those used during the acquisition session (cued fear conditioning). Long-term memory was tested in the same way 24 hr after the acquisition session. In all the experiments, the freezing response was recorded and its duration was taken as a fear index. Freezing was defined as the complete absence of somatic motility, except for respiratory movements.

Conditioned Taste Aversion

Control and mutant mice were deprived of water for one day. After this period, the mice were trained for 2 days to drink their daily supply of water during a 20 min interval in the drinking box, equipped with two calibrated pipettes. On day 3, the mice were offered 0.1% solution of sodium saccharine in all pipettes immediately followed by an intraperitoneal injection of lithium chloride (LiCl, 0.15 M, 2% b.wt.). On day 4, the animals were again offered water. For retrieval test on day 5, one pipette was filled with water and the other with saccharine. The individual preference scores for each mouse were expressed as the percentage of saccharine versus water intake from the total fluid consumption.

Open Field Test

The open field consists of a plastic opaque box (40 $\times$ 40 $\times$ 44 cm). The mice were put in the center and their behavior (time spent in the center and number of rectangles crossed in the center and in the periphery) was observed for 10 min.

Light-Dark Box

This apparatus consists of a dark compartment and a light compartment connected by a 7 $\times$ 6 cm opening. The mice were placed in the light compartment, and we measured the first step-through latency to enter the dark compartment, the number of entries into this compartment, and the total time spent in both the lit and the dark compartments during a 10 min period.

All the behavioral procedures were performed between 10:00 a.m. and 1:00 p.m. to minimize circadian influence. The experimental plan was designed in accordance with Italian Law for care and use of experimental animals (DL116/92) and approved by the Italian Ministry of Health.

Slice Preparation

Brain slices were prepared 10 min or 24 hr after the acquisition session from conditioned, unpaired, and naive groups. Briefly, the rats were decapitated and the vermis of the cerebellum was removed and quickly cooled by dipping in ice-cold artificial cerebrospinal fluid (ACSF; in mM: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$, 20 glucose) saturated with 95% O_2 and 5% CO_2. Parasagittal (for patch-clamp recordings) or frontal (for extracellular

recordings) (200 μm thick) slices were cut in a vibratome and left to recover 1 hr in ACFS at 25°C. Then, one slice at a time was transferred to a recording chamber and constantly perfused with ACSF at room temperature (22°C–26°C).

Electrophysiology

Extracellular Recordings

The PF volley was recorded as extracellular field potential with an ACSF-filled pipette (3–5 MΩ) placed in the molecular layer 400–600 μm away from the stimulus site. Negative current pulses ranging from 20 to 80 μA with a duration of 100 μs were delivered in ascending and descending order at 20 s intervals. PF stimulation produced a well-characterized extracellular potential, in which the triphasic potential (p1-n1-p2) corresponds to the current generated by propagating action potential PFs. The signals were amplified 1000× and filtered 1–10,000 Hz.

Whole-Cell Recordings

All recordings were performed with the patch-clamp technique in whole-cell configuration, using an EPC-7 patch-clamp amplifier. Patch-clamp pipettes were pulled from borosilicate glass (2–3 MΩ). The internal electrode solution contained in mM: 120 CsCl, 20 TEA, 10 HEPES, 4 Na_2ATP, 0.4 Na_3GTP, 2 $MgCl_2$, 10 EGTA (pH 7.3) adjusted with CsOH. We employed Cs^+ instead of K^+ to facilitate the measurement of synaptic currents. Cs^+ blocks outward current through K^+ channels and thus simplifies interpretation of the records. The PC soma was visualized using a 40× water-immersion objective of an upright microscope. The criteria to select cell recording were current leakage less than 200 pA, continuous compensation of capacitative transient and series resistance with less 10% variation throughout the recording period, and reversal potential less than 10 mA.

In order to stimulate PFs, a micropipette pulled from sodalime glass (about 10 μm tip diameter) and filled with ACSF was placed in the molecular layer at about two-thirds of the distance between the PC layer and the pial surface. The holding potential was set at −70 mV. In order to block inhibitory GABAergic input, recordings were performed in the presence of bicuculline (20 μM). Negative current pulses ranging from 3 to 15 μA with a duration of 100 μs were delivered in ascending and descending order at 20 s intervals. EPSC amplitude was measured as the difference between the current baseline level before the stimulus artifact and the peak of the EPSC. For each stimulus intensity, a single EPSC value was calculated as the mean of the responses evoked by ascending and descending stimulus intensities. Typically, 2–3 cells were recorded per animal. The values of every cell, recorded within each group of rats, were used to calculate means, SEMs, and statistical tests. One-way ANOVA test was performed on the slope values of the linear fits obtained in each cell for the first three points of the stimulus-response curve. PPF was elicited by twin pulses at different time intervals (50, 100, 150, 200 ms). The PPF was expressed as the difference between the second and the first EPSC divided by the size of the first EPSC.

In order to elicit CF responses, the stimulating micropipette was gently moved from molecular layer to granule cells or white matter. Since the large size of CF-PC EPSCs could produce significant voltage errors, the quality of the clamp was estimated for each cell by evoking CF-PC EPSCs at different membrane potentials between −40 mV and +40 mV and estimating the current-voltage relationship. Only recordings with a reversal potential less than +10 mV were accepted. At any voltage, three EPSCs were collected and averaged. PPD was elicited by twin pulses (100 ms) and expressed as the percentage of the ratio between the second and the first EPSC amplitude.

Acknowledgments

We are grateful to E. Kandel for critically reading the manuscript and T. Sacco for continuous support and advice. This work was supported by grants of Italian MIUR, CNR, and Ministry of Health.

Received: February 3, 2003
Revised: March 10, 2004
Accepted: May 13, 2004
Published: June 23, 2004

References

Albus, J.S. (1971). A theory of cerebellar function. Math. Biosci. *10*, 25–61.

Attwell, P.J., Cooke, S.F., and Yeo, C.H. (2002). Cerebellar function in consolidation of a motor memory. Neuron *34*, 1011–1020.

Bravin, M., Morando, L., Vercelli, A., Rossi, F., and Strata, P. (1999). Control of spine formation by electrical activity in the adult rat cerebellum. Proc. Natl. Acad. Sci. USA *96*, 1704–1709.

Bredt, D.S., and Nicoll, R.A. (2003). AMPA receptor trafficking at excitatory synapses. Neuron *40*, 361–379.

Bures, J., Bermudez-Rattoni, F., and Yamamoto, T. (1998). Conditioned Taste Aversion: Memory of a Special Kind (Oxford: Oxford University Press).

Carey, M., and Lisberger, S. (2002). Embarrassed, but not depressed: eye opening lessons for cerebellar learning. Neuron *35*, 223–226.

Cesa, R., Morando, L., and Strata, P. (2003). Glutamate receptor delta2 subunit in activity-dependent heterologous synaptic competition. J. Neurosci. *23*, 2363–2370.

Fendt, M., and Fanselow, M.S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. Neurosci. Biobehav. Rev. *23*, 743–760.

Hansel, C., and Linden, D.J. (2000). Long-term depression of the cerebellar climbing fiber-Purkinje neuron synapse. Neuron *26*, 473–482.

Heath, R.G., Rouchell, A.M., and Goethe, J.W. (1981). Cerebellar stimulation in treating intractable behavior disorders. Curr. Psychiatr. Ther. *20*, 329–336.

Hollmann, H., and Heinemann, S. (1994). Cloned glutamate receptors. Annu. Rev. Neurosci. *17*, 31–108.

Huang, C.M., Liu, G., and Huang, R. (1982). Projections from the cochlear nucleus to the cerebellum. Brain Res. *244*, 1–8.

Ito, M. (1984). The Cerebellum and Neural Control (New York: Raven).

Ito, M. (2001). Cerebellar long-term depression: characterization, signal transduction, and functional roles. Physiol. Rev. *81*, 1143–1195.

Jacoby, S., Sims, R.E., and Hartell, N.A. (2001). Nitric oxide is required for the induction and heterosynaptic spread of long-term potentiation in rat cerebellar slices. J. Physiol. *535*, 825–839.

Johansson, B., Halldner, L., Dunwiddie, T.V., Masino, S.A., Poelchen, W., Gimenez-Llort, L., Escorihuela, R.M., Fernandez-Teruel, A., Wiesenfeld-Hallin, Z., Xu, X.J., et al. (2001). Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A1 receptor. Proc. Natl. Acad. Sci. USA *98*, 9407–9412.

Kim, J.J., and Fanselow, M.S. (1992). Modality-specific retrograde amnesia of fear. Science *256*, 675–677.

Kim, J.J., and Thompson, R.F. (1997). Cerebellar circuits and synaptic mechanisms involved in classical eyeblink conditioning. Trends Neurosci. *20*, 177–181.

Kleim, J.A., Vij, K., Ballard, D.H., and Greenough, W.T. (1997). Learning-dependent synaptic modifications in the cerebellar cortex of the adult rat persist for at least four weeks. J. Neurosci. *17*, 717–721.

Koekkoek, S.K., Hulscher, H.C., Dortland, B.R., Hensbroek, R.A., Elgersma, Y., Ruigrok, T.J., and De Zeeuw, C.I. (2003). Cerebellar LTD and learning-dependent timing of conditioned eyelid responses. Science *301*, 1736–1739.

Konnerth, A., Llano, I., and Armstrong, C.M. (1990). Synaptic currents in cerebellar Purkinje cells. Proc. Natl. Acad. Sci. USA *87*, 2662–2665.

LeDoux, J.E. (2000). Emotion circuits in the brain. Annu. Rev. Neurosci. *23*, 155–184.

Levenes, C., Daniel, H., Soubrie, P., and Crepel, F. (1998). Cannabinoids decrease excitatory synaptic transmission and impair long-

term depression in rat cerebellar Purkinje cells. J. Physiol. *510*, 867–879.

Lev-Ram, V., Wong, S.T., Storm, D.R., and Tsien, R.G. (2002). A new form of cerebellar long-term potentiation is postsynaptic and depends on nitric oxide but not cAMP. Proc. Natl. Acad. Sci. USA *99*, 8389–8393.

Lev-Ram, V., Mehta, S.B., Kleinfeld, D., and Tsien, R.G. (2003). Reversing cerebellar long-term depression. Proc. Natl. Acad. Sci. USA *100*, 15989–15993.

Marr, D. (1969). A theory of cerebellar cortex. J. Physiol. *202*, 437–470.

Maschke, M., Schugens, M., Kindsvater, K., Drepper, J., Kolb, F.P., Diener, H.C., Daum, I., and Timmann, D. (2002). Fear conditioned changes of heart rate in patients with medial cerebellar lesions. J. Neurol. Neurosurg. Psychiatry *72*, 116–118.

McGaugh, J.L. (2000). Memory—a century of consolidation. Science *287*, 248–251.

McKernan, M.G., and Shinnick-Gallagher, P. (1997). Fear conditioning induces a lasting potentiation of synaptic currents in vitro. Nature *390*, 607–611.

Mediavilla, C., Molina, F., and Puerto, A. (1999). Inferior olive lesions impair concurrent taste aversion learning in rats. Neurobiol. Learn. Mem. *72*, 13–27.

Medina, J.F., Nores, W.L., Ohyama, T., and Mauk, M.D. (2000). Mechanisms of cerebellar learning suggested by eyelid conditioning. Curr. Opin. Neurobiol. *10*, 717–724.

Medina, J.F., Nores, W.L., and Mauk, M.D. (2002). Inhibition of climbing fibres is a signal for the extinction of conditioned eyelid responses. Nature *416*, 330–333.

Moye, T.B., and Rudy, J.W. (1987). Ontogenesis of trace conditioning in young rats: dissociation of associative and memory processes. Dev. Psychobiol. *20*, 405–414.

Moyer, J.R., Thompson, L.T., and Disterhoft, J.F. (1996). Trace eyeblink conditioning increases CA1 excitability in a transient and learning-specific manner. J. Neurosci. *16*, 5536–5546.

Oliet, S.H., Malenka, R.C., and Nicoll, R.A. (1996). Bidirectional control of quantal size by synaptic activity in the hippocampus. Science *271*, 1294–1297.

Parvizi, J., Anderson, S.W., Martin, C.O., Damasio, H., and Damasio, A.R. (2001). Pathological laughter and crying: a link to the cerebellum. Brain *124*, 1708–1719.

Perkel, D.J., Hestrin, S., Sah, P., and Nicoll, R.A. (1990). Excitatory synaptic currents in Purkinje cells. Philos. Trans. R. Soc. Lond. B Biol. Sci. *241*, 116–121.

Rioult-Pedotti, M.S., Friedman, D., and Donoghue, J.P. (2000). Learning-induced LTP in neocortex. Science *290*, 533–536.

Rogan, M.T., Staubli, U.V., and LeDoux, J.E. (1997). Fear conditioning induces associative long-term potentiation in the amygdala. Nature *390*, 604–607.

Saab, C.Y., and Willis, W.D. (2003). The cerebellum: organization, functions and its role in nociception. Brain Res. Brain Res. Rev. *42*, 85–95.

Sacchetti, B., Ambrogi Lorenzini, C., Baldi, E., Tassoni, G., and Bucherelli, C. (1999). Auditory thalamus, dorsal hippocampus, basolateral amygdala, and perirhinal cortex role in the consolidation of conditioned freezing to context and to acoustic conditioned stimulus in the rat. J. Neurosci. *19*, 9570–9578.

Sacchetti, B., Ambrogi Lorenzini, C., Baldi, E., Bucherelli, C., Roberto, M., Tassoni, G., and Brunelli, M. (2001). Long-lasting hippocampal potentiation and contextual memory consolidation. Eur. J. Neurosci. *13*, 2291–2298.

Sacchetti, B., Ambrogi Lorenzini, C., Baldi, E., Bucherelli, C., Roberto, M., Tassoni, G., and Brunelli, M. (2002a). Time-dependent inhibition of hippocampal LTP in vitro following contextual fear conditioning in the rat. Eur. J. Neurosci. *15*, 143–150.

Sacchetti, B., Baldi, E., Ambrogi Lorenzini, C., and Bucherelli, C. (2002b). Cerebellar role in fear-conditioning consolidation. Proc. Natl. Acad. Sci. USA *99*, 8406–8411.

Salin, P., Malenka, R.C., and Nicoll, R.A. (1996). Cyclic AMP mediates a presynaptic form of LTP at cerebellar parallel fiber synapses. Neuron *16*, 797–803.

Sanders, M.J., Wiltgen, B.J., and Fanselow, M.S. (2003). The place of the hippocampus in fear conditioning. Eur. J. Pharmacol. *463*, 217–223.

Schmahmann, J.D., and Sherman, J.C. (1998). The cerebellar cognitive affective syndrome. Brain *121*, 561–579.

Schreurs, B.G., Tomsic, D., Gusev, P.A., and Alkon, D.L. (1997). Dendritic excitability microzones and occluded long-term depression after classical conditioning of the rabbit's nictitating membrane response. J. Neurophysiol. *77*, 86–92.

Schreurs, B.G., Tomsic, D., Gusev, P.A., Alkon, D.L., and Shi, T. (1998). Intracellular correlates of acquisition and long-term memory of classical conditioning in Purkinje cell dendrites in slices of rabbit cerebellar lobule HVI. J. Neurosci. *18*, 5498–5507.

Sebastiani, L., La Noce, A., Paton, J.F.R., and Ghelarducci, B. (1992). Influence of the cerebellar posterior vermis on the acquisition of the classically conditioned bradycardic response in the rabbit. Exp. Brain Res. *88*, 193–198.

Snider, R.S., and Maiti, A. (1976). Cerebellar contributions to the Papez circuit. J. Neurosci. Res. *2*, 133–146.

Snider, R.S., and Stowell, A. (1944). Receiving areas of the tactile auditory and visual systems in the cerebellum. J. Neurophysiol. *7*, 331–358.

Strata, P. (2002). Dendritic spines in Purkinje cells. Cerebellum *1*, 230–232.

Strata, P., and Rossi, F. (1998). Plasticity of the olivocerebellar pathway. Trends Neurosci. *21*, 407–413.

Supple, W.F., and Kapp, B.S. (1993). The anterior cerebellar vermis: essential involvement in classically conditioned bradycardia in the rabbit. J. Neurosci. *13*, 3705–3711.

Supple, W.F., and Leaton, R.N. (1990). Lesions of the cerebellar vermis and cerebellar hemispheres: effects on heart rate conditioning in rats. Behav. Neurosci. *104*, 934–947.

Supple, W.F., Leaton, R.N., and Fanselow, M.S. (1987). Effects of cerebellar vermal lesions on species-specific fear responses, neophobia, and taste-aversion learning in rats. Physiol. Behav. *39*, 579–586.

Supple, W.F., Cranney, J., and Leaton, R.N. (1988). Effects of lesions of the cerebellar vermis on VMH lesion-induced hyperdefensiveness, spontaneous mouse killing, and freezing in rats. Physiol. Behav. *42*, 145–153.

Tempia, F., Kano, M., Schneggenburger, R., Schirra, C., Garaschuk, O., Plant, T., and Konnerth, A. (1996). Fractional calcium current through neuronal AMPA-receptor channels with a low calcium permeability. J. Neurosci. *16*, 456–466.

Walker, D.L., and Davis, M. (2002). The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction. Pharmacol. Biochem. Behav. *71*, 379–392.

Yuzaki, M. (2003). The delta2 glutamate receptor: 10 years later. Neurosci. Res. *46*, 11–22.