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Synaptic inhibition of Purkinje cells mediates consolidation of vestibulo-cerebellar motor learning

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Abstract

Although feed-forward inhibition onto Purkinje cells was first documented forty years ago, we still understand little of how inhibitory interneurons contribute to cerebellar function in behaving animals. Using a mouse line (*PC-Δγ2*) in which GABA_A receptor-mediated synaptic inhibition was selectively removed from Purkinje cells, we examined how feed-forward inhibition from molecular layer interneurons regulates adaptation of the vestibulo-ocular reflex. Whereas impairment of baseline motor performance was relatively mild, the ability to adapt the phase of the vestibulo-ocular reflex and to consolidate gain adaptations, was strongly compromised. Purkinje cells showed abnormal patterns of simple spikes, both during and in the absence of evoked compensatory eye movements. Based on modeling of the experimental data, we propose that feed-forward inhibition, by controlling the fine scale patterns of Purkinje cell activity, enables the induction of plasticity in neurons of the cerebellar and vestibular nuclei.

Introduction

Feed-forward inhibitory microcircuits, in which interneurons and their target principal cells receive common excitatory input, enhance network performance in many brain regions^{1,2}. In the hippocampus, feed-forward inhibition, by reducing the time window of synaptic integration, increases the precision of spike timing in CA1 pyramidal neurons³, and plasticity of feed-forward inhibition is required to maintain the fidelity of information processing⁴. In the cerebellum, molecular layer interneurons (stellate and basket cells)

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Author contributions

P.W. developed the mouse model and helped co-ordinate the project; M.S. and A.B. designed and performed VOR experiments and *in vivo* electrophysiology; M.F. and M.R. designed, performed and analyzed the *in vitro* electrophysiology; P.W., L.V. and M.S.P. performed quantitative anatomical studies; Z.G. and F.E.H. performed LTP and LTD experiments; S.V.D. designed and implemented the model; W.W. initiated the project and co-ordinated collaborations between groups; C.I.D.Z. designed experiments and guided the project; P.W., M.S., M.R., S.V.D., W.W., M.F., and C.I.D.Z. co-wrote the manuscript.

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control Purkinje cells by powerful feed-forward inhibition^{5,6,7,8,9} (Supplementary Fig. 1). Additionally, subsets of Purkinje cells sparsely inhibit each other via axon collaterals¹⁰. Purkinje cells provide the only output of the cerebellar cortex and project to the cerebellar and vestibular nuclei. They fire complex spikes in response to climbing fiber activity¹¹, and simple spikes that reflect the integration of intrinsic pacemaker activity with excitatory and inhibitory synaptic inputs from parallel fibers and molecular layer interneurons^{8,12,13,14,15}.

Although feed-forward inhibition onto Purkinje cells was documented more than four decades ago⁵, we still know little about how it contributes to cerebellar function in behaving animals. Fast synaptic inhibition at molecular layer interneuron to Purkinje cell synapses is mediated by $\alpha 1\beta 2/3\gamma 2$ -type GABA_A receptors¹⁶. The $\gamma 2$ subunit is required to target the receptors to the postsynaptic membrane¹⁷. Thus, to investigate the role of GABA_A receptor-mediated feed-forward inhibition we selectively ablated the $\gamma 2$ subunit, and thereby synaptic GABA_A receptors, from Purkinje cells (*PC-Δγ2* mice). The resulting changes in Purkinje cell simple spike activity and motor behaviour implicate molecular layer interneurons as essential regulators of cerebellar signal coding and memory formation.

Results

Purkinje cell-specific removal of synaptic GABA_A receptors

To remove GABA_A receptor-mediated feed-forward inhibition onto Purkinje cells, we selectively deleted the GABA_A receptor $\gamma 2$ subunit using the Cre/loxP-system (**Methods**). Cre recombinase, under the control of the *L7* promoter, induced a Purkinje cell-specific deletion of the *floxed* $\gamma 2$ subunit gene starting in the second postnatal week^{16,18}. Ablation of synaptic GABA_A receptors from Purkinje cells caused no anatomical alterations of the cerebellar circuitry (Fig. 1).

Patch-clamp recordings in acute slices of cerebellar vermis from adult animals showed spontaneous fast inhibitory postsynaptic currents (sIPSCs) at high frequency in all Purkinje cells ($n = 21$) from control mice (Fig. 2a), which could be blocked by the GABA_A receptor antagonist SR-95531 (20 μ M; data not shown). By contrast, sIPSCs were absent from all Purkinje cells ($n = 19$) of *PC-Δγ2* mice (Fig. 2b). In some *PC-Δγ2* cells (12 of 19) occasional small, slow-rising currents remained. However, these produced on average less than 2% of the control synaptic charge (Fig. 2), and likely reflect spillover of synaptically released GABA onto extrasynaptic α and β subunit-containing receptors^{19,20} (Supplementary Fig. 2). Consistent with a complete loss of synaptic GABA_A receptors, recordings from *PC-Δγ2* mice in the presence of TTX confirmed the absence of miniature IPSCs (mIPSCs) (Fig. 2c,d). The loss of synaptic GABA_A receptors was restricted to Purkinje cells: mIPSCs in molecular layer interneurons were unaltered in *PC-Δγ2* mice (Supplementary Fig. 3).

PC-Δγ2 mice show altered simple spike patterning

Feed-forward inhibition via molecular layer interneurons is rapidly (~ 1 ms) recruited by parallel fiber activation and curtails the parallel fiber-evoked excitatory postsynaptic potential (EPSP) in Purkinje cells^{7,21}. To determine how absence of synaptic GABA_A receptors affected Purkinje cells response to parallel fiber stimulation, we analyzed the temporal dispersion (jitter) of evoked Purkinje cell simple spikes (Fig. 3a). The jitter, quantified as the standard deviation of spike latency in a 10 ms window following stimulation (10V, 100 μ s), was strongly increased in *PC-Δγ2* Purkinje cells (control: 0.81 ± 0.14 ms; *PC-Δγ2*: 1.80 ± 0.10 ms, $p < 0.0001$, $n = 12$ and 11 , respectively). Acute blockade of GABA_A receptors with SR-95531 significantly increased spike jitter in cells from control

mice (to 1.45 ± 0.14 ms, $p = 0.0011$; see also Ref. 7), but, as expected, had no effect in *PC-Δγ2* cells (1.76 ± 0.10 ms, $p = 0.605$). We also determined the number of spikes evoked by parallel fiber stimulation (Fig. 3a, lower panels) (see **Methods**). On average, 0.60 ± 0.04 spikes were evoked in the 60 ms following each stimulus in control cells and 0.41 ± 0.05 spikes in *PC-Δγ2* cells ($n = 17$ and 13 , respectively; $p = 0.0069$). This smaller evoked response is consistent with a reduced parallel fiber excitatory input (see Supplementary Fig. 4 and **Discussion**). Consistent with the complete loss of GABA_A receptor-mediated inhibition in *PC-Δγ2* cells, SR-95531 increased the number of evoked spikes only in control cells (0.61 ± 0.05 to 0.76 ± 0.08 , $n = 11$, $p = 0.0248$; *PC-Δγ2* cells 0.41 ± 0.05 to 0.45 ± 0.06 , $n = 13$, $p = 0.3199$). Thus, loss of molecular layer interneuron-mediated feed-forward inhibition in *PC-Δγ2* mice results in altered simple spike responses to parallel fiber inputs.

Purkinje cells in cerebellar slices from *PC-Δγ2* mice showed a significant increase in simple spike firing regularity compared with controls (Fig. 3b). The mean firing rate at room temperature was not different between groups (control 12.3 ± 1.6 vs *PC-Δγ2* 13.7 ± 0.6 Hz, $n = 26$ and 9 ; $p = 0.062$, Mann-Whitney *U*-test), but the coefficient of variation (CV; SD/mean) of the inter-spike interval (ISI) was reduced in *PC-Δγ2* mice (0.20 ± 0.03 in control vs 0.10 ± 0.01 in *PC-Δγ2*; $p = 0.018$, Mann-Whitney *U*-test). The coefficient of variation of adjacent intervals (CV₂; mean value of $2 |ISI_{n+1} - ISI_n| / (ISI_{n+1} + ISI_n)$; a measure for the regularity of firing on small timescales²²) also differed. CV₂ was 0.19 ± 0.02 in control vs 0.10 ± 0.01 in *PC-Δγ2* mice ($p = 0.018$; Mann-Whitney *U*-test). Blockade of GABA_A receptors with SR-95531 in control Purkinje cells decreased the CV of the ISI (0.20 ± 0.04 vs 0.13 ± 0.02 in SR-95531; $p = 0.024$, $n = 8$) to a value comparable to that found in *PC-Δγ2* mice (see also Refs. ^{12,15,23}). As expected, SR-95531 failed to alter the CV of the ISI in cells from *PC-Δγ2* mice (0.13 ± 0.02 vs 0.13 ± 0.04 , $n = 3$). Importantly, similar results were obtained at near-physiological temperature (34–35°C), with no change in mean rate (51.3 ± 9.1 in control vs 50.0 ± 3.5 Hz in *PC-Δγ2*, $n = 9$ and 7 ; $p = 0.61$; Mann-Whitney *U*-test), but a significant decrease in the CV (0.14 ± 0.01 in control vs 0.06 ± 0.01 in *PC-Δγ2*; $p = 0.001$; Mann-Whitney *U*-test) and CV₂ (0.15 ± 0.02 vs 0.06 ± 0.01 ; $p = 0.0099$).

Finally, we examined whether loss of inhibition onto Purkinje cells modified long-term plasticity at parallel fiber to Purkinje cell synapses. Neither parallel fiber LTD nor LTP (see **Methods**) were significantly impaired in *PC-Δγ2* mice compared with controls ($p = 0.624$ and $p = 0.257$, respectively) (Fig. 3c,d).

PC-Δγ2 mice display little impairment in motor performance

PC-Δγ2 mice showed no obvious neurological abnormality¹⁶. To assess cerebellar performance we analyzed compensatory eye movements in male *PC-Δγ2* mice ($n = 9$) and littermate controls ($n = 8$). Mice were exposed to whole-field visual stimuli to determine the amplitude (gain) and timing (phase) of their optokinetic reflex (OKR) and/or tested with turntable stimulation to investigate the same parameters for the vestibulo-ocular reflex in the dark (VOR) and light (visual VOR or VVOR). During OKR, *PC-Δγ2* mice showed a relatively small, but significant, deficit, evident as a reduction in gain and a lag in phase compared to controls ($p = 0.018$ and $p = 0.012$, respectively; two-way repeated-measures ANOVA) (Supplementary Fig. 5a). During VOR the gain values and phase leads of *PC-Δγ2* mice were larger and smaller, respectively, than those of controls ($p = 0.012$ and $p = 0.030$; two-way repeated-measures ANOVA) (Supplementary Fig. 5b). By contrast, no significant differences were observed during VVOR ($p = 0.43$ and $p = 0.63$, for gain and phase values, respectively) (Supplementary Fig. 5c). Thus, *PC-Δγ2* mice show small, but significant, abnormalities in motor performance when visual and vestibular systems are investigated separately, but not when they operate together, as under natural conditions or during visuo-vestibular training.

PC-Δγ2 mice show marked deficits in learning and consolidation

Loss of inhibition onto Purkinje cells had more profound effects on cerebellar motor learning. We studied gain and phase learning by applying a protocol aimed at reducing the gain of the VOR on day 1 (5×10 min sinusoidal, *in phase* drum and table rotation at 0.6 Hz, both with an amplitude of 5°) and subsequently shifting its phase on days 2, 3 and 4 (5×10 min sinusoidal *in phase* drum and table rotation at 0.6 Hz, but with drum amplitudes of 7.5° on day 2 and 10° on days 3 and 4, while the table amplitude remained 5°). Animals were kept in the dark between the recording days.

Gain-decrease learning of PC-Δγ2 ($n = 9$) and control mice ($n = 10$) on day 1 was similar ($p = 0.11$; two-way repeated-measures ANOVA) (Fig. 4a,b). However, when the measurements were resumed the next day, the degree of gain reduction carried forward from the previous day's learning was significantly smaller in PC-Δγ2 mice than in controls ($p = 0.001$) (Fig. 4b, upper panel). This consolidation deficit was apparent at a wide range of frequencies (Fig. 4c, upper panel). To exclude non-specific effects (habituation during gain-decrease learning) we tested PC-Δγ2 and control mice in non-adapting VOR paradigms; importantly, mice of both genotypes showed no significant decreases in VOR over consecutive days (last gain value of session 1 *vs* first value of session 2; $p = 0.610$ for controls and 0.551 for PC-Δγ2 mice) (see Supplementary Fig. 6).

Deficits in gain consolidation were also seen when the drum rotation amplitude was kept constant (3×10 min of sinusoidal *in phase* drum and table rotation at 0.6 Hz, both with an amplitude of 5°) (Fig. 4d, lower panel). Here too, the initial level of learning was not significantly affected (PC-Δγ2 mice *vs* controls; $p = 0.61$; $n = 6$ and 5 , respectively), whereas the level of consolidation was significantly reduced (gain day $1 \rightarrow 2$, $p = 0.034$; gain day $2 \rightarrow 3$, $p = 0.046$). Moreover, gain consolidation deficits in PC-Δγ2 mice did not depend on the direction of learning. With a gain-increase paradigm (5×10 min sinusoidal *out of phase* drum and table rotation at 1.0 Hz, both with an amplitude of 1.6°) no significant consolidation was present in the PC-Δγ2 mice (gain day $1 \rightarrow 2$, $p = 0.744$, $n = 6$; One-Sample *t*-test). By contrast, consolidation in controls was present and was significantly stronger than in PC-Δγ2 mice ($p = 0.002$) (Fig. 4d, lower panel). Notably, the level of gain increase learning in PC-Δγ2 mice was not significantly different from that in controls ($p = 0.800$). Thus, deficits in consolidation of learned gain changes, during both gain decrease and increase training paradigms, were not due to differences in baseline performance.

The adaptation paradigm provided on days 2, 3 and 4 immediately revealed significant deficits in phase learning in PC-Δγ2 mice, starting 10 - 20 min after the initiation of visuo-vestibular training (e.g. at 20 min $p = 0.009$) (Fig. 4b, lower panel). These deficits in phase change acquisition were followed by clear differences in consolidation (e.g. from day 2 to day 3 $p = 0.0008$) (Fig. 4d, upper panel). Phase adaptation deficits also occurred at a wide range of frequencies (Fig. 4c, lower panel) and were not caused by visual problems in PC-Δγ2 mice, as eye movement recordings during the adaptation sessions showed that PC-Δγ2 mice were capable of full phase reversal (Supplementary Fig. 7). In short, PC-Δγ2 mice showed a relatively normal capacity for acquisition during gain-decrease and gain-increase motor learning, but a profound deficit in acquisition during phase adaptation learning and a general deficit in consolidation of gain and phase adaptation.

Abnormal temporal patterns of Purkinje cell simple spikes

Since the flocculus controls adaptation of compensatory eye movements^{24,25,26} and Purkinje cells provide the sole output of the cerebellar cortex (Supplementary Fig. 1), we analyzed floccular Purkinje cell activity during optokinetic stimulation (Fig. 5a). Single units of Purkinje cells that responded optimally to stimulation around the vertical axis were

identified by creating tuning curves of their complex spike responses and by identifying a clean climbing fiber pause^{26,27}. The average climbing fiber pause in *PC-Δγ2* mice and controls was 15.3 ± 0.8 and 18.6 ± 1.3 ms, respectively (55 and 60 *PC-Δγ2* and control cells, respectively; $p = 0.029$). The average simple spike firing frequency, phase relative-to-stimulus and modulation amplitude, were similar in *PC-Δγ2* and control mice (Fig. 5b). However, as predicted by the in vitro recordings, the regularity of Purkinje cell firing was affected. For floccular simple spike activities, the CV of the ISIs was significantly reduced during visual stimulation in *PC-Δγ2* mice ($p = 0.008$; *PC-Δγ2*: $n = 55$, controls: $n = 60$) (Fig. 5c). This difference reflected specific changes in temporal patterning, as CV2 was significantly lower in *PC-Δγ2* mice ($p < 0.0001$) (Fig. 5c; see also Supplementary Fig. 8). If these differences in Purkinje cell firing patterns contribute to consolidation deficits in *PC-Δγ2* mice, we would also expect to find them outside periods of optokinetic stimulation. Indeed, both CV and CV2 of ISIs were significantly reduced in the absence of stimulation ($p = 0.022$ and $p < 0.0001$, respectively; *PC-Δγ2*: $n = 41$, controls: $n = 43$) (Fig. 5c). By contrast, the patterns of complex spike activities of Purkinje cells did not differ between *PC-Δγ2* and control mice (Fig. 5a,c; see also Supplementary Fig. 9). Also the antiphasic modulation of complex and simple spikes was unchanged (Fig. 5a), arguing against a critical involvement of molecular layer interneurons in this phenomenon⁸.

Model and simulations

We interpreted the experimental data from the 4-day gain decrease - phase adaptation routine using a 'distributed memory' model (Fig. 6). Short-term adaptation is assumed to take place in the cerebellar cortex, and is expressed as adaptation of the phase and gain of modulation of Purkinje cell simple spikes, which in turn modulate the activity of target neurons in the vestibular nucleus; this process underlies the rapid VOR gain adaptation observed in both *PC-Δγ2* and control mice. On a longer timescale, the learned Purkinje cell activity guides plasticity at the target neurons in the vestibular nuclei^{28,29}, the polarity of which is presumably regulated by the precise timing of simple spikes relative to input from mossy fiber collaterals³⁰. Simultaneously, a partial extinction of the previously learned changes at the level of the Purkinje cells takes place³¹. The memory is thus partially transferred to the target nuclei, potentially underlying long-term consolidation^{29,32}. After several days of training, this form of 'systems-consolidation' ensures that the cerebellar cortex is no longer responsible for the expression of the learned behavior, but mainly regulates the precise timing (phase). In *PC-Δγ2* mice, the altered temporal patterns of Purkinje cell simple spikes could impair the induction of plasticity in the nuclei and thus consolidation.

Given this working hypothesis, we examined whether deficits in VOR gain consolidation and phase adaptation in *PC-Δγ2* mice could be replicated in a conceptual model of the idealized VOR circuit (Supplementary Material online). We modeled the modulation of Purkinje cell simple spike firing during head movement as resulting from linear summation of sinusoidal excitatory (parallel fiber) and inhibitory (interneuron) inputs⁶. We assumed the gain and phase of such modulation to be regulated through bidirectional plasticity of the inputs³³.

Purkinje cells and mossy fiber collaterals modulate, by linear summation of their activity, the firing of cells in the vestibular nuclei, which in turn control eye movement. Panels a-f in Fig. 6 depict an overview of the gains and phases of sinusoidal modulation that can be attained by the elements in the simulation, drawn as positions in a polar plot in which 0° represents modulation in phase with head movement (increased activation during ipsilateral head velocity). The order of the panels follows the signal flow through the vestibulo-cerebellar system from the modeled activation of parallel fibers and interneurons (Fig. 6a,b), to the simple spike modulation attainable by appropriate depression and potentiation of these

excitatory and inhibitory inputs (Fig. 6c), to the modulation of target vestibular nucleus neurons (Fig. 6d,e), and ultimately the resulting limits on eye movement (Fig. 6f). Due to the absence of inhibition in *PC-Δγ2* mice, the simple spike activation required for adequate modulation of the vestibular nucleus is out of range of the normal plasticity mechanisms in the cerebellar cortex (Fig. 6c). In addition, impaired plasticity of the inputs to the vestibular nucleus (Fig. 6e) both abolishes consolidation and excludes the possibility of extreme phase adaptations (Fig. 6f).

Data from four training sessions, each followed by an overnight period (Fig. 6g), were simulated using the upper bounds on sinusoidal modulation as depicted in panels a-f. Adaptation of modulation (**Fig. 6h I and II**) was simulated as an exponential decay from the start position in the polar plot (defined by the initial gain and phase) towards a new position determined by the experimental paradigm (black horizontal bars in Fig. 6g). As detailed in the Supplementary Material, simulation parameters were chosen to mimic the rate of adaptation observed experimentally. Under these conditions, both control and *PC-Δγ2* Purkinje cells rapidly reached the required modulation during short-term VOR adaptation (Fig. 6h I). However, impairments in both VOR gain consolidation and phase adaptation, can be generated if we assume disrupted plasticity in the vestibular nucleus is caused by poor timing of simple spikes. (Figs. 3, 5, 6h).

Discussion

Signal coding and plasticity in cerebellar learning

Although inhibitory interneurons in the molecular layer of the cerebellum have been studied extensively^{5,6,7,8}, their behavioural relevance has remained enigmatic. Here, we show that these interneurons shape the temporal patterns of Purkinje cell simple spikes and suggest that this process could be essential for plasticity and consolidation in the cerebellar and vestibular nuclei.

Floccular Purkinje cells control the adaptation of compensatory eye movements by modulating the activity of vestibular nucleus neurons (Supplementary Fig. 1). To adapt the VOR, two things should happen within the framework of a ‘distributed memory’ model. First, Purkinje cells should ‘learn’ the correct simple spike modulation and express it at sufficient gain in order to modulate the vestibular nuclei. Second, the input from the direct vestibular pathway to the vestibular nuclei should be suppressed, as it only allows modulation in phase with ipsilateral head movement. The first of these processes is thought to reflect complementary inhibitory and excitatory actions, with plasticity at parallel fiber to Purkinje cell and parallel fiber to interneuron synapses, both under climbing fiber control^{13,24,25}. The second is thought to occur through plasticity at mossy fiber to vestibular nuclei synapses^{25,28}. In *PC-Δγ2* mice the temporal fidelity of Purkinje cell firing is disrupted and consolidation of learned VOR adaptations is severely compromised (Figs. 4 and 6g). As induction of various forms of plasticity in the vestibular and cerebellar nuclei could depend on the precise timing of inhibitory and excitatory input from Purkinje cells and mossy fiber collaterals (Supplementary Material), disruption of this timing would impair transfer of plasticity to the nuclei and thus ‘systems consolidation’.

Simple spike trains in Purkinje cells show significantly more temporal patterns than expected from random activation, and these patterns are influenced by natural stimuli²². Both the electrical coupling among interneurons and the sagittal orientation of their axons^{34,35} (Supplementary Fig. 1) will enhance the effects of feed-forward inhibition by promoting common firing patterns in ensembles of Purkinje cells within individual zones, known to project to the same nucleus²⁶. The activity patterns of individual Purkinje cells in an ensemble might thus interact with each other and/or with those of mossy fiber and/or

climbing fiber collaterals to facilitate the induction of plasticity in the cerebellar and vestibular nuclei^{25,28,36,37}. We therefore propose that the vestibular nuclei are the locus for consolidation (see also Ref. ^{29,32}). Alternatively, both initial learning and consolidation could occur in the cerebellar cortex and the consolidation signal could be preserved in the average simple spike frequency of a particular Purkinje cell. In fact, changes in simple spike frequencies in the flocculus of monkeys are sufficient to drive changes in eye velocity during trial-by-trial motor learning³⁸. To determine the extent to which spatiotemporal patterns of simple spikes contribute to consolidation, and whether this consolidation occurs in the nuclei, future experiments will require simultaneous multi-unit recording from ensembles of Purkinje cells and cerebellar or vestibular nuclei neurons during learning.

Previous studies have identified long-term changes at the parallel fiber to Purkinje cell synapse as a potential plasticity mechanism during cerebellar learning, and some mouse lines with disrupted LTD induction at this synapse indeed show impaired motor learning^{39,40,41}. However, neither parallel fiber LTD nor LTP were impaired in *PC-Δγ2* mice. Notably, the motor learning deficits in *PC-Δγ2* mice differed from those seen in mouse lines in which LTD was impaired by blocking PKC, PKG, or alpha-CaMKII activity in Purkinje cells. Furthermore, in the latter mouse lines acute learning was affected more severely than in *PC-Δγ2* mice, whereas learning over multiple days of training was less affected^{39,40,41,42}.

GABAergic interneurons in the cerebellar cortex have ample possibilities to induce and express plasticity at both the synaptic input and output level^{1,9,13,43,44,45}. Simultaneous induction of LTP at molecular layer interneuron and parallel fiber to Purkinje cell synapses is required for associative fear conditioning⁹. In this scenario, the potentiation of GABAergic synapses may balance the LTP of excitatory inputs in a form of scaling to preserve coincidence detection of parallel fiber inputs^{7,9}. Loss of this scaling mechanism in *PC-Δγ2* mice might contribute to the observed phenotype.

Inhibition is essential for spike patterning and learning

Although *PC-Δγ2* mice showed marked deficits in cerebellar motor learning, baseline motor performance was only moderately affected. Despite the lack of synaptic GABA_A receptors on *PC-Δγ2* Purkinje cells we found their average simple spike frequency to be normal. This could reflect enhancement of another inhibitory input (e.g. GABA_B receptors) and/or reduced parallel fiber excitatory input. Whereas GABA_B receptor-mediated inhibition of *PC-Δγ2* Purkinje cells was unchanged (Supplementary Fig. 10), we found a significant decrease in AMPA receptor-mediated EPSC charge transfer after parallel fiber stimulation (Supplementary Fig. 4). This might allow Purkinje cells to maintain their excitability in a normal operational range in the absence of fast inhibition. By contrast, the loss of temporal fidelity in Purkinje cell responses to parallel fiber stimulation and the increase in simple spike regularity in *PC-Δγ2* mice, were comparable to the changes seen after acute pharmacological blockade of GABA_A receptors^{7,12} (Figs. 3 and 5). The cerebellum may thus compensate for the loss of certain functions of molecular layer interneurons, but these interneurons are essential for the temporal control of Purkinje cell activity and for both phase adaptation learning and consolidation of gain adaptations.

Purkinje cell collaterals

By deleting synaptic GABA_A receptors from Purkinje cells in *PC-Δγ2* mice we also disrupted any inhibition mediated by recurrent collaterals of Purkinje cell axons^{10,46}. However, as GABAergic terminals from basket and stellate cells onto Purkinje cells vastly outnumber those from recurrent collaterals, and as Purkinje-Purkinje contacts in mice appear restricted to young animals⁴⁶, the phenotype we observe is most likely caused by the loss of

inhibition from molecular layer interneurons. Although it has been proposed that Purkinje cell axon collaterals contribute to fast cerebellar oscillations in adult rats⁴⁷, such oscillations have not been recorded in wild-type mice¹⁰.

General functional implications

Studies on learning and memory have focused largely on the role of plasticity at excitatory synapses onto projecting neurons. However, GABAergic interneurons also express plasticity, which increases the computational capacity of their microcircuit^{1,2,9}. Here we examined the role of fast synaptic inhibition in cerebellar motor learning using genetic dissection of the circuit and suggest that feed-forward inhibition is essential for specific aspects of procedural learning. Can our findings be extrapolated to other brain regions? Feed-forward inhibition is a common motif throughout the CNS. In the amygdala it mediates extinction learning of conditioned fear responses⁴⁸. In cortical circuits some interneuron types may serve functions similar to those we have identified in the cerebellum. For example, feed-forward inhibitory interneurons in the hippocampus may promote the temporal fidelity of synaptic integration and action potential generation in pyramidal cells necessary for encoding declarative memories^{2,3}. Thus, feed-forward inhibition might be an operational necessity for memory formation in different brain circuits.

Methods

Procedures involving mice were performed in accordance with regulations of the United Kingdom Animals (Scientific Procedures) Act 1986, the Animal Care and Use Committee of Turin University, the Dutch Ethical Committee for animal experiments..

Generation of *PC-Δγ2* mice

We generated $\gamma 2I77lox$ mice by flanking exon 4 of the GABA_A receptor $\gamma 2$ subunit gene with *loxP* sites¹⁶. Homozygous $\gamma 2I77lox$ mice were crossed with mice heterozygous for $\gamma 2I77lox$ and hemizygous for an *L7Cre* transgene^{16,18}. Littermates of the following genotypes were used: $\gamma 2I77lox/\gamma 2I77lox/L7Cre$ (*PC-Δγ2*) and $\gamma 2I77lox/\gamma 2I77lox$ (controls). Mice were genotyped by PCR analysis of genomic DNA using the following primer pairs: $\gamma 2lx5'$ (5'-GTCATGCTAAATATCCTACAGTGG-3') plus $\gamma 2lx5'$ (5'-GGATAGTGCATCAGCAGACAATAG-3') to test for the $\gamma 2I77lox$ allele (213 bp control; 250 bp $\gamma 2I77lox$), and: Cre1 (5'-GACCAGGTTTCGTTCACTCATGG-3') plus Cre2 (5'-AGGCTAAGTGCCTTCTCTACAC-3') to test for the *Cre* transgene (250 bp *L7Cre*).

Morphology

Adult mice were anaesthetized by intraperitoneal injection of ketamine/xylazine and perfused with 4% paraformaldehyde in phosphate-buffered saline (PBS; pH7.4). Cerebella were cryoprotected in sucrose (10%, 20% and 30% in PBS) and cut into 16μm coronal sections with a cryostat. Following blocking in normal goat serum (10% in PBS with 0.5% Triton X-100), sections were incubated with antibodies against calbindin (1:10000; Swant), anti-VGAT (1:3000), anti-VGLUT1 (1:1000), or anti-VGLUT2 (1:500; all Synaptic Systems). Sections were rinsed and incubated with secondary antibodies conjugated to Alexa 488 (Molecular Probes) or Cy3 (Jackson ImmunoResearch). Sections were examined with a laser scanning confocal microscope (Zeiss LSM5 Pascal). Stacks of 5-15 sections spaced by 250-350nm were acquired (pinhole: 1 Airy unit). Quantification of VGLUT2-positive puncta was done on segmented images spanning the molecular layer; 8 confocal fields (13225 μm²/field) were counted per animal (n = 4). For VGLUT1 and VGAT, images acquired at a magnification of 8.1×10⁻³ μm²/pixel (512 × 512 pixels) were segmented using a threshold that maximized the selection of immunofluorescent puncta over background. The number and density of puncta were calculated with ImageJ software (<http://>

rsbweb.nih.gov/ij/). For VGLUT1 and VGAT, 6 and 8 fields ($2125 \mu\text{m}^2/\text{field}$) were counted per animal ($n = 3$ and 4 , respectively). To quantify the number of Purkinje cells, a line was placed through the Purkinje cell layer and all calbindin-positive cells on the line were counted (4 sections per animal, $n = 4$). The density of molecular layer interneurons was calculated in 3-6 fields ($5000 \mu\text{m}^2/\text{field}$) of 3-5 Nissl-stained sections per animal ($n = 4$).

For electron microscopy, adult mice ($n = 2$ per genotype) were perfused with 4% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer (0.1M, pH 7.4). Cerebella were postfixed in the same solution overnight. Blocks of tissue were postfixed in 1% osmium tetroxide (in 0.1M cacodylate buffer), dehydrated in ethanol and embedded in Epon-Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and analyzed with a JEM-1010 transmission electron microscope (Jeol) equipped with a side-mounted CCD camera (Mega View III, Soft Imaging System). In each mouse, 90 electron micrographs were taken randomly from the neuropil of the molecular layer at a magnification of $\times 30000$ ($15.7 \mu\text{m}^2/\text{micrograph}$) to compare the density of parallel fiber to Purkinje cell synapses.

***In vitro* electrophysiology**

Mice (10 – 25 weeks old) were anaesthetized with isoflurane (IVAX Pharmaceuticals) and parasagittal slices (250-300 μm) were cut from the cerebellar vermis/paravermis (HM 650V; Microm International GmbH) as previously described¹⁶. Slices were transferred to a submerged recording chamber and perfused (1.5-2.5 ml/min) with an ‘external’ solution containing (in mM): 125 NaCl, 2.5 KCl, 2 CaCl_2 , 1 MgCl_2 , 25 NaHCO_3 , 1.25 NaH_2PO_4 , and 25 D-glucose; pH 7.4, when bubbled with 95% O_2 and 5% CO_2 . Patch-clamp recordings were made with Axopatch-200A or -200B amplifiers (Molecular Devices Corporation) from Purkinje cells visualized under infrared differential interference contrast optics (Zeiss Axioscop or Olympus BX51 WI). Whole-cell and single-channel currents were recorded at room temperature ($25 \pm 1^\circ\text{C}$). Simple spike activity was recorded at both room and near-physiological temperature ($34 \pm 2^\circ\text{C}$), in loose cell-attached mode with external solution in the recording pipette. Firing was recorded in voltage- or current-clamp with the pipette current set to zero¹².

For whole-cell and loose cell-attached recordings, pipettes were pulled from thin-walled borosilicate glass tubing (1.5 mm o.d., 1.17 mm i.d; G150TF-3; Warner Instr. Inc.). For patch recordings, thick-walled borosilicate glass tubing (1.5 mm o.d., 0.86 mm i.d; GC-150F; Harvard Apparatus Ltd) was used. Pipettes were coated with Sylgard resin (Dow Corning 184) and fire polished to give a final resistance of 2-6 M Ω (whole-cell and loose cell-attached) or 10-15 M Ω (single-channel). The internal solution contained (in mM): CsCl, 140; NaCl, 4; CaCl_2 , 0.5; N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES), 10; ethyleneglycol-bis (β -aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 5; Mg-ATP, 2; pH 7.3 with CsOH. Ionotropic glutamate receptors were blocked with 10 μM D-AP5 and 5 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). mIPSCs were recorded in the presence of 0.5-1 μM tetrodotoxin (TTX).

Parallel fiber-evoked responses were recorded in loose cell-attached mode during molecular layer stimulation (glass pipette containing external solution placed in fixed position ~100-150 μm from the recorded Purkinje cell soma). Stimuli of 5-10 V and 100 μs duration were delivered at 0.5 Hz (Digitimer DS2 isolated stimulator). Recordings were made in the absence of drugs. The effect of GABA_A receptor blockade was tested using SR-95531 (40 μM). In all cases tested, NBQX completely blocked evoked responses (data not shown).

Long-term plasticity at parallel fiber-Purkinje cell synapses was examined as previously described⁴¹. 200 μm thick parasagittal slices were cut in ice-cold ACSF (containing in mM:

124 NaCl, 5 KCl, 1.25 Na₂PO₄, 2 MgSO₄, 2 CaCl₂, 26 NaHCO₃ and 15 D-glucose, bubbled with 95% O₂ and 5% CO₂). Experiments were made at room temperature with GABA_A receptors blocked (100 μM picrotoxin). Whole-cell patch-clamp recordings of Purkinje cells were performed using an EPC-10 amplifier (HEKA electronics). Pipette resistance was 4–5 MΩ when filled with intracellular solution containing (in mM): 120 K-gluconate, 9 KCl, 10 KOH, 3.48 MgCl₂, 4 NaCl, 10 HEPES, 4 Na₂ATP, 0.4 Na₃GTP and 17.5 sucrose (pH 7.25). LTP was induced by parallel fiber stimulation at 1 Hz for 5 min in current-clamp mode and measured by test responses recorded in voltage-clamp mode. LTD was induced using combined parallel and climbing fiber stimulation⁴¹. All drugs were obtained from Tocris Bioscience, Ascent Scientific or Sigma.

Eye movement recordings

Mice (12 – 30 weeks old) were surgically prepared under general anesthesia with isoflurane. A construct with two nuts was attached to the frontal and parietal bones using Optibond (Kerr) and Charisma (Heraeus Kulzer). After 5 days recovery, mice were placed in a restrainer, with their heads bolted to a bar. The restrainer was fixed onto the turntable. A cylindrical screen (diameter 63 cm) with a random-dotted pattern (each element 2°) surrounded the turntable (diameter 60 cm). OKR and (V)VOR were evoked by independently rotating the screen and turntable (5° amplitude at different frequencies; AC servo-motors, Harmonic Drive AG). The table and drum position were measured by potentiometers and the signal digitized (CED Limited) and stored for off-line analysis. Eye movements were recorded using an infrared CCD camera fixed to the turntable (240 Hz; ISCAN Inc.). Video calibrations and eye movement computations were performed as described previously^{27,49}.

In vivo electrophysiology

Mice (15 – 40 weeks old) were surgically prepared under general anesthesia by mounting a pedestal as described above²⁷. A recording chamber was built around craniotomies in left and right occipital bones. Extracellular Purkinje cell activity was recorded using borosilicate glass electrodes (OD 2.0 mm, ID 1.16 mm, 2 M NaCl, 4–8 MΩ). Electrodes were advanced by a hydraulic micro-drive (Narishige). Recordings were made from left and right Crus I and II, paramedian lobule, and (para)flocculus (recordings during optokinetic stimulation were from floccular Purkinje cells). Purkinje cells were identified by the brief pause in simple spike activity following each complex spike. The raw signal was amplified, filtered (CyberAmp, CED), digitized (CED) and stored for off-line analysis. Following each recording session the brain was covered with gramicidin-containing ointment and the chamber was sealed with bone wax.

Data Analysis

During *in vitro* experiments signals were recorded onto digital audiotape (DTR-1204; BioLogic; DC to 20 kHz); for analysis, replayed signals were filtered at 2 or 5 kHz (whole-cell and single-channel or loose cell-attached recordings, respectively; -3dB, 8-pole lowpass Bessel) and digitised at 10 kHz (Digidata 1200; Axotape, Molecular Devices). mIPSCs were detected using a scaled template detection method⁵⁰ implemented in IGOR Pro 5.0 (Wavemetrics) with NeuroMatic 1.91 (<http://www.neuromatic.thinkrandom.com>)¹⁶. The decay of synaptic currents was best described by the sum of two exponential functions according to:

$$y = A_1 \exp\left(\frac{-(x - x_0)}{\tau_1}\right) + A_2 \exp\left(\frac{-(x - x_0)}{\tau_2}\right)$$

where x_0 is the decay onset, τ_1 and τ_2 are the decay time constants of the fast and slow components, and A_1 and A_2 are their respective amplitudes. The weighted time constant of decay (τ_w) was calculated according to:

$$\tau_w = \tau_1 \left(\frac{A_1}{A_1 + A_2} \right) + \tau_2 \left(\frac{A_2}{A_1 + A_2} \right)$$

Purkinje cell simple spikes were detected by threshold-crossing. Inter-spike interval (ISI) and peristimulus-time histograms (PSTHs) were generated using NeuroMatic and IGOR Pro 5.0 for spontaneous and parallel fiber-evoked responses, respectively. To determine the number of extra spikes evoked by stimulation, PSTHs were integrated. A linear fit to the pre-stimulus section was extrapolated over the full duration of the integral and subtracted to yield the cumulative spike probability corrected for baseline firing⁷. The number of additional spikes evoked by each stimulus was determined by averaging over a 0-60 ms period.

Off-line analysis of eye movements and *in vivo* recordings was performed in Matlab (MathWorks)²⁷. Gain and phase of eye movements were determined by fitting sine functions to the slow-phase eye velocity traces. Gain was computed as the ratio of eye velocity to stimulus velocity, whereas phase was expressed as the difference (in degrees) between eye velocity and stimulus velocity traces.

In vivo simple spikes and complex spikes were discriminated using custom-made routines based on principal component analysis. Simple spike PSTHs (100 bins/cycle) were compiled at each stimulus frequency and fitted by a sine function. Epochs containing quick phases were deleted from the trace (−50 to +150 ms). Modulation was calculated by dividing the amplitude of the fitted sine wave by its offset. Phase difference was calculated as the difference between the phase of the fitted sine wave and the optokinetic stimulus.

Models and simulations

A phenomenological model of an idealized VOR circuit was created to elucidate the potential role of vestibular nuclei in a phase adaptation paradigm. Elements in the VOR circuit were characterized by the gain and phase of their sinusoidal modulation, which define the coordinates of a position on a polar plot. Plasticity rules were employed phenomenologically, as exponential decay of the modulation along a trajectory in a polar plot, with the target gain and phase (new position in the polar plot) defined by the vestibular mismatch. Equations were solved numerically using Matlab. See Supplementary Material for detail.

Statistical analyses

Statistical tests were performed with GraphPad (Prism 3.0, GraphPad Software Inc.) or with SPSS 11 (SPSS Inc.). Unless stated otherwise, data were compared with two-tailed paired or un-paired Student's *t*-tests, as appropriate. We also used two-way repeated-measures ANOVA, and where data were non-normally distributed (Shapiro-Wilk test), the Mann-Whitney *U*-test. The level of significance was set at $p < 0.05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Reference List

1. Smith SL, Otis TS. Pattern-dependent, simultaneous plasticity differentially transforms the input-output relationship of a feedforward circuit. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102:14901–14906. [PubMed: 16199519]
2. Kullmann DM, Lamsa KP. Long-term synaptic plasticity in hippocampal interneurons. *Nature reviews*. 2007; 8:687–699.
3. Pouille, F.; Scanziani, M. *Science*. Vol. 293. New York, N.Y.: 2001. Enforcement of temporal fidelity in pyramidal cells by somatic feed-forward inhibition; p. 1159–1163.
4. Lamsa K, Heeroma JH, Kullmann DM. Hebbian LTP in feed-forward inhibitory interneurons and the temporal fidelity of input discrimination. *Nature neuroscience*. 2005; 8:916–924.
5. Eccles, JC.; Ito, M.; Szentagothai, J. *The cerebellum as a neuronal machine*. springer; 1967.
6. Miyashita Y, Nagao S. Contribution of cerebellar intracortical inhibition to Purkinje cell response during vestibulo-ocular reflex of alert rabbits. *The Journal of physiology*. 1984; 351:251–262. [PubMed: 6611408]
7. Mittmann W, Koch U, Hausser M. Feed-forward inhibition shapes the spike output of cerebellar Purkinje cells. *The Journal of physiology*. 2005; 563:369–378. [PubMed: 15613376]
8. Barmack NH, Yakhnitsa V. Functions of interneurons in mouse cerebellum. *J Neurosci*. 2008; 28:1140–1152. [PubMed: 18234892]
9. Scelfo B, Sacchetti B, Strata P. Learning-related long-term potentiation of inhibitory synapses in the cerebellar cortex. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:769–774. [PubMed: 18184813]
10. Orduz D, Llano I. Recurrent axon collaterals underlie facilitating synapses between cerebellar Purkinje cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:17831–17836. [PubMed: 17965230]
11. Davie JT, Clark BA, Hausser M. The origin of the complex spike in cerebellar Purkinje cells. *J Neurosci*. 2008; 28:7599–7609. [PubMed: 18650337]
12. Hausser M, Clark BA. Tonic synaptic inhibition modulates neuronal output pattern and spatiotemporal synaptic integration. *Neuron*. 1997; 19:665–678. [PubMed: 9331356]
13. Jorntell 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]
14. Santamaria F, Tripp PG, Bower JM. Feedforward inhibition controls the spread of granule cell-induced Purkinje cell activity in the cerebellar cortex. *Journal of neurophysiology*. 2007; 97:248–263. [PubMed: 17050824]
15. Raman IM, Bean BP. Resurgent sodium current and action potential formation in dissociated cerebellar Purkinje neurons. *J Neurosci*. 1997; 17:4517–4526. [PubMed: 9169512]
16. Wulff P, et al. From synapse to behavior: rapid modulation of defined neuronal types with engineered GABAA receptors. *Nature neuroscience*. 2007; 10:923–929.
17. Schweizer C, et al. The gamma 2 subunit of GABA(A) receptors is required for maintenance of receptors at mature synapses. *Molecular and cellular neurosciences*. 2003; 24:442–450. [PubMed: 14572465]
18. Barski JJ, Dethleffsen K, Meyer M. Cre recombinase expression in cerebellar Purkinje cells. *Genesis*. 2000; 28:93–98. [PubMed: 11105049]

19. Brickley SG, Cull-Candy SG, Farrant M. Single-channel properties of synaptic and extrasynaptic GABAA receptors suggest differential targeting of receptor subtypes. *J Neurosci.* 1999; 19:2960–2973. [PubMed: 10191314]
20. Lorez M, Benke D, Luscher B, Mohler H, Benson JA. Single-channel properties of neuronal GABAA receptors from mice lacking the gamma2 subunit. *The Journal of physiology.* 2000; 527(Pt 1):11–31. [PubMed: 10944167]
21. Brunel N, Hakim V, Isope P, Nadal JP, Barbour B. Optimal information storage and the distribution of synaptic weights: perceptron versus Purkinje cell. *Neuron.* 2004; 43:745–757. [PubMed: 15339654]
22. Shin SL, et al. Regular patterns in cerebellar Purkinje cell simple spike trains. *PLoS ONE.* 2007; 2:e485. [PubMed: 17534435]
23. Walter JT, Alvina K, Womack MD, Chevez C, Khodakhah K. Decreases in the precision of Purkinje cell pacemaking cause cerebellar dysfunction and ataxia. *Nature neuroscience.* 2006; 9:389–397.
24. Ito M. Cerebellar flocculus hypothesis [letter]. *Nature.* 1993; 363:24–25. [PubMed: 8479532]
25. Lisberger SG. Cerebellar LTD: A molecular mechanism of behavioral learning? *Cell.* 1998; 92:701–704. [PubMed: 9529245]
26. Schonewille M, et al. Zonal organization of the mouse flocculus: physiology, input, and output. *The Journal of comparative neurology.* 2006; 497:670–682. [PubMed: 16739198]
27. Hoebeek FE, et al. Increased noise level of purkinje cell activities minimizes impact of their modulation during sensorimotor control. *Neuron.* 2005; 45:953–965. [PubMed: 15797555]
28. Gittis AH, du Lac S. Intrinsic and synaptic plasticity in the vestibular system. *Current opinion in neurobiology.* 2006; 16:385–390. [PubMed: 16842990]
29. Kassardjian CD, et al. The site of a motor memory shifts with consolidation. *J Neurosci.* 2005; 25:7979–7985. [PubMed: 16135754]
30. Medina JF, Mauk MD. Computer simulation of cerebellar information processing. *Nature neuroscience.* 2000; 3(Suppl):1205–1211.
31. Medina JF, Norez WL, Mauk MD. Inhibition of climbing fibres is a signal for the extinction of conditioned eyelid responses. *Nature.* 2002; 416:330–333. [PubMed: 11907580]
32. Shutoh F, Ohki M, Kitazawa H, Itohara S, Nagao S. Memory trace of motor learning shifts transsynaptically from cerebellar cortex to nuclei for consolidation. *Neuroscience.* 2006; 139:767–777. [PubMed: 16458438]
33. 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]
34. Mann-Metzer P, Yarom Y. Electrotonic coupling interacts with intrinsic properties to generate synchronized activity in cerebellar networks of inhibitory interneurons. *J Neurosci.* 1999; 19:3298–3306. [PubMed: 10212289]
35. Van Der Giessen RS, Maxeiner S, French PJ, Willecke K, De Zeeuw CI. Spatiotemporal distribution of Connexin45 in the olivocerebellar system. *The Journal of comparative neurology.* 2006; 495:173–184. [PubMed: 16435305]
36. Steuber V, et al. Cerebellar LTD and pattern recognition by Purkinje cells. *Neuron.* 2007; 54:121–136. [PubMed: 17408582]
37. Blazquez PM, Hirata Y, Highstein SM. Chronic changes in inputs to dorsal Y neurons accompany VOR motor learning. *Journal of neurophysiology.* 2006; 95:1812–1825. [PubMed: 16319196]
38. Medina JF, Lisberger SG. Links from complex spikes to local plasticity and motor learning in the cerebellum of awake-behaving monkeys. *Nature neuroscience.* 2008; 11:1185–1192.
39. De Zeeuw CI, et al. Expression of a protein kinase C inhibitor in Purkinje cells blocks cerebellar LTD and adaptation of the vestibulo-ocular reflex. *Neuron.* 1998; 20:495–508. [PubMed: 9539124]
40. Feil R, et al. Impairment of LTD and cerebellar learning by Purkinje cell-specific ablation of cGMP-dependent protein kinase I. *J Cell Biol.* 2003; 163:295–302. [PubMed: 14568994]
41. Hansel C, et al. alphaCaMKII Is essential for cerebellar LTD and motor learning. *Neuron.* 2006; 51:835–843. [PubMed: 16982427]

42. Boyden ES, et al. Selective engagement of plasticity mechanisms for motor memory storage. *Neuron*. 2006; 51:823–834. [PubMed: 16982426]
43. 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]
44. Duguid IC, Smart TG. Retrograde activation of presynaptic NMDA receptors enhances GABA release at cerebellar interneuron-Purkinje cell synapses. *Nature neuroscience*. 2004; 7:525–533.
45. Mittmann W, Hausser M. Linking synaptic plasticity and spike output at excitatory and inhibitory synapses onto cerebellar Purkinje cells. *J Neurosci*. 2007; 27:5559–5570. [PubMed: 17522301]
46. Watt AJ, et al. Traveling waves in developing cerebellar cortex mediated by asymmetrical Purkinje cell connectivity. *Nature neuroscience*. 2009; 12:463–473.
47. de Solages C, et al. High-frequency organization and synchrony of activity in the purkinje cell layer of the cerebellum. *Neuron*. 2008; 58:775–788. [PubMed: 18549788]
48. Likhtik E, Popa D, Apergis-Schoute J, Fidacaro GA, Pare D. Amygdala intercalated neurons are required for expression of fear extinction. *Nature*. 2008; 454:642–645. [PubMed: 18615014]
49. Stahl JS, van Alphen AM, De Zeeuw CI. A comparison of video and magnetic search coil recordings of mouse eye movements. *J Neurosci Methods*. 2000; 99:101–110. [PubMed: 10936649]
50. Clements JD, Bekkers JM. Detection of spontaneous synaptic events with an optimally scaled template. *Biophysical journal*. 1997; 73:220–229. [PubMed: 9199786]

Figure 1.

PC-Δγ2 mice show normal cerebellar morphology and synaptic organization. (a, b) Nissl stains of sections through vermis (sagittal) and flocculus (coronal) revealed no differences between control (a) and *PC-Δγ2* (b) mice, and the number of Purkinje cells (24.5 ± 2.0 vs 23.9 ± 2.5 cells/1000 μm^2 ; $p = 0.75$) and molecular layer interneurons (2.36 ± 0.19 vs 2.28 ± 0.18 cells/1000 μm^2 ; $p = 0.53$) were similar in both groups. Cb1-10, lobules 1-10; Mol, molecular layer; PC, Purkinje cell layer; Gr, granule cell layer, C, cochlear nucleus. (c-e) Immunofluorescence labelling in the flocculus showed no differences in the distribution of GABAergic terminals (vesicular γ -aminobutyric acid transporter; VGAT) (c), climbing fiber terminals (vesicular glutamate transporter 2; VGLUT2) (d) and parallel fiber terminals (VGLUT1) (e). Quantification of puncta per 1000 μm^2 revealed no difference ($p = 0.27$, 0.62 and 0.68, respectively; $n = 4$). (f-h) Electron microscopy showed no obvious morphological changes of parallel and climbing fiber synapses. (f) Asymmetric synapses between parallel fibers and Purkinje cell spines (asterisks). The density of parallel fiber to Purkinje cell synapses was unchanged (33.0 vs 32.9 synapses/100 μm^2 in *PC-Δγ2* and control; see **Methods**). (g) Asymmetric synapses made by climbing fibers (cf). (h) Symmetric synapses (arrowheads) made by basket cells (BC) onto the cell body of Purkinje cells (PC). Scale bars: (a and b) 250 μm and 50 μm ; (c, d) 20 μm ; (e) 5 μm ; (f) 500 nm; (g) 360 nm; (h) 440 nm.

Figure 2.

Loss of fast synaptic inhibition from Purkinje cells in *PC-Δγ2* mice. **(a)** Representative contiguous segments of whole-cell recording (-70 mV) from a Purkinje cell of a control mouse. Ionotropic glutamate receptors were blocked with CNQX and D-AP5. Lower panel shows quantification of mean synaptic charge in a different Purkinje cell, with a 2.5 s record of sIPSCs and corresponding all-point amplitude histogram. The left-hand peak (most positive current values), corresponding to the baseline current noise, is fitted with a single-sided Gaussian (white). The peak of the histogram is taken as the zero current value (dotted line in inset). The filled grey area corresponds to all sample points other than those within the baseline noise, and thus represents the current produced by phasic synaptic events. In this cell, the mean synaptic charge was 25.9 pC. **(b)** Corresponding data from two *PC-Δγ2* mice. sIPSCs were seen in all cells from control mice but in none from *PC-Δγ2* mice. Slow SR-95531-sensitive currents were seen in ~60% of *PC-Δγ2* cells. For the cell shown in the lower panel, phasic charge transfer was 2.2 pC. On average, the charge transfer was reduced from 59.8 ± 18.4 pC in control ($n = 8$) to 1.0 ± 0.5 pC in *PC-Δγ2* cells ($n = 15$; $p < 0.0002$; Mann-Whitney U -test). **(c)** and **(d)** Corresponding data recorded in the presence of TTX. Note the different scaling of the current record and the abscissa of the all-point histogram and the complete absence of mIPSCs in *PC-Δγ2* cells.

Figure 3.

PC-Δγ2 mice display altered parallel fiber-evoked and spontaneous simple spike firing in vitro and unaltered parallel fiber-Purkinje cell LTP and LTD. **(a)** Simple spikes evoked by parallel fiber activation. Upper and middle panels are raster plots (400 sweeps at 0.5 Hz) and corresponding PSTHs (0.5 ms bin-width). Arrows and dashed lines denote stimulation. Insets show SD of spike latency in a 10 ms window (red bars; 12 control, 11 *PC-Δγ2* cells). Here, and throughout, error bars denote s.e.m. Jitter was greater in *PC-Δγ2* (red) than in control (blue) cells (* $p < 0.0001$). SR-95531 increased jitter in control (* $p = 0.0011$) but not in *PC-Δγ2* cells ($p = 0.605$). Lower panels show global averages of baseline-corrected cumulative spike probability (see **Methods**). Shaded areas denote s.e.m.; 17 control, 13 *PC-Δγ2* cells. Stimulation evoked fewer spikes in *PC-Δγ2* cells (averaged between 0 and 60 ms, $p = 0.0069$). SR-95531 (40 μ M; black line, grey shading) increased spikes in control ($n = 11$; $p = 0.0248$) but not in *PC-Δγ2* cells ($n = 13$; $p = 0.3199$). **(b)** Representative simple spikes (room temperature) and corresponding ISI histograms. Right panels show pooled data (26 control, 9 *PC-Δγ2* cells). Mean firing rate was not significantly different. However, the CV and CV₂ of ISIs differed significantly (* $p < 0.05$) (see text for details). **(c)** Pooled data showing parallel fiber-Purkinje cell LTP in control ($n = 7$, blue) and *PC-Δγ2* cells ($n = 4$, red); EPSC amplitude was similarly increased in the strains (both $p < 0.005$; control vs *PC-Δγ2* $p = 0.257$). **(d)** Parallel fiber-Purkinje cell LTD was similar in *PC-Δγ2* ($n = 5$) and control ($n = 4$) cells (both $p < 0.05$; control vs *PC-Δγ2* $p = 0.624$).

Figure 4.

Motor learning is severely affected in *PC-Δγ2* mice. **(a)** Illustration of drum and table rotation during the training paradigm. Traces show sinusoidal drum rotation (black) and examples of eye movement (control, blue; *PC-Δγ2*, red). Gain and phase parameters were evaluated 5 times at 10 min intervals. **(b)** On day 1 *PC-Δγ2* and control mice showed similar gain reduction ($p = 0.11$), but the first test on day 2 revealed clear differences ($p = 0.001$) (upper panel). During phase reversal training, control mice learned better than *PC-Δγ2* mice (day 4 $p < 0.00001$) (lower panel). **(c)** Differences in gain consolidation and phase reversal occurred over a wide range frequencies. “Day x before” and “Day x after” indicate values before and after training on day “ x ”; “24hr after” indicates the value on the next day, just before a new measurement. **(d)** Upper panel: differences in consolidation (percentage change carried forward from the previous day) for gain decrease (day 1 to 2) and phase reversal (day 2 to 3 and day 3 to 4). Lower panel: differences in gain consolidation were also seen with constant *in phase* drum and table rotation (gain decrease; two histograms on the left), and with constant *out of phase* drum and table rotation (gain increase; histogram on the right). For the lower panel in **(d)** data are from 5 control and 6 *PC-Δγ2* mice, for all other panels, data are from 10 control and 9 *PC-Δγ2* mice. Error bars denote s.e.m.; * and ** $p < 0.05$ and 0.01 .

Figure 5.

Temporal patterns of simple spike activities of floccular Purkinje cells are specifically affected in *PC-Δγ2* mice, both during compensatory eye movement behaviour and during spontaneous behaviour. (a) Representative single unit activity recorded from Purkinje cells in the flocculus of a control and a *PC-Δγ2* mouse during fixed velocity (8°/s, 0.2 Hz) OKR stimulation. The visual stimulus and eye position are shown together with histograms of simple spike and complex spike frequencies and corresponding raster plots. (b) Firing frequency, phase relative to stimulus and amplitude of modulation (see **Methods**) of floccular simple spike activities during optokinetic stimulation (8°/s, 0.1 - 1.6 Hz) were not significantly different among *PC-Δγ2* and control mice. (c) Although average firing frequency of simple and complex spike activity did not differ between *PC-Δγ2* and control mice, the coefficient of variation (CV) of simple spikes in *PC-Δγ2* mice was significantly reduced in recordings both with and without visual stimuli ($p = 0.008$ and $p = 0.022$, respectively). Also, CV_2 values of simple spikes were significantly lower than those of controls in both conditions. Error bars denote s.e.m., * denotes $p < 0.05$, ** $p < 0.01$ and *** $p < 0.0001$.

Figure 6.

Interpretation of VOR adaptation data using a 'distributed memory' model. (a) Modelled activation of parallel fibers and interneurons plotted in polar coordinates. Most parallel fibers modulate in phase with ipsilateral head movement (0°), while a fraction responds to input from the contralateral horizontal canal (180°). Interneurons are modeled similarly, but with opposite sign representing their inhibitory nature. (b) Same as (a), but for *PC-Δγ2* mice lacking inhibition. (c) Maximum simple spike modulation attainable by appropriate depression and potentiation of excitatory and inhibitory inputs shown in (a) and (b) for control (blue) and *PC-Δγ2* (red) mice (based on linear input summation). (d) Modulation of target vestibular nucleus neurons attainable by linear summation of mossy fiber inputs (blue arrow, in phase with head movement) and Purkinje cell inputs (panel (c), blue curve) in control mice. The black arrow represents the efficacy of mossy fiber input prior to training. (e) Same as (d), but for *PC-Δγ2* mice, where the efficacy of plasticity at the mossy fiber synapses is presumably impaired. (f) Limited simple spike modulation and mossy fiber plasticity restrain eye movements in *PC-Δγ2* mice (red curve) as compared to control mice (blue curve). The control curve also covers the area of VOR phase reversal, from out-of-phase with head movement (180° in this figure) to in-phase (0°). (g) Experimental data; squares represent VOR gain and dashed lines represent VOR phase relative to the head (shifted by 180°, for ease of illustration) (see also Fig. 4). For each session, after initial adaptation, the learned Purkinje cell signal determines the new 'desired' phase and gain state for the vestibular nucleus neurons (dashed and solid black bars, respectively). The superimposed blue (control) and red (*PC-Δγ2*) arrows indicate the direction of change. (h) Simulation of the training paradigm shown in g. During training, Purkinje cells rapidly approach their target modulation (I), reflecting short-term VOR adaptation. Purkinje cell-guided plasticity of mossy fiber input to vestibular nuclei (II) allows control mice to gradually adapt the phase of their VOR during prolonged training (III). In *PC-Δγ2* mice, loss of vestibular nucleus consolidation impairs phase adaptation. For simplicity, adaptation in the vestibular nuclei and partial extinction of cortical memory were simulated to occur between training sessions (grey bars).