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Nothing can be coincidence: synaptic inhibition and plasticity in the cerebellar nuclei

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Abstract

Many cerebellar neurons fire spontaneously, generating 10–100 action potentials per second even without synaptic input. This high basal activity correlates with information-coding mechanisms that differ from those of cells that are quiescent until excited synaptically. For example, in the deep cerebellar nuclei, Hebbian patterns of coincident synaptic excitation and postsynaptic firing fail to induce long-term increases in the strength of excitatory inputs. Instead, excitatory synaptic currents are potentiated by combinations of inhibition and excitation that resemble the activity of Purkinje and mossy fiber afferents that is predicted to occur during cerebellar associative learning tasks. Such results indicate that circuits with intrinsically active neurons have rules for information transfer and storage that distinguish them from other brain regions.

Introduction

Neurons and synapses throughout the vertebrate brain share many common properties, which have given rise to the useful generalizations taught in introductory neuroscience courses: neurons rest near -65 mV; voltage-gated Na^+ channels inactivate after opening; depletion of a presynaptic neurotransmitter produces synaptic depression; and long-term potentiation (LTP) results from coincident presynaptic glutamate release and postsynaptic depolarization. Current research, however, is revealing the diversity of electrical and chemical signaling mechanisms employed by neurons in different brain regions. One such area is the cerebellum, particularly regarding signaling from Purkinje neurons to their target neurons in the medial, interpositus and lateral cerebellar nuclei. Purkinje neurons, located in the cerebellar cortex, are among the most studied cells in the vertebrate brain. By contrast, despite being the oblique referent of the frequently quoted statements that Purkinje neurons ‘are the sole output of the cerebellar cortex’ and ‘exert a powerful inhibitory control on their targets’, cerebellar nuclear neurons have been the focus of far fewer studies, and exploration of their properties at the cellular level is only beginning to become widespread. Recent work has demonstrated that these neurons and the synapses onto them provide an exception to all the generalizations just mentioned. These deviations from the norm are interesting, not only because they demand that we re-examine the bases for common assumptions but also because they reveal mechanisms underlying specific, well-characterized forms of cerebellar learning.

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Spontaneous firing in cerebellar neurons

Neither Purkinje neurons nor cerebellar nuclear cells have a true resting potential. Instead, both cell types are active in the basal state, firing tens of action potentials per second *in vivo* when animals are not engaged in cerebellar behaviors [1,2]. This activity is intrinsically generated: the neurons continue to fire spontaneously *in vitro* even in the absence of synaptic input [3–9]. This high level of basal activity keeps the firing rates of the neurons in the middle of their dynamic range because these rates can be increased by synaptic excitation and decreased by synaptic inhibition. The conservation of spontaneous firing in cerebellar neurons across vertebrate species, from turtles to primates [2,3,7,10,11], indicates that this feature has an adaptive role in cerebellar regulation of coordinated movements. Moreover, because Purkinje neurons release γ -aminobutyric acid (GABA), the cerebellum is unusual in that its principal neurons are intrinsically active cells that communicate by inhibition, in contrast to the cerebral cortex and hippocampus, in which generally silent principal cells communicate by excitation.

Spontaneous firing in Purkinje cells (studied in mice and rats) arises from specialized ionic currents including voltage-gated Na^+ currents with a ‘resurgent’ component and high-voltage-activated K^+ currents [6,11,12]. During the upstroke of an action potential, Purkinje Na^+ channels open with depolarization but are rapidly blocked by an endogenous protein. The blocking protein occludes current flow but protects channels from fast inactivation. On the downstroke of the action potential, the blocker is expelled from Na^+ channels, which pass current briefly before closing. The process of voltage-dependent block and unblock during a spike thereby largely bypasses fast inactivation, increases Na^+ channel availability and shortens the absolute refractory period of the cell. Meanwhile, the very large K^+ currents, which flow primarily through $\text{K}_{\text{V}3}$ channels [13], provide a strong, rapid repolarization. These K^+ currents deactivate rapidly after each spike, however, quickly restoring a high input resistance. The small Na^+ currents that remain active at interspike potentials can therefore depolarize the cell and initiate another action potential [11,13]. The interaction among these intrinsic currents leads Purkinje cells to generate ~50 action potentials per second in the absence of synaptic input.

An interesting consequence of the high spontaneous firing rate of Purkinje neurons is that cerebellar nuclear neurons receive far more basal inhibition than excitation. This observation raises the question of how they manage to fire at all. Most excitatory input to cerebellar nuclear neurons arises from mossy fibers, the intrinsic activity of which is low [14]. By contrast, each cerebellar nuclear cell receives extensive inhibitory synaptic contacts on its soma and proximal dendrites from tens to hundreds of Purkinje cells [15]. Given that spontaneous spikes in Purkinje cells propagate reliably along the axons [16,17], each nuclear cell potentially receives thousands of inhibitory postsynaptic potentials (IPSPs) per second. Despite this tremendous amount of basal inhibition, cerebellar nuclear neurons persist in firing tens of spikes per second *in vivo* [2,18]. A simple explanation for how these cells maintain spontaneous firing is provided by studies of cerebellar nuclear neurons that have been acutely isolated from the mouse cerebellum. Recordings from these neuronal somata, which continue to fire spontaneously in isolation, demonstrate that the cells have few K^+ channels open at subthreshold potentials. Instead, the primary intrinsic conductance that is active at hyperpolarized voltages is a small, voltage-independent, non-specific cationic leak with a reversal potential near -30 mV, which is referred to as the ‘tonic cation current’ or ‘undercurrent’ [9]. The molecular identity of the channel responsible for this current is not known, but it shares many properties with the recently identified channel *NACLN1* [19]. When cerebellar nuclear neurons are bathed in tetrodotoxin (TTX), the tiny K^+ currents and larger undercurrent lead cells to rest near -40 mV, which is a voltage that is normally suprathreshold (Figure 1a,b). In physiological solutions, therefore, as currents equilibrate after each spike, voltage-gated Na^+ channels are activated, threshold is crossed and another action potential is generated. Thus, spontaneous firing is maintained in a

simpler manner than that employed by Purkinje cells and one suited to neurons faced with chronic inhibition: cerebellar nuclear cells simply try to rest above threshold.

Moreover, in many nuclear neurons, the voltage-gated K^+ currents that open during an action potential deactivate rapidly. As a result, the voltage during the interspike interval remains near -50 mV. These shallow interspike potentials permit voltage-gated Na^+ channels to recover only modestly from fast inactivation [9]. Because Na^+ channels in nuclear cells are also particularly prone to slow inactivation, the neurons operate with a low availability of Na^+ channels despite the presence of resurgent Na^+ current [20]. In slice preparations, although spontaneous firing persists, even a short bout of rapid firing evoked by current injection or synaptic excitation can easily drive these cells into a state of depolarization block that outlasts the depolarizing stimulus, and firing usually resumes only after an active hyperpolarization (Figure 1c). In other words, cerebellar nuclear neurons often need to be *hyperpolarized* to threshold; under physiological conditions, it is likely to be the tonic inhibition from Purkinje cells that keeps them firing.

Minimizing inhibitory synaptic depression in spontaneously firing neurons

During cerebellar behaviors, increased activity in Purkinje neurons can and does suppress firing by nuclear neurons [21,22]. Studies in cerebellar slice preparations indicate that silencing of nuclear cells requires a consistent inhibitory shunt, which might be achieved when Purkinje cell afferents fire coherently and/or at rates exceeding the basal level. Conversely, any lapse of the inhibitory conductance leads to the resumption of firing [22–24].

The constant basal and driven activity of Purkinje cells raises the question of how Purkinje terminals avoid chronic depletion of neurotransmitter. When Purkinje axons are stimulated at and above basal firing rates, the peak amplitudes of inhibitory postsynaptic currents (IPSCs) recorded in cerebellar nuclear neurons depress only modestly even when stimulation continues for more than a second [23]. Interestingly, the terminals of Purkinje neurons have unusual features that have long been noticed in biochemical and electron microscopic studies: they fail to take up tritiated GABA and have many presynaptic densities per bouton [15,25,26]. The former property is a reflection of the fact that they, like their nuclear cell targets, lack GABA transporters; the only GABA transporters in the vicinity of the nuclei are in astrocytes that surround the boutons without intervening between densities [25,26]. As a result, upon fusion of a single vesicle, GABA reaches not only the postsynaptic receptors immediately opposite the release site but also receptors in neighboring postsynaptic densities, before being taken up by astrocytic transporters. Because of this ‘spillover’ of neurotransmitter, even a very low release probability of each presynaptic vesicle generates a high probability that GABA_A receptors at most postsynaptic densities opposite a single bouton will be exposed to GABA on the time scale of the rising phase of the IPSC [26,27]. Thus, each release site takes its turn at activating receptors in multiple postsynaptic densities, which conserves vesicles and delays depletion (Figure 2a,b).

Rebound bursts of cerebellar nuclear cells

A much-explored feature of nuclear cells is their tendency to fire action potentials after periods of hyperpolarization [8,28,29]. These ‘rebound spikes’ often consist of a short series of action potentials produced at a higher rate than those occurring before the hyperpolarization.

Alternatively, they sometimes form a true burst of high-frequency action potentials. Although the ionic mechanisms underlying the accelerated firing or the true burst are likely to differ, in both cases the rebound spikes ride on a ‘rebound depolarization’ [8] that directly or indirectly elicits an increase in intracellular Ca^{2+} . This Ca^{2+} rise has been implicated in plasticity of both intrinsic excitability and IPSCs [30,31]. A likely candidate for a voltage-gated Ca^{2+} source that is recruited by periods of hyperpolarization is T-type Ca^{2+} current. Consistent with this

idea, imaging studies indicate that low-voltage-activated currents, which include but need not be limited to T-type currents, generate Ca^{2+} (or Ba^{2+}) increases that are particularly large in nuclear cell dendrites [32,33]. The expression of specific Ca_v3 (T-type) channel subunits also correlates with the propensity of nuclear cells to fire rebound bursts [34]. Moreover, recent work that includes voltage-clamp studies of the ‘rebound currents’ underlying the depolarization has shown that nuclear neurons with stronger (higher frequency) bursts tend to have larger T-type Ca^{2+} currents [35], and, in some cases, rebound bursts can be blocked by drugs that can block T-type channels [36] (see also Ref. [8]).

An open question, however, is the extent to which synaptic inhibition alone can recruit T-type currents. In most published studies to date, the hyperpolarizations preceding the rebound have been evoked by current injection, with or without concurrent synaptic inhibition. As a result, the membrane potential was hyperpolarized further during the conditioning step than is predicted from GABA_A -receptor-mediated synaptic inhibition. Sharp microelectrode recordings from cerebellar nuclear neurons estimate the Cl^- equilibrium potential (E_{Cl}) to be near -75 mV [8,37], and GABA_B receptors are apparently not activated even by high-frequency trains of inhibition [5,26,38]; in fact, when high-frequency IPSP trains have been recorded without current injection, the membrane potential falls short of E_{Cl} by 6–10 mV [23,39,40]. Because most T-type currents remain largely inactivated at voltages more depolarized than -70 mV [41], the contribution of T-type currents to post-inhibitory rebound responses, at least as recruited by fast synaptic inhibition, might be small.

Consistent with this idea, recent work indicates that stimulation of Purkinje afferents *in vivo* does not always activate post-inhibitory bursts of action potentials [40]. Nevertheless, the high incidence of T-type Ca^{2+} channels targeted to the dendrites of nuclear cells indicates that physiological conditions that favor their activation must exist. It is perhaps relevant that whether or not rebound bursts are generated depends strongly on the preparation, recording conditions and the particular cells selected for recording. Adding to the number of variables, rebound bursts are likely to be influenced by the availability and activation of multiple currents, including Na^+ channels [20], high-voltage-activated Ca^+ channels [42], SK channels [8] and/or hyperpolarization-activated cation currents. The current debates in the field, regarding conditions that elicit post-inhibitory bursts and the role of specific channel types in these bursts, are likely to be resolved by future studies that explore and explain both the natural and the experimentally induced heterogeneity of rebound firing.

Information coding in cerebellar circuits

The question of how information is encoded in a circuit with substantial ongoing activity has been approached in many ways, starting with an analysis of the anatomy of cerebellar circuits and the ‘sign’ of synaptic transmission. Purkinje neurons, nuclear neurons and inferior olivary neurons in the brainstem form a trisynaptic, functionally inhibitory loop with the activity of Purkinje and nuclear neurons modulated by excitatory mossy fiber afferents [43,44] (Figure 3a). Specifically, spontaneously firing Purkinje neurons can be further excited by parallel fiber afferents from granule cells, which are excited by mossy fibers. Purkinje cells inhibit the spontaneously firing neurons of the cerebellar nuclei. Cerebellar nuclear neurons can be further excited by mossy fibers and, to a lesser extent, by inferior olivary fibers. Cerebellar nuclear cells form the output of the cerebellum, with glutamatergic nuclear neurons projecting to premotor areas, such as the red nucleus, and GABAergic nuclear neurons projecting back to the inferior olive. Olivary cells fire spontaneously at ~ 1 –10 Hz [45]; their axons form climbing fibers, which form synapses onto Purkinje cells, the activity of which leads to long-term depression (LTD) of parallel fibers [46]. By depressing the primary source of synaptic excitation to Purkinje cells, climbing fiber activity has a net inhibitory effect on Purkinje cells.

This network thus forms a negative feedback loop: perturbations that increase the activity of Purkinje cells will increase the inhibition of nuclear cells thereby disinhibiting olivary cells and promoting LTD of parallel fibers. The decreased drive from parallel fibers shifts Purkinje cell activity back toward basal levels. Thus, the network can self-regulate until it reaches a new equilibrium, at which a given output can be generated by a different set of stimuli than before the perturbation. These ideas have been elaborated explicitly in models of delay eyelid conditioning [43] (see also Ref. [47]). In this cerebellar associative learning task, animals learn to associate a neutral 'conditioned' stimulus, such as a tone, with a reflex-eliciting 'unconditioned' stimulus, such as a puff of air to the eye that elicits eyelid closure. After repeated pairings of tone and puff, the animal learns to close its eyelid late in the tone, just before the puff [48]. Conditioned- and unconditioned-stimulus information is carried by mossy fibers and climbing fibers, respectively [49–51]. Because firing by cerebellar nuclear cells promotes eyelid closure, learning must ultimately increase the responsiveness of nuclear neurons to mossy fiber activity.

Behavioral and modeling studies indicate that this learning process occurs in two stages, with synaptic and/or excitability changes occurring first at the level of Purkinje cells [52,53] and second at the level of nuclear cells [54–56]. The nature of the long-term plasticity in the cerebellar nuclei (or indeed whether it occurs at all) has been a matter of debate [57,58]. One proposal with considerable experimental support from whole-animal studies is that learning involves an instructive signal from Purkinje cells that ultimately strengthens mossy fiber inputs onto nuclear neurons [43].

Coincidence detection in spontaneously active neurons

This hypothesis (the Medina-Mauk model) raises the question of how inhibition might generate a signal detected by excitatory synapses and, more generally, what synaptic, spike-related and biochemical events can trigger a long-lasting modulation of cerebellar output. In other brain regions, LTP of excitatory synaptic responses results from coincidence detection of signals that, together, permit a substantial Ca^{2+} influx. Perhaps the best studied example is provided by CA1 hippocampal pyramidal cells: when presynaptic release of glutamate temporally overlaps with postsynaptic spiking, *N*-methyl-D-aspartic acid (NMDA) receptors pass Ca^{2+} because they are simultaneously activated by glutamate and relieved of Mg^{2+} block by depolarization. The resultant rise in intracellular Ca^{2+} triggers the processes that ultimately increase synaptic strength [59].

The properties of cerebellar nuclear cells present multiple complicating factors for a CA1-like plasticity rule. The most salient is that, because of spontaneous firing, any mossy-fiber-dependent excitatory postsynaptic currents (EPSPs) has a reasonable chance of overlapping temporally with an action potential in a nuclear cell. Therefore, the Hebbian condition of correlated pre- and postsynaptic spiking arises randomly, which is predicted to yield a noisy signal. Furthermore, the NMDA receptors themselves are unusual. Cerebellar nuclear cells express the NR2D subunit, which generates channels that are blocked only weakly by Mg^{2+} [60]. In physiological solutions, therefore, NMDA-receptor-mediated current is evoked even at negative potentials [30,61–63], particularly during trains of EPSCs [64]. Thus, postsynaptic spiking does not gate NMDA-receptor-mediated synaptic Ca^{2+} influx: NMDA receptors are not coincidence detectors in the cerebellar nuclei as they are elsewhere in the brain.

These peculiarities constrain the possible learning rules but fit neatly into the activity that is present in the circuit. During acquisition and expression of conditioned responses, conditioned and unconditioned stimuli probably do not simply produce a strong activation of nuclear cells. Instead, stimulus-induced mossy fiber activity is likely to excite nuclear cells directly while simultaneously inhibiting them via the granule–Purkinje–nuclear cell pathway. If synaptic

strengths in the cerebellar nuclei are indeed changed during cerebellar learning [43,65], it is possible that the appropriate trigger for the modulation of mossy-fiber-mediated EPSCs might be coincident excitation and inhibition, approximating the pattern of activity predicted during training.

Synaptic plasticity at mossy fiber synapses onto nuclear cells

This possibility was tested by recording EPSCs from nuclear cells in mouse cerebellar slices before and after application of a variety of combinations of excitatory and inhibitory stimuli [64]. The putative induction protocols included a high-frequency train of EPSPs evoked by stimulation of mossy fibers, with or without a period of hyperpolarization (induced by current injection) followed by rebound spiking. Consistent with the Medina-Mauk model of cerebellar learning, trains of action-potential-generating EPSPs alone do not induce long-term plasticity [64]; in fact, separate studies indicate that patterns of synaptic excitation and postsynaptic depolarization can generate LTD [66]. EPSCs are potentiated by high-frequency synaptic excitation applied before hyperpolarizing steps that permit a post-inhibitory rebound depolarization [64]. The effective sequence of stimulation remains constant whether or not cells are permitted to fire spontaneous action potentials and whether or not 'real' synaptic inhibition replaces the hyperpolarizing current injection [67] (Figure 3b). Therefore, potentiation of EPSCs in cerebellar nuclear cells follows non-Hebbian plasticity rules that are compatible with spontaneous firing.

Despite the requirement for inhibition, this form of potentiation resembles CA1 LTP in that it requires NMDA receptor activation and is blocked by postsynaptic BAPTA [1,2-bis(o-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid], which indicates the need for postsynaptic Ca^{2+} influx [64]. Unlike CA1 LTP, however, drugs that prevent the activation of calcineurin also block the induction of potentiation [67]. The involvement of this Ca^{2+} -dependent phosphatase, which is often engaged by NMDA receptor activation, provides an interesting parallel with plasticity in the cerebellar cortex. Potentiation of parallel fiber EPSCs in Purkinje cells is also calcineurin dependent [68]; this is an observation that has motivated the suggestion that the cerebellum uses coding schemes that are inverted with respect to the hippocampus and cerebral cortex [69]. In the cerebellar nuclei, however, plasticity is also blocked by drugs that interfere with calcium/calmodulin-dependent protein kinase II (CaMKII) [67], indicative of a link with the sister synapses of the cerebellar nuclei, namely Purkinje cell targets in the medial vestibular nuclei. In those neurons, CaMKII is tonically activated by Ca^{2+} influx evoked by spontaneous firing. Synaptic inhibition reduces CaMKII activity, thereby modulating intrinsic excitability [70]. Whether a comparable activation and suppression of CaMKII occurs in the cerebellar nuclei is unknown; nevertheless, the sensitivity of potentiation to CaMKII blockade indicates that the induction of plasticity requires multiple biochemical cascades. One possibility is that NMDA-receptor-mediated Ca^{2+} influx provides a local signal at synapses (possibly by activating calcineurin), whereas the hyperpolarization and/or rebound provide a global signal (possibly by modulating CaMKII) that enables potentiation to proceed only at previously marked synapses [67].

Relating cellular plasticity to behavioral studies

The effective induction protocols in the cerebellar nuclei conform in many respects to predicted patterns of excitation and inhibition predicted to take place during cerebellar learning. First, plasticity is synapse specific [67], consistent with the lack of stimulus generalization of conditioned responses in rabbits, even after cerebellar cortical control is eliminated [71]. Second, synaptic excitation must occur in a specific time window to induce potentiation, preceding the hyperpolarization and rebound by no more than a few hundred milliseconds. Similarly, during eyelid conditioning, concurrent excitation and inhibition last for one to a few

hundred milliseconds during the initial portion of the conditioned stimulus, whereas a relief of inhibition occurs during the late part of the conditioned stimulus and possibly the unconditioned stimulus [21,54]. Finally, strengthening of mossy fiber EPSCs, which is likely to facilitate firing in response to the conditioned stimulus alone, is the type of change that is expected to permit the expression of conditioned responses, including the ill-timed, short-latency responses that persist after Purkinje cells are lesioned after training [72]. These points of correspondence between the cellular plasticity and the behavior make this form of potentiation an attractive possibility as a mechanism that contributes to learning.

Nevertheless, linking these synaptic changes to the behavioral changes evident *in vivo* will require more precise measurements *in vitro*. For instance, a specific region of the cerebellar cortex required for acquisition of conditioned eyelid responses has been identified in whole-animal experiments [54,72]. Ideally, cellular studies will eventually focus on the targets of these Purkinje cells. Such work will probably necessitate labeling of specific subsets of nuclear cells; at present, few slice studies have distinguished cerebellar nuclear neurons anatomically, beyond localizing them to a particular nucleus. As a first step toward recording from identified cerebellar nuclear cells, mice with green-fluorescent-protein-labeled inhibitory neurons have recently been developed, and studies of these animals are revealing differences between glutamatergic neurons and inhibitory cells that project back to the inferior olive [73,74].

Concluding remarks

The cellular characteristics of cerebellar nuclear neurons are distinct from those of many other principal cells. They fire spontaneously by seeking a suprathreshold resting potential, and the basal activity of a multitude of Purkinje afferents makes Cl^- influx through GABA_A receptors act as a virtual intrinsic current that keeps nuclear cells in the middle of their dynamic range. The non-Hebbian rules for potentiation of EPSCs in cerebellar nuclear neurons, which require synaptic excitation and inhibition to coincide, therefore seem to be compatible with both the intrinsic firing properties and the intense inhibitory innervation of nuclear cells. This correlation raises the question of whether this form of potentiation is a quirk of the cerebellar nuclear circuit or whether similar mechanisms might be present in other spontaneously active neurons that receive strong inhibitory inputs. At least two classes of neurons emerge as candidates for such plasticity: neurons of the medial vestibular nuclei, which are also Purkinje target neurons, and neurons of the subthalamic nuclei, which share many intrinsic properties with cerebellar nuclear cells [75].

More generally, the negative-feedback nature of the trisynaptic olivo-cerebellar loop indicates that both long- and short-term plasticity are likely to be the rule rather than the exception for cells in this circuit: modulation of activity in one set of synapses is expected to lead to measurable compensation elsewhere. In this context, it is interesting that ion channel mutations that disrupt basal activity of Purkinje cells correlate with movement deficits and also with compromised cerebellar learning [76–78]. These observations support the proposal that the modulation that usually occurs during motor control is sensitive to spontaneous firing in Purkinje cells. Studies of synaptic inhibition and potentiation in the cerebellar nuclei of animals with movement disorders have the potential to provide insight into the relationship between cellular plasticity and motor behavior under both normal and pathophysiological conditions.

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References

1. Granit R, Phillips CG. Excitatory and inhibitory processes acting upon individual Purkinje cells of the cerebellum in cats. *J Physiol* 1956;133:520–547. [PubMed: 13368102]
2. Thach WT. Discharge of Purkinje and cerebellar nuclear neurons during rapidly alternating arm movements in the monkey. *J Neurophysiol* 1968;31:785–797. [PubMed: 4974877]
3. Llinás R, Sugimori M. Electrophysiological properties of *in vitro* Purkinje cell somata in mammalian cerebellar slices. *J Physiol* 1980;305:171–195. [PubMed: 7441552]
4. Jahnsen H. Electrophysiological characteristics of neurones in the guinea pig deep cerebellar nuclei *in vitro*. *J Physiol* 1986;372:129–147. [PubMed: 3723407]
5. Mougnot D, Gahwiler BH. Characterization of synaptic connections between cortex and deep nuclei of the rat cerebellum *in vitro*. *Neuroscience* 1995;64:699–712. [PubMed: 7715782]
6. Raman IM, Bean BP. Resurgent sodium current and action potential formation in dissociated cerebellar Purkinje neurons. *J Neurosci* 1997;17:4517–4526. [PubMed: 9169512]
7. Häusser M, Clark BA. Tonic synaptic inhibition modulates neuronal output pattern and spatiotemporal synaptic integration. *Neuron* 1997;19:665–678. [PubMed: 9331356]
8. Aizenman CD, Linden DJ. Regulation of the rebound depolarization and spontaneous firing patterns of deep nuclear neurons in slices of rat cerebellum. *J Neurophysiol* 1999;82:1697–1709. [PubMed: 10515960]
9. Raman IM, et al. Ionic currents and spontaneous firing in neurons isolated from the cerebellar nuclei. *J Neurosci* 2000;20:9004–9016. [PubMed: 11124976]
10. Hounsgaard J. Pacemaker properties of mammalian Purkinje cells. *Acta Physiol Scand* 1979;106:91–92. [PubMed: 463582]
11. Raman IM, Bean BP. Ionic currents underlying spontaneous action potentials in isolated cerebellar Purkinje neurons. *J Neurosci* 1999;19:1663–1674. [PubMed: 10024353]
12. Khaliq ZM, et al. The contribution of resurgent sodium current to high-frequency firing in Purkinje neurons: an experimental and modeling study. *J Neurosci* 2003;23:4899–4912. [PubMed: 12832512]
13. Akemann W, Knöpfel T. Interaction of Kv3 potassium channels and resurgent sodium current influences the rate of spontaneous firing of Purkinje neurons. *J Neurosci* 2006;26:4602–4612. [PubMed: 16641240]
14. Shinoda Y, et al. Functional significance of excitatory projections from the precerebellar nuclei to interpositus and dentate nucleus neurons for mediating motor, premotor, and parietal cortical inputs. *Prog Brain Res* 1997;114:193–207. [PubMed: 9193145]
15. Chan-Palay, V. Organization, Cytology, and Transmitters. Springer-Verlag; 1977. Cerebellar Dentate Nucleus.
16. Khaliq ZM, Raman IM. Axonal propagation of simple and complex spikes in cerebellar Purkinje neurons. *J Neurosci* 2005;25:454–463. [PubMed: 15647489]
17. Monsivais P, et al. Determinants of action potential propagation in cerebellar Purkinje cell axons. *J Neurosci* 2005;25:464–472. [PubMed: 15647490]
18. McDevitt CJ, et al. Relationships between simultaneously recorded Purkinje cells and nuclear neurons. *Brain Res* 1987;425:1–13. [PubMed: 3427412]
19. Lu B, et al. The neuronal channel NALCN contributes resting sodium permeability and is required for normal respiratory rhythm. *Cell* 2007;129:371–383. [PubMed: 17448995]
20. Aman TK, Raman IM. Subunit dependence of Na channel open-channel block and slow inactivation in cerebellar neurons. *Biophys J* 2007;92:1938–1951. [PubMed: 17189307]
21. Medina JF, et al. Mechanisms of cerebellar learning suggested by eyelid conditioning. *Curr Opin Neurobiol* 2000;10:717–724. [PubMed: 11240280]
22. Gauck V, Jaeger D. The control of rate and timing of spikes in the deep cerebellar nuclei by inhibition. *J Neurosci* 2000;20:3006–3016. [PubMed: 10751453]
23. Telgkamp P, Raman IM. Depression of inhibitory synaptic transmission between Purkinje cells and neurons of the cerebellar nuclei. *J Neurosci* 2002;22:8447–8457. [PubMed: 12351719]
24. Gauck V, Jaeger D. The contribution of NMDA and AMPA conductances to the control of spiking in neurons of the deep cerebellar nuclei. *J Neurosci* 2003;23:8109–8118. [PubMed: 12954873]

25. Ribak CE, et al. Astrocytic processes compensate for the apparent lack of GABA transporters in the axon terminals of cerebellar Purkinje cells. *Anat Embryol (Berl)* 1996;194:379–390. [PubMed: 8896702]
26. Telgkamp P, et al. Maintenance of high-frequency inhibitory transmission at Purkinje to cerebellar nuclear synapses by spillover from boutons with multiple release sites. *Neuron* 2004;41:113–126. [PubMed: 14715139]
27. Pugh JR, Raman IM. GABA_A receptor kinetics in the cerebellar nuclei: evidence for detection of transmitter from distant release sites. *Biophys J* 2005;88:1740–1754. [PubMed: 15626699]
28. Llinás R, Mühlethaler M. Electrophysiology of guinea-pig cerebellar nuclear cells in the *in vitro* brain stem-cerebellar preparation. *J Physiol* 1988;404:241–258. [PubMed: 2855348]
29. Czubayko U, et al. Two types of neurons in the rat cerebellar nuclei as distinguished by membrane potentials and intracellular fillings. *J Neurophysiol* 2001;85:2017–2029. [PubMed: 11353018]
30. Aizenman CD, Linden DJ. Rapid, synaptically driven increases in the intrinsic excitability of cerebellar deep nuclear neurons. *Nat Neurosci* 2000;3:109–111. [PubMed: 10649564]
31. Aizenman CD, et al. Polarity of long-term synaptic gain change is related to postsynaptic spike firing at a cerebellar inhibitory synapse. *Neuron* 1998;21:827–835. [PubMed: 9808468]
32. Muri R, Knöpfel T. Activity induced elevations of intracellular calcium concentration in neurons of the deep cerebellar nuclei. *J Neurophysiol* 1994;71:420–428. [PubMed: 8158239]
33. Gauck V, et al. Spatial distribution of low- and high-voltage-activated calcium currents in neurons of the deep cerebellar nuclei. *J Neurosci* 2001;21:RC158. [PubMed: 11459879]
34. Molineux ML, et al. Specific T-type calcium channel isoforms are associated with distinct burst phenotypes in deep cerebellar nuclear neurons. *Proc Natl Acad Sci U S A* 2006;103:5555–5560. [PubMed: 16567615]
35. Molineux ML, et al. Ionic factors governing rebound burst phenotype in rat deep cerebellar neurons. *J Neurophysiol* 2008;100:2684–2701. [PubMed: 18768644]
36. Alviña K, et al. T-type calcium channels mediate rebound firing in intact deep cerebellar neurons. *Neuroscience*. 2008. (www.sciencedirect.com)
37. Jahnsen H. Extracellular activation and membrane conductances of neurones in the guinea pig deep cerebellar nuclei *in vitro*. *J Physiol* 1986b;372:149–168. [PubMed: 2425083]
38. Morishita W, Sastry BR. Pharmacological characterization of pre- and postsynaptic GABA_B receptors in the deep nuclei of rat cerebellar slices. *Neuroscience* 1995;68:1127–1137. [PubMed: 8544987]
39. McKay BE, et al. Kv1 K⁺ channels control Purkinje cell output to facilitate postsynaptic rebound discharge in deep cerebellar neurons. *J Neurosci* 2005;25:1481–1492. [PubMed: 15703402]
40. Alviña K, et al. Questioning the role of rebound firing in the cerebellum. *Nat Neurosci* 2008;11:1256–1258. [PubMed: 18820695]
41. Perez-Reyes E. Molecular physiology of low-voltage-activated t-type calcium channels. *Physiol Rev* 2003;83:117–161. [PubMed: 12506128]
42. Alviña K, Khodakhah K. Selective regulation of spontaneous activity of neurons of the deep cerebellar nuclei by N-type calcium channels in juvenile rats. *J Physiol* 2008;586:2523–2538. [PubMed: 18372310]
43. Medina JF, Mauk MD. Simulations of cerebellar motor learning: computational analysis of plasticity at the mossy fiber to deep nucleus synapse. *J Neurosci* 1999;19:7140–7151. [PubMed: 10436067]
44. Medina JF, et al. Inhibition of climbing fibres is a signal for the extinction of conditioned eyelid responses. *Nature* 2002;416:330–333. [PubMed: 11907580]
45. Llinás R, Yarom Y. Electrophysiology of mammalian inferior olivary neurones *in vitro*. Different types of voltage-dependent ionic conductances. *J Physiol* 1981;315:549–567. [PubMed: 6273544]
46. Ito M. Cerebellar long-term depression: characterization, signal transduction, and functional roles. *Physiol Rev* 2001;81:1143–1195. [PubMed: 11427694]
47. Miles FA, Lisberger SG. Plasticity in the vestibulo-ocular reflex: a new hypothesis. *Annu Rev Neurosci* 1981;4:273–299. [PubMed: 6784658]
48. McCormick DA, Thompson RF. Cerebellum: essential involvement in the classically conditioned eyelid response. *Science* 1984;223:296–299. [PubMed: 6701513]

49. Mauk MD, et al. Classical conditioning using stimulation of the inferior olive as the unconditioned stimulus. *Proc Natl Acad Sci U S A* 1986;83:5349–5353. [PubMed: 3460097]
50. Steinmetz JE, et al. Classical conditioning in rabbits using pontine nucleus stimulation as a conditioned stimulus and inferior olive stimulation as an unconditioned stimulus. *Synapse* 1989;3:225–233. [PubMed: 2718098]
51. Hesslow G, et al. Learned movements elicited by direct stimulation of cerebellar mossy fiber afferents. *Neuron* 1999;24:179–185. [PubMed: 10677036]
52. Hesslow G. Inhibition of classically conditioned eyeblink responses by stimulation of the cerebellar cortex in the decerebrate cat. *J Physiol* 1994;476:245–256. [PubMed: 8046641]
53. Jirenhed DA, et al. Acquisition, extinction, and reacquisition of a cerebellar cortical memory trace. *J Neurosci* 2007;27:2493–2502. [PubMed: 17344387]
54. Garcia KS, Mauk MD. Pharmacological analysis of cerebellar contributions to the timing and expression of conditioned eyelid responses. *Neuropharmacology* 1998;37:471–480. [PubMed: 9704988]
55. Ohyama T, Mauk M. Latent acquisition of timed responses in cerebellar cortex. *J Neurosci* 2001;21:682–690. [PubMed: 11160447]
56. Ohyama T, et al. Learning-induced plasticity in deep cerebellar nucleus. *J Neurosci* 2006;26:12656–12663. [PubMed: 17151268]
57. Attwell PJ, et al. Cerebellar mechanisms in eyeblink conditioning. *Ann N Y Acad Sci* 2002;978:79–92. [PubMed: 12582043]
58. Lavond DG. Role of the nuclei in eyeblink conditioning. *Ann N Y Acad Sci* 2002;978:93–105. [PubMed: 12582044]
59. Malenka RC, Nicoll RA. Long-term potentiation – a decade of progress? *Science* 1999;285:1870–1874. [PubMed: 10489359]
60. Akazawa C, et al. Differential expression of five N-methyl-D-aspartate receptor subunit mRNAs in the cerebellum of developing and adult rats. *J Comp Neurol* 1994;347:150–160. [PubMed: 7798379]
61. Audinat E, et al. Responses to excitatory amino acids of Purkinje cells and neurones of the deep nuclei in cerebellar slice cultures. *J Physiol* 1990;430:297–313. [PubMed: 1982313]
62. Audinat E, et al. Excitatory synaptic potentials in neurons of the deep nuclei in olivo-cerebellar slice cultures. *Neuroscience* 1992;49:903–911. [PubMed: 1359457]
63. Anchisi D, et al. Postsynaptic currents in deep cerebellar nuclei. *J Neurophysiol* 2001;85:323–331. [PubMed: 11152732]
64. Pugh JR, Raman IM. Potentiation of mossy fiber EPSCs in the cerebellar nuclei by NMDA receptor activation followed by postinhibitory rebound current. *Neuron* 2006;51:113–123. [PubMed: 16815336]
65. Kleim JA, et al. Synapse formation is associated with memory storage in the cerebellum. *Proc Natl Acad Sci U S A* 2002;99:13228–13231. [PubMed: 12235373]
66. Zhang W, Linden DJ. Long-term depression at the mossy fiber-deep cerebellar nucleus synapse. *J Neurosci* 2006;26:6935–6944. [PubMed: 16807323]
67. Pugh JR, Raman IM. Mechanisms of potentiation of mossy fiber EPSCs in the cerebellar nuclei by coincident synaptic excitation and inhibition. *J Neurosci* 2008;28:10549–10560. [PubMed: 18923031]
68. Belmeguenai A, Hansel C. A role for protein phosphatases 1, 2A, and 2B in cerebellar long-term potentiation. *J Neurosci* 2005;25:10768–10772. [PubMed: 16291950]
69. Jörmell H, Hansel C. Synaptic memories upside down: bidirectional plasticity at cerebellar parallel fiber-Purkinje cell synapses. *Neuron* 2006;52:227–238. [PubMed: 17046686]
70. Nelson AB, et al. Decreases in CaMKII activity trigger persistent potentiation of intrinsic excitability in spontaneously firing vestibular nucleus neurons. *Neuron* 2005;46:623–631. [PubMed: 15944130]
71. Ohyama T, et al. Stimulus generalization of conditioned eyelid responses produced without cerebellar cortex: implications for plasticity in the cerebellar nuclei. *Learn Mem* 2003;10:346–354. [PubMed: 14557607]
- 72G. Garcia KS, et al. Cerebellar cortex lesions prevent acquisition of conditioned eyelid responses 1999;19:10940–10947.

73. Uusisaari M, et al. Morphological and electrophysiological properties of GABAergic and non-GABAergic cells in the deep cerebellar nuclei. *J Neurophysiol* 2007;97:901–911. [PubMed: 17093116]
74. Uusisaari M, Knöpfel T. GABAergic synaptic communication in the GABAergic and non-GABAergic cells in the deep cerebellar nuclei. *Neuroscience* 2008;156:537–549. [PubMed: 18755250]
75. Do MT, Bean BP. Subthreshold sodium currents and pacemaking of subthalamic neurons: modulation by slow inactivation. *Neuron* 2003;39:109–120. [PubMed: 12848936]
76. Walter JT, et al. Decreases in the precision of Purkinje cell pacemaking cause cerebellar dysfunction and ataxia. *Nat Neurosci* 2006;9:389–397. [PubMed: 16474392]
77. Levin SI, et al. Impaired motor function in mice with cell-specific knockout of sodium channel Scn8a (NaV1.6) in cerebellar Purkinje neurons and granule cells. *J Neurophysiol* 2006;96:785–793. [PubMed: 16687615]
78. Woodruff-Pak DS, et al. Inactivation of sodium channel Scn8A (NaV1.6) in Purkinje neurons impairs learning in Morris water maze and delay but not trace eyeblink classical conditioning. *Behav Neurosci* 2006;120:229–240. [PubMed: 16719687]

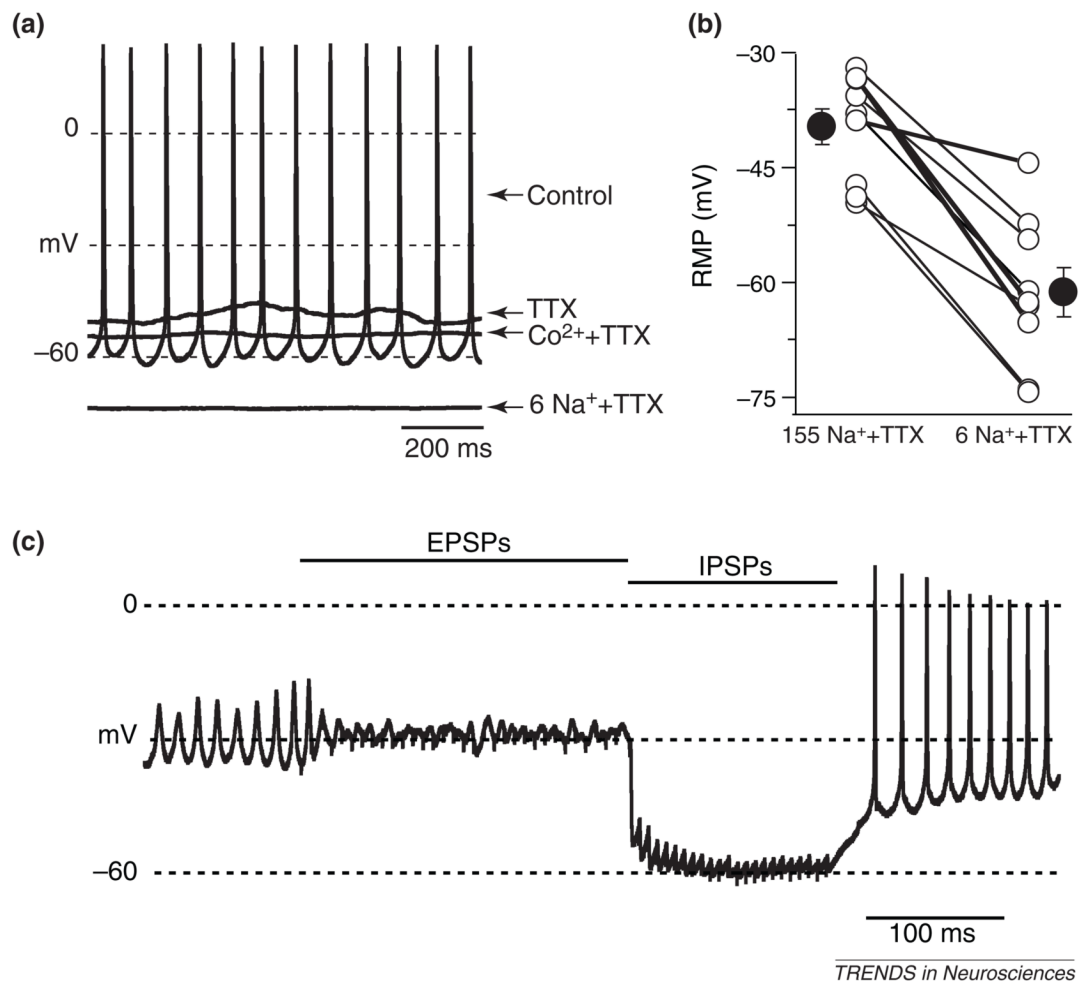
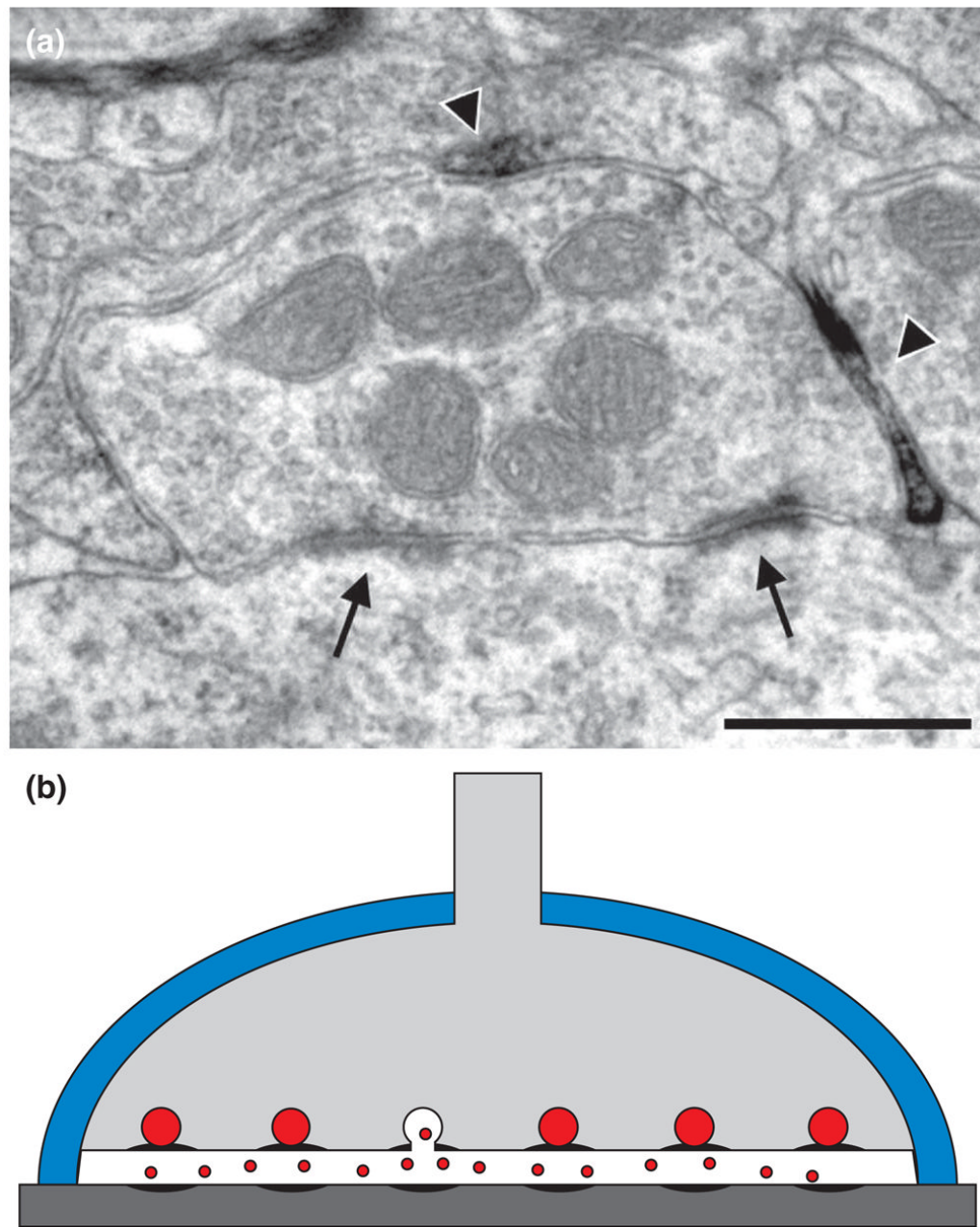


Figure 1.

Spontaneous firing, depolarization block and inhibition in cerebellar nuclear cells. **(a)** Spontaneous firing in a cerebellar nuclear cell isolated from the mouse cerebellum, in quasi-physiological extracellular and intracellular solutions (control). With voltage-gated Na⁺ channels blocked by TTX, the same cell rests at -47 mV (TTX). This depolarized resting membrane potential in TTX depends slightly on Ca²⁺ flux because the cell hyperpolarizes to -54 mV when the 2 mM Ca²⁺ in the bath is replaced with 2 mM Co²⁺ (Co²⁺+TTX). When 155 mM Na⁺ in the control solution is reduced to 6 mM Na⁺ (6 Na⁺+TTX; equimolar substitution with *N*-methyl-D-glucamine⁺, 2 Ca²⁺ present), the membrane hyperpolarizes strongly to -74 mV. **(b)** Group data for resting membrane potential (RMP) in TTX for nine cells treated as in (a) (open symbols). Mean RMP (filled symbols) was -40 ± 2 mV in 155 mM Na⁺ (control solutions) and -61 ± 3 mV in 6 mM Na⁺. These data support the idea that a TTX-insensitive tonic cation current brings cerebellar nuclear cells above threshold. (a) and (b) are modified, with permission, from Ref. [9]. **(c)** A recording from a cerebellar nuclear neuron in a cerebellar slice from mouse with quasi-physiological solutions (35°C). The neuron was originally firing spontaneously (not shown), but it depolarized to produce the oscillatory behavior evident at the beginning of the trace. A 250 ms stimulation of mossy fiber excitatory afferents at 100 Hz (EPSPs) drives the cell further into depolarization block. A 150 ms stimulation of Purkinje axons at 100 Hz (IPSPs) hyperpolarizes the cell to a voltage near -60 mV. Spontaneous firing resumes after the train, illustrating that synaptic inhibition can restore the activity of cerebellar

nuclear neurons that have entered depolarization block (J.R.P. and I.M.R., unpublished; recording conditions as in Ref. [67]).



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Figure 2.

Specializations for spillover-mediated transmission by Purkinje neurons. **(a)** Immuno-electron microscopy reveals multiple presynaptic densities per bouton (arrows) and the presence of GAT-3 GABA transporters in astrocytes (arrowheads) surrounding boutons. 3D reconstructions of boutons indicated an average of nine presynaptic densities per bouton without astrocytes intervening between densities. Data taken, with permission, from Ref. [26]. **(b)** Schematic of spillover-mediated transmission in a Purkinje bouton. Vesicles (red circles) are shown at six presynaptic densities. An astrocytic process (blue) surrounds the bouton (gray). With a low vesicular release probability, fusion occurs at only a subset of release sites in response to an action potential. Transmitter release (red dots) from a single fusion event can

reach postsynaptic receptors in multiple postsynaptic densities (black) on the postsynaptic cerebellar nuclear cell membrane (dark gray) [26,27].

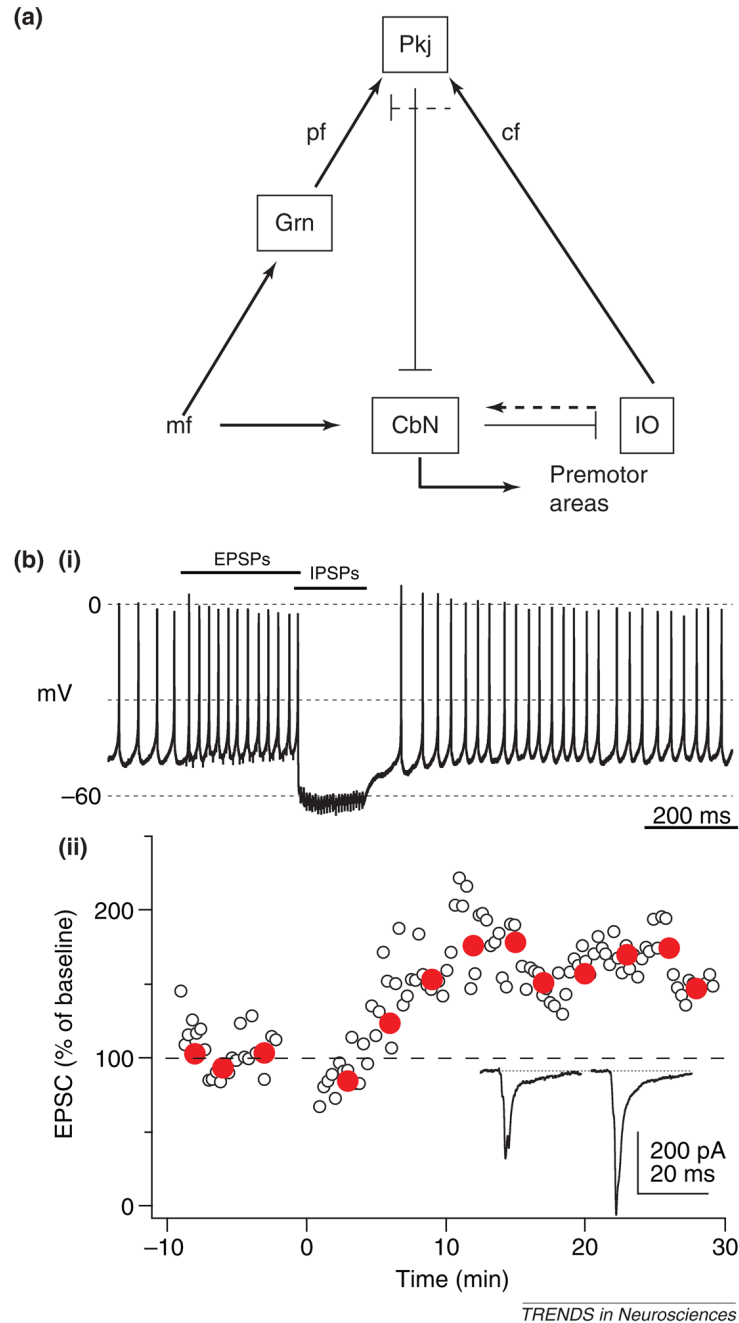


Figure 3. Plasticity of EPSCs in cerebellar nuclear neurons. **(a)** Simplified diagram of the olivo-cerebellar circuit. Arrows indicate excitatory synapses; perpendiculars indicate inhibitory synapses. Broken line perpendicular from cf to pf indicates cf-dependent LTD of pf synapses. Broken arrow indicates relatively weak excitation of CbN by IO. Abbreviations: Pkj, Purkinje; CbN, cerebellar nuclei; IO, inferior olive; Grn, granule cell; mf, mossy fiber; pf, parallel fiber; cf, climbing fiber. **(b) (i)** Response to a stimulus protocol that induces LTP of mossy fiber EPSCs. A postsynaptic cerebellar nuclear neuron is current clamped and allowed to fire spontaneous action potentials. High-frequency stimulation of one set of mossy fiber afferents for 250 ms (EPSPs) is followed by a high-frequency stimulation of a separate set of afferents

that are dominated by Purkinje axons (IPSPs). (ii) EPSCs (open symbols) recorded in the mossy fiber pathway every 15 s before and after the induction protocol was applied thirty times [at time 0; same cell as in part (i)]. Red symbols indicate averages of ten surrounding EPSCs. Inset: mean of ten EPSCs before the induction protocol (left) and after EPSCs have stabilized after induction (right). Data are from the same cell illustrated in Ref. [67], obtained with permission.