



Published in final edited form as:

Cerebellum. 2010 March ; 9(1): 56–66. doi:10.1007/s12311-009-0140-6.

Synaptic Inhibition, Excitation, and Plasticity in Neurons of the Cerebellar Nuclei

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Abstract

Neurons of the cerebellar nuclei generate the non-vestibular output of the cerebellum. Like other neurons, they integrate excitatory and inhibitory synaptic inputs and filter them through their intrinsic properties to produce patterns of action potential output. The synaptic and intrinsic features of cerebellar nuclear cells are unusual in several respects, however: these neurons receive an overwhelming amount of basal and driven inhibition from Purkinje neurons, but are also spontaneously active, producing action potentials even without excitation. Moreover, not only is spiking by nuclear cells sensitive to the amount of inhibition, but the strength of inhibition is also sensitive to the amount of spiking, through multiple forms of long-term plasticity. Here, we review the properties of synaptic excitation and inhibition, their short-term plasticity, and their influence on action potential firing of cerebellar nuclear neurons, as well as the interactions among excitation, inhibition, and spiking that produce long-term changes in synaptic strength. The data provide evidence that electrical and synaptic signaling in the cerebellar circuit is both plastic and resilient: the strength of IPSPs and EPSPs readily changes as the activity of cerebellar nuclear cells is modified. Notably, however, many of the identified forms of plasticity have an apparently homeostatic effect, responding to perturbations of input by restoring cerebellar output toward pre-perturbation values. Such forms of self-regulation appear consistent with the role of cerebellar output in coordinating movements. In contrast, other forms of plasticity in nuclear cells, including a long-term potentiation of excitatory postsynaptic currents (EPSCs) and excitation-driven increases in intrinsic excitability, are non-homeostatic, and instead appear suited to bring the circuit to a new set point. Interestingly, the combinations of inhibitory and excitatory stimuli that potentiate EPSCs resemble patterns of activity predicted to occur during eyelid conditioning, suggesting that this form long-term potentiation, perhaps amplified by intrinsic plasticity, may represent a cellular mechanism that is engaged during cerebellar learning.

Keywords

Purkinje; Deep cerebellar nuclei; Rebound; Delay eyeblink; Long-term potentiation (LTP); Long-term depression (LTD)

Introduction

The output of the cerebellum, with the exception of vestibular information, is carried exclusively by neurons of the cerebellar nuclei. A subset of neurons, primarily with large diameter somata ($>15\ \mu\text{m}$), project to premotor areas, thereby directly influencing motor control, while other, smaller nuclear neurons project to the inferior olive, providing a negative feedback that likely corrects errors of movement (Fig. 1; [1–5]). In the cerebellar nuclei, therefore, converging excitatory and inhibitory synaptic inputs must be integrated and transformed by the intrinsic properties of nuclear neurons to yield the final output of the cerebellum. An understanding of the cellular mechanisms of this encoding of information by the cerebellum ideally includes identifying what the properties of synaptic inputs to the nuclei are, how the excitability of nuclear neurons is affected by these stimuli, and which patterns of activity modify synaptic inputs and spike outputs to yield motor coordination and learning. The synaptic inputs to the nuclei, and the sites and signs of plasticity discussed in this review are illustrated in Fig. 1.

Spontaneous Firing and Synaptic Inputs

Neurons of the cerebellar nuclei belong to the class of neurons that fire action potentials spontaneously, even without synaptic input. Recordings from *in vitro* preparations from rodents indicate that the intrinsic firing rates of these cells generally lie between 10 and 50 Hz [6–12]. Thus, like any other spontaneously active neuron, the basal activity of cerebellar nuclear cells lies in the middle of their dynamic range, from which it can be reduced by inhibition, or increased by excitation. What distinguishes cerebellar nuclear cells from most other neurons, however, is the amount of inhibition that they receive. Although cerebellar nuclear neurons are diverse in their morphologies, transmitter contents, and projections, they all appear to be targets of Purkinje cells of the cerebellar cortex, which are GABAergic [1,13–17]. Both large (primarily excitatory) and small (primarily inhibitory) nuclear cells receive input from dozens of Purkinje cells, which form dense, inhibitory synaptic contacts on nuclear cell somata and proximal dendrites (Fig. 1; [1,18]). Purkinje cells, too, are spontaneously active, firing ~50 spikes per second *in vitro* with synaptic transmission blocked or removed [19–21], as well as *in vivo* when the animal is not actively engaged in cerebellar behaviors [22,23]. Since somatic spikes in Purkinje cells propagate reliably for several hundred microns along myelinated axons [24,25], most are likely to invade the synaptic terminal where they can trigger release of GABA. Thus, in the basal state, each nuclear cell is subject to a barrage of more than a thousand inhibitory postsynaptic potentials (IPSPs) per second.

In addition to this large amount of inhibitory input, nuclear cells receive excitatory contacts from both mossy fibers and inferior olivary fibers (Fig. 1). The anatomy of these synaptic connections, however, leads to the prediction that elevated excitatory input to nuclear cells will frequently coincide with elevated inhibitory input. Mossy fibers have a low basal activity, but during cerebellar behaviors, can fire at rates exceeding 100 Hz [26]. These inputs directly excite nuclear cells, but also indirectly excite Purkinje cells, via granule cells. Thus, mossy fiber activity is likely to increase both excitation and inhibition of nuclear neurons nearly simultaneously. An additional source of excitation comes from inferior olivary fibers, which split into the massive climbing fiber contact onto Purkinje cells, which elicits complex spikes and the subsequent pauses, and an anatomically less impressive excitatory contact onto nuclear cells [1].

It is striking, therefore, that although inhibition frequently dominates excitation, especially in the basal state, activity of cerebellar nuclear neurons persists *in vivo* [22,27,28], indicating that the tendency of nuclear neurons to fire can resist even substantial inhibition. This maintenance of firing is in part supported by the intrinsic leak currents of cerebellar nuclear cells: in contrast to many neurons whose resting membrane potential is set largely by K channels, cerebellar

nuclear cells primarily express voltage-independent non-selective cationic leak channels that reverse near -30 mV [10], whose properties resemble NALCN channels [29]. As a consequence, cells continually seek a potential that is suprathreshold, eliciting spikes whenever spontaneous synaptic inhibition transiently drops (e.g. reference [11], discussed below).

Firing rates of cerebellar nuclear cells are modulated during specific motor behaviors, however, consistent with their participation in regulating movements [22,27,30]; at certain times during a behavior, excitation dominates, whereas at other times, inhibition becomes more effective. Importantly, motor learning can modify patterns of nuclear cell firing [31], likely owing to plasticity of both synaptic inputs and intrinsic conductances. Such observations raise the question of how excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs) change in the short term and long term, interact in nuclear cells, and regulate the rate and timing of cerebellar nuclear action potentials.

Short-Term Synaptic Excitation

Remarkably few studies have focused directly on the properties of excitatory synaptic transmission in the cerebellar nuclei. Most of the existing research on synaptic transmission has concentrated on the larger nuclear cells, which include, but are not limited to, glutamatergic neurons that project to premotor areas [13,17]. During prolonged stimulus trains at ≥ 100 Hz, excitatory postsynaptic potentials (EPSPs) in these cells consistently raise firing above spontaneous rates, suggesting that excitation remains effective for several hundred milliseconds [32]. Voltage-clamp studies illustrate that the short-term plasticity of underlying EPSCs is variable, with some cells depressing and others facilitating during 100-Hz trains of stimulation; the average response across cells shows nearly no net change in EPSC amplitude [32]. A distinctive feature of these synapses is that *N*-methyl-D-aspartic acid (NMDA) receptors contribute a substantial component of the total synaptic current even at negative voltages [33,34], owing to the expression of NR2D NMDA receptor subunits, which generate channels that are only weakly blocked by Mg^{++} [35–37]. The contribution of NMDA receptors is particularly evident during trains of evoked EPSCs, which permit the NMDA-receptor-mediated component to summate; after 20 stimuli at 100 Hz, its amplitude exceeds 60% of the AMPA-receptor mediated component, even at -65 mV in 1 mM Mg^{++} [32]. Consequently, NMDA receptors in the cerebellar nuclei permit substantial Ca influx even when cells are not spiking [38], a feature that may be significant to long-term plasticity (discussed below).

Short-Term Synaptic Inhibition

Purkinje-mediated IPSCs in large cerebellar nuclear cells are plastic but remain robust during trains of stimuli. Their plasticity can be conveniently measured in cerebellar slices, in which most Purkinje axons are severed from their spontaneously firing somata. Consequently, IPSCs can be elicited without a background of ongoing inhibition or a history of either facilitation or depression. Under these conditions, stimulating Purkinje axons elicits synaptic currents that are apparently mediated exclusively by GABA_A receptors. When IPSCs are evoked at 50 Hz, they fall to about half their initial amplitude over the course of a second ([39]; Fig. 2a), suggesting that spontaneous activity of Purkinje cells tonically depresses IPSCs by about 50% in the intact cerebellum. Each synaptic current decays rapidly, with a time constant of about 5 ms near physiological temperatures, so that little tonic (residual) current remains between IPSCs evoked at 50 Hz (Fig. 2b); lapses of the inhibitory conductance between release events from afferent Purkinje neurons may provide a temporal window in which spikes are generated [11]. Consistent with this idea, action potentials in these cerebellar nuclear cells often persist during 50-Hz IPSPs elicited by coherent activation of about five to ten afferents (Fig. 2c). Increasing the stimulation rate above 50 Hz, as is expected to occur during cerebellar behaviors, mildly increases the amount of depression of IPSCs. Nevertheless, inhibition becomes more effective, for the simple reason that the synaptic current does not decay completely between

stimuli, thus shunting action potential firing (Fig. 2a–d,[39]); a similar shunt can probably be achieved by asynchronous inhibitory inputs [11]. Mechanistically, the resistance of IPSCs to strong synaptic depression at high stimulus rates results from an effective spillover-mediated transmission, owing to release of GABA from boutons with multiple release sites but without GABA transporters [40,41]. These features allow a low vesicular release probability, which minimizes depletion, to evoke a postsynaptic response with high probability. Functionally, it permits the efficacy of inhibition to vary with the firing rate of afferent Purkinje cells, in a manner that is relatively independent of short-term depression [8].

Disinhibition and Voltage-Gated Na Currents

Although inhibition most obviously acts to reduce firing by nuclear cells, a long-standing question is how nuclear cells respond to the offset of IPSPs [7,42]. As mentioned above, because of the intrinsic tendency of nuclear cells to spike, whenever inhibition subsides, firing invariably resumes [8,10,11]. Elevated inhibition that interrupts firing for a few hundred milliseconds, however, can change the availability of channels, e.g., permitting the recovery of voltage-gated Na or Ca channels that have inactivated during spiking, deactivating K_{Ca} or other channels that require tonic Ca influx to open, or activating HCN channels. If these changes favor the availability and/or activation of depolarizing currents—as they seem likely to do—then firing will not only resume but may also be accelerated whenever a prolonged inhibition is abated.

One current that is highly sensitive to the mean membrane potential in cerebellar nuclear cells is voltage-gated Na current. Voltage-clamped Na currents of both large and small nuclear neurons have a transient, persistent, and resurgent component (Fig. 3a); the transient component has a relatively depolarized half-inactivation voltage of -52 mV and a marked tendency for slow inactivation [10,43,44]. During spiking, the rapidly deactivating voltage-gated K currents prevent deep after-hyperpolarizations, keeping most interspike intervals above -65 mV and mean membrane potentials near -50 mV [6,8–12,16,39]. As a result, spontaneously firing nuclear cells operate with a remarkably low availability of Na channels [10,44]. Even interruptions of firing on the order of a few hundred milliseconds, however, can permit recovery of Na channels from fast and slow inactivation. This effect is evident when tetrodotoxin-sensitive Na currents are evoked by 20-Hz trains of brief, spike-like depolarizations, applied from -60 mV. The amplitude of the transient Na current decreases by more than 50% over the first ten stimuli. A gap of 250 ms at -70 mV, however, can restore the current to 80% of its initial amplitude. Such recovery is likely to facilitate firing immediately upon disinhibition ([44], Fig. 3b). Moreover, the availability of Na channels is likely to be increased for a few hundred milliseconds after firing resumes. Evidence for this idea comes from holding cells at -68 mV and then releasing the voltage clamp to allow spiking to occur. Plotting the time derivative of the resulting action potentials illustrates that the maximal rate of rise of each spike drops with successive spikes, reflecting the decreasing Na channel availability over the course of about half a second (Fig. 3c). Indeed, after trains of IPSPs (at 100 Hz for 500 ms), the rate of spontaneous action potential firing in large neurons in slices is increased by about 20 Hz for at least 300 ms, regardless of the initial rate of firing (Fig. 3d, [45]). Thus, Na channels form one class of channels that is sensitive to periods of inhibition, and which is likely to influence firing patterns after inhibition is relieved.

Disinhibition and T-Type Ca Currents

After experimentally applied hyperpolarizations to strongly negative potentials, both small and large cerebellar nuclear cells in slice preparations often generate bursts of spikes that are superimposed on an underlying depolarization [7,9,12,46]. The term “rebound” has come to be applied to the depolarization, the spike burst, and occasionally the period of prolonged firing following hyperpolarization. The rebound response elicited after hyperpolarizations beyond

about -90 mV is likely to result in part from activation of recovered T-type Ca channels, which are present at high densities on nuclear cell dendrites [38,45,47,48], and whose density correlates with the number of spikes in the post-hyperpolarization burst [46,49].

Whether rebound bursts with a similar ionic mechanism are activated by GABA_A-mediated synaptic inhibition, however, is still debated (e.g. [7,42,45,50,51]). The recruitment of T-type or any other channels necessarily depends on the duration and extent of hyperpolarization by inhibition. Activation of GABA_A receptors can hyperpolarize cells no further than E_{Cl} , which has repeatedly been measured to be near -75 mV in these neurons, in different species (guinea pig, rat, and mouse) and at different ages (from P5 to young adult) [9,42,45,50,52]. The voltage-dependence of T-type channels (measured in large neurons), however, makes it unlikely that they recover substantially even at E_{Cl} . Moreover, imaging studies reveal no evidence of large, brief T-type Ca transients even in dendrites after trains of IPSPs (Fig. 4a). Instead, when spontaneous firing is interrupted by synaptic inhibition, the Ca signal drops, and when firing resumes, it is restored to baseline levels. Thus, it is difficult to conceive of a scenario in which T-type currents are recruited by GABA_A-mediated inhibition alone. Only when cells are hyperpolarized by current injections beyond E_{Cl} does the Ca signal overshoot the basal level [45]. These results are consistent with previous work demonstrating that post-hyperpolarization Ca signals are roughly proportional to firing rate in these cells, indicating that most of the Ca influx is generated by high-voltage-activated (HVA) Ca currents activated during spiking [53].

In some preparations, the post-inhibitory propensity to burst has been reported to increase with the strength of stimulation of Purkinje afferents [51]. Since the depth of hyperpolarization does not change significantly with increased synaptic inhibition, this observation raises the interesting possibility that stronger stimulation facilitates post-inhibitory bursts by engaging additional mechanisms, such as modulation of T-type currents or activation of other ionic conductances. In fact, the elevations of firing rate observed after strong inhibition persists for several hundred milliseconds, far outlasting the duration of T-type current, which decays within 50 ms [32,45,54], possibly indicative of multiple underlying processes.

Nevertheless, the presence of T-type currents in nuclear cells suggests that they are indeed activated by specific stimuli, and it is of interest to identify what these stimuli may be. Substantial recovery of T-type currents may well require sustained activation of K currents, which can hyperpolarize cells beyond E_{Cl} . Which K currents may be involved is yet unknown; inward rectifiers that are stimulated by GABA_B receptors seem a likely candidate, but GABA_B receptors are not readily activated even with prolonged Purkinje cell stimulation, at least in slice studies [8,40,55]. Another possibility is that E_{Cl} itself becomes more negative under certain conditions. During development, E_{Cl} does appear to shift negatively as a result of changes in the relative density of chloride transporters NKCC1 and KCC2. In cerebellar slices from very young (P2-P3) rats, E_{Cl} of nuclear neurons is relatively depolarized (-55 mV) and shifts about 10 mV negative in response to experimentally induced synaptic activity that drives expression of the potassium chloride co-transporter KCC2 [56]. The shift appears to be complete by P5, however, making it seem unlikely that further developmental shifts bring E_{Cl} significantly below the value of -75 mV. A relevant point has recently been raised by research on neocortical neurons, however, namely, that young (nursing) mammals often rely on ketone bodies rather than glucose for metabolism. Application of ketone bodies to neocortical slices from P4-P8 rats, before pyramidal cells express KCC2, shifts E_{Cl} from about -55 mV to about -80 mV, apparently by actions on the chloride/bicarbonate transporter [57]. If chloride/bicarbonate transporters influence E_{Cl} in the cerebellar nuclei even after expression of KCC2, it is possible that GABA_A receptor activation *in vivo* may hyperpolarize cells more strongly than *in vitro*, in which ACSF is usually supplemented only by glucose. Notably,

however, relief of 500-ms hyperpolarizations even to -80 mV fails to engage significant T-type current in cerebellar nuclear cell dendrites (Fig. 4b,[45]).

The Long-Term Influence of Excitation on Inhibition

Because NMDA receptors in the nuclei are only weakly blocked by Mg^{++} as described above, an NMDA-receptor-dependent Ca influx is not restricted to periods of postsynaptic depolarization. These receptors therefore likely do not carry out their common role as coincidence detectors that drive plasticity when presynaptic and post-synaptic spiking temporally overlap. Nevertheless, NMDA receptors in the nuclei do indeed participate in plasticity. For instance, in nuclear cells studied without selection based on size, a variety of protocols that elicit moderate Ca influx, including low-frequency stimulation of NMDA receptors, induce a long-term depression (LTD) of inhibitory synaptic currents; depolarizations that activate L-type Ca channels can substitute for the NMDA receptor activation. This form of LTD does not require activation of $GABA_A$ receptors, and consequently, it is not synapse-specific but cell-wide [52,58]. Moreover, LTD appears to be postsynaptically expressed, since it reduces the sensitivity to $GABA_A$ agonists. LTD does not, however, change the chloride reversal potential or the kinetics of IPSCs [58].

Conversely, protocols that generate a large Ca influx, such as 100-Hz stimulation of NMDA receptors, induce a cell-wide long-term potentiation (LTP) of IPSCs that depends on postsynaptic exocytosis, implicating $GABA_A$ receptor insertion [59]. Like LTD, however, the LTP of IPSCs in large and small cells does not have an absolute requirement for NMDA receptor activation. With fast glutamatergic transmission blocked, LTP of IPSPs can be evoked by 10-pulse, 100-Hz trains of IPSPs evoked twice per second, a protocol that produces relatively large postsynaptic Ca influx via post-inhibitory action potential firing: more action potentials generate a larger intracellular Ca signal and a more consistent potentiation [53]. Interestingly, this same protocol induces LTD if the number of post-inhibitory action potentials, as well as the corresponding Ca signal, is reduced by tonic hyperpolarization [53]. As mentioned above, more recent imaging studies in large cells confirm that the amplitude of Ca signals in nuclear neurons correlates with the rate of action potential firing [45], and that these spike-related signals result from flux through HVA Ca channels [45,54,60]. Regarding the role of specific Ca channels in inducing plasticity, the LTD-induction protocol likely evokes more T-type than HVA Ca channel current, because tonic hyperpolarization is expected to permit T-type channel recovery, and the low number of post-inhibitory action potentials are expected to minimize HVA Ca channel activation. In contrast, the LTP-induction protocol favors activation of HVA Ca channels over T-type channels, because cells are relatively more depolarized both during and after inhibition, and higher post-inhibitory firing rates are attained. L-type channels in particular, unlike other HVA channels, do not contribute strongly to dendritic Ca influx, but are present at high densities in the somata, making them well placed to interact with $GABA_A$ receptors [45].

Together, these data on LTD and LTP provide insight into the regulation of inhibition in the cerebellar nuclei. The olivocerebellar circuit forms a negative feedback loop [5]: a drop in spiking by small nuclear cells, e.g. because of a reduction in excitatory drive from mossy fibers, decreases the inhibitory drive onto inferior olivary neurons, which increases climbing fiber activity, thereby evoking complex spikes in Purkinje cells. Because complex spikes induce LTD of parallel fiber synapses onto Purkinje cells, they have the effect of reducing excitation and slowing the driven rate of Purkinje cells [61]. The resulting decrease in inhibition from Purkinje cells will have the tendency to restore firing in the nuclei. The negative feedback is incomplete, however, since reductions in the driven firing of Purkinje cells by definition do not influence spontaneous firing of Purkinje cells. Thus, the LTD of Purkinje IPSCs is well suited to carry out the missing component of the negative feedback, by making nuclear cells

less responsive even to basal inhibition. Conversely, high-frequency excitation that drives rapid firing in the nuclei may exert a homeostatic strengthening of inhibition via the negative feedback circuit as well as by LTP of individual inhibitory synapses. Thus, multiple negative feedback systems are engaged that apparently interact to stabilize nuclear cell output to a new set point of incoming excitation.

The Long-Term Influence of Inhibition on Excitation

Based on systems and behavioral studies, cerebellar models have long predicted that motor learning requires Purkinje-mediated inhibition to regulate the strength of synaptic excitation and the consequent spike output of Purkinje target neurons [62,63]. Cellular support for this hypothesis has come from cerebellar slice studies of large nuclear neurons, which demonstrate that specific combinations of excitation and inhibition can induce long-term changes in excitatory synaptic strength in cerebellar nuclear cells. Specifically, when high-frequency trains of synaptic excitation precede a period of inhibition and disinhibition, EPSCs undergo a synapse-specific LTP (Fig. 5,[32,38]). What makes this pattern of plasticity of interest is that the induction protocols resemble the patterns of stimulation that are expected to occur during associative learning in the cerebellum ([63]; reviewed in [64]). Shifting the relative timing of the EPSPs and inhibition changes the polarity of plasticity: if the train of excitation is too early relative to disinhibition, EPSCs tend to depress weakly. Likewise, if excitation is superimposed on the post-hyperpolarization action potentials, depression is favored (Fig. 5,[38]). A similar LTD of EPSCs can be induced by simultaneous synaptic excitation and depolarization, in a manner that depends on metabotropic glutamate receptor activation [65]. Both these forms of LTD (which may be convergent in their mechanisms) are suggestive of another negative feedback-like form of plasticity in the nuclei, since coincident synaptic excitation and postsynaptic spiking tends to diminish excitatory synaptic strength.

The dependence of the LTP of EPSCs on a fixed temporal pattern excitation and inhibition raises the question of the mechanisms underlying the plasticity. To induce LTP effectively, the period of excitation must activate NMDA receptors, and the disinhibition—which can be generated either by current injection or stimulation of Purkinje afferents—must activate voltage-gated Ca channels. Although voltage-clamp experiments demonstrate that activation of T-type Ca currents are sufficient to generate the disinhibition-dependent Ca signal [32], they do not appear to be necessary, since potentiation can be induced by real synaptic inhibition that activates high-voltage-activated Ca channels but not T-type currents [38,45].

The requirement for at least two sources of Ca to induce LTP suggests that multiple signaling cascades are engaged by the induction protocol. In fact, this form of LTP is consistently prevented by blockade of the Ca-dependent phosphatase calcineurin, which unmasks an LTD driven by the same stimulus protocol. The plasticity is also prevented, however, by blockade of the Ca-dependent kinase CaMKII [38]. Thus, both dephosphorylation and phosphorylation appear necessary for potentiation of EPSCs. A possible scenario involves activation of at least one of these enzymes, e.g. calcineurin, by NMDA receptor activation and another enzyme, e.g. CaMKII, by disinhibition, although it is equally possible that an additional signaling mechanism is activated by the drop in Ca resulting from the suppression of spiking during inhibition [45]. Such a situation exists in medial vestibular neurons, which are also targets of Purkinje cells, and in which CaMKII activity is down-regulated by synaptic inhibition [66, 67]. Thus, synaptic excitation may serve to prime individual synapses for potentiation, which is subsequently triggered if and only if the priming event is followed relatively rapidly by inhibition that interrupts firing [38].

The most appealing interpretation of this form of potentiation is that it may form part of the cellular mechanism of cerebellar learning, such as delay eyelid conditioning ([68], reviewed in [69]). Whole-animal studies demonstrate that during training, Purkinje neurons change their

responses to mossy fiber input that carries the information associated with the conditioned stimulus [70]: initially they fire throughout the conditioned stimulus, but after repeated exposure to paired conditioned and unconditioned stimuli, they fire during the early phase but less during the late phase of the conditioned stimulus [71]. At the level of the cerebellar nuclei, such a situation predicts that the excitation of cerebellar nuclei (via the mossy fibers that are active during the conditioned stimulus) overlaps with a sequence of inhibition and disinhibition, which could potentiate EPSCs. In this way, plasticity in the cortex would drive plasticity in the nuclei, as predicted by the Medina–Mauk model [63]. Disinhibition of cerebellar nuclear cells that project to premotor areas, combined with the increased sensitivity to excitatory input from mossy fibers, might be sufficient to drive conditioned responses, i.e., to evoke the reflex-like eyelid closure in response to the conditioned stimulus alone.

Such an increased responsiveness to mossy fiber input may be further augmented by an unusual form of intrinsic plasticity that is present in the cerebellar nuclei. Bursts of high-frequency EPSPs that evoke Ca influx through NMDA receptors induce neurons to become more excitable in response to fixed current injections [72,73]. Along with the LTP of EPSCs, this form of plasticity is one of the few examples of a positive feedback in the cerebellar nuclei, as stimuli that (intermittently) facilitate firing actually increase excitability. Strikingly, however, IPSP bursts that permit post-inhibitory spiking and an associated intracellular Ca rise can also induce this intrinsic activity [73], suggesting that the response is not necessarily sensitive to the polarity of synaptic input, as long as firing is increased. Whatever the means by which excitability is modulated, it is possible that intrinsic plasticity may amplify synaptic plasticity, making the response of nuclear cells to mossy fiber input even more robust.

Pathophysiological Plasticity of Synaptic Inhibition

Studies of mutant mice with disrupted Purkinje cell input provide interesting parallels with the plasticity of inhibitory inputs onto cerebellar nuclear cells. For instance, in heterozygous Lurcher mice (*Lc/+*), in which the *GluR δ 2* gene is mutated, Purkinje cells degenerate between P8 and P26, rendering animals ataxic [74–76]. Multiple changes occur at the level of the cerebellar nuclei, however, that are suggestive of compensation for reduced inhibitory input. For example, inhibitory boutons enlarge, and even the number of cerebellar nuclear cells that co-label for GABA and glycine increases [77]. Consistent with these anatomical changes, during the period of Purkinje cell degeneration, the amplitude of spontaneous and miniature IPSCs increases [78]. In addition, aggregates of gephyrin, which clusters GABA_A receptors at synapses, increase in size [79]. Together, these data are consistent with the idea that strong regulatory mechanisms operate to keep inhibition onto cerebellar nuclear cells within some operationally acceptable range: low levels of inhibition, both physiological and pathophysiological, trigger multiple compensatory processes that raise the sensitivity to inhibitory input.

Complementary data come from studies of dystonic rats [80]. This work not only illustrates pathophysiological plasticity but also provides information that may address the question of whether bursting contributes to signals involved in motor control. In these animals, basal firing rates of Purkinje cells are increased [81]. Climbing fiber input also decreases, which is expected to correlate with a loss of long-term depression of excitatory parallel fiber inputs, and therefore a further increase in Purkinje neuronal activity [81]; these changes are likely to increase inhibition of cerebellar nuclear cells. As in Lurcher mice, however, examination of GABA_A receptors reveals compensatory changes: autoradiographic studies of dystonic rats indicate that the density of GABA receptors in the nuclei is reduced [82], suggestive of a down-regulation of sensitivity to GABA that may offset increased Purkinje-mediated inhibition. Intriguingly, however, measurements of the changes in firing by cerebellar nuclear neurons in these animals reveals neither a net increase nor a decrease in firing; instead the primary modification is that

spike output of nuclear cells consists of more burst-like clusters of action potentials [83]. Most strikingly, the symptoms of dystonia are relieved by ablation of the cerebellum, suggesting that this aberrant cerebellar output generates the pathological co-contraction of opposing muscle groups [80]. Consistent with the idea that excessively abrupt changes of nuclear cell spike rate disrupt motor coordination, studies of mice with mutations of P-type Ca channels have shown that irregularities in firing by Purkinje cells correlate with ataxia. Moreover, the motor disorders in these mice can be largely reversed by application of SK agonists that regularize Purkinje cell firing [84]. Together, these studies raise the interesting point that smooth modulation of spike output of cerebellar nuclear neurons may be regulated by Purkinje cells and may constitute an important component of normal motor control.

Acknowledgments

We would like to acknowledge all the members of the laboratory whose research is reviewed in this article: Fatemeh Afshari, Teresa Aman, Amy Gustafson, Zayd Khaliq, Dan Padgett, Jason Pugh, and Petra Telgkamp. We are especially grateful to Jason Bant and Teresa Aman for their contributions to Figures. Supported by NIH NS39395 (IMR).

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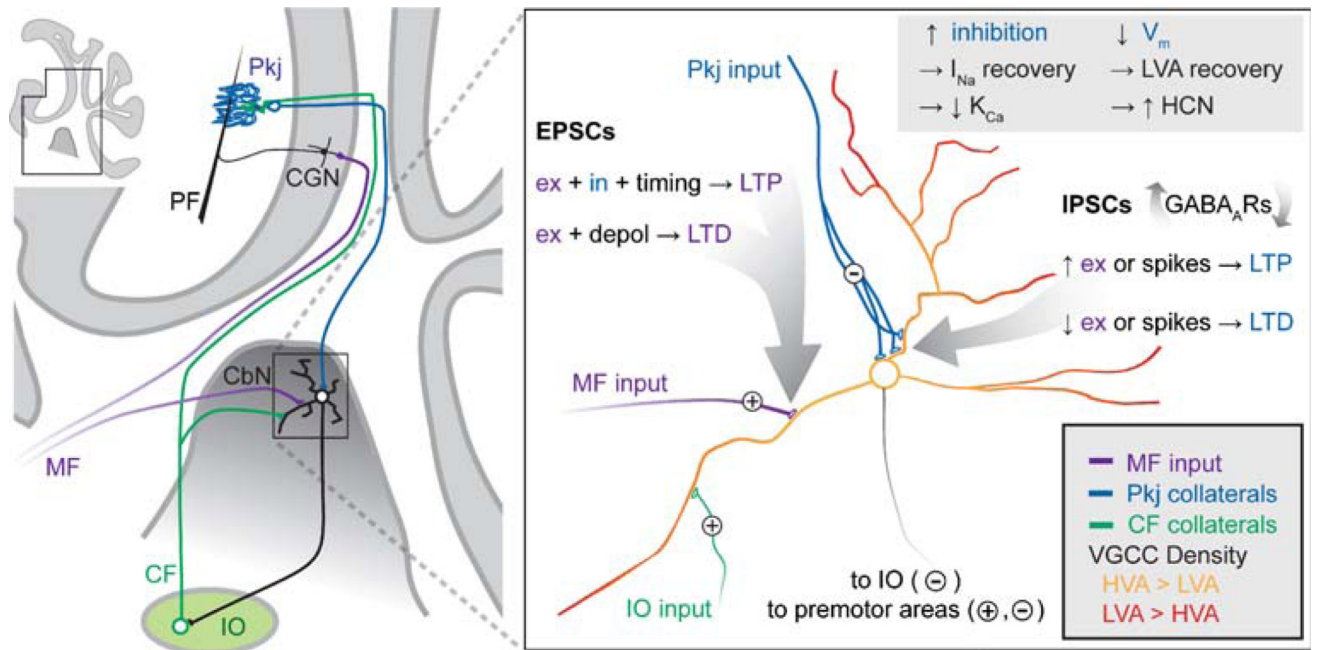


Fig. 1. Schematic of sites of plasticity in the cerebellar nuclei. *Left* Schematic of a parasagittal section of the cerebellum (*inset*) with expanded region indicating that cerebellar nuclear neurons (*CbN*) project either to premotor areas (not shown) or to the inferior olive (*IO*). The *IO* forms the climbing fiber (*CF*) that targets Purkinje neurons (*Pkj*), with collaterals excite the *CbN* cells. Mossy fibers (*MF*) excite *CbN* cells as well as cerebellar granule neurons (*CGN*), which in turn form the parallel fibers (*PF*) that excite *Pkj* cells. *Right* Expansion of the cerebellar nuclear cell and its inputs, along with the sites and bases of plasticity. Note that small, usually inhibitory, cells project to the *IO* and large, usually excitatory cells project to premotor areas. V_m membrane voltage, I_{Na} voltage-gated Na current, *LVA* low-voltage activated (T-type) Ca current, K_{Ca} Ca-activated K current, *HCN* hyperpolarization-gated cyclic nucleotide-gated channels, *VGCC* voltage-gated Ca channels, *HVA* high-voltage activated Ca channels, *ex* excitatory input, *in* inhibitory input, *depol* depolarization. Figure by J. S. Bant

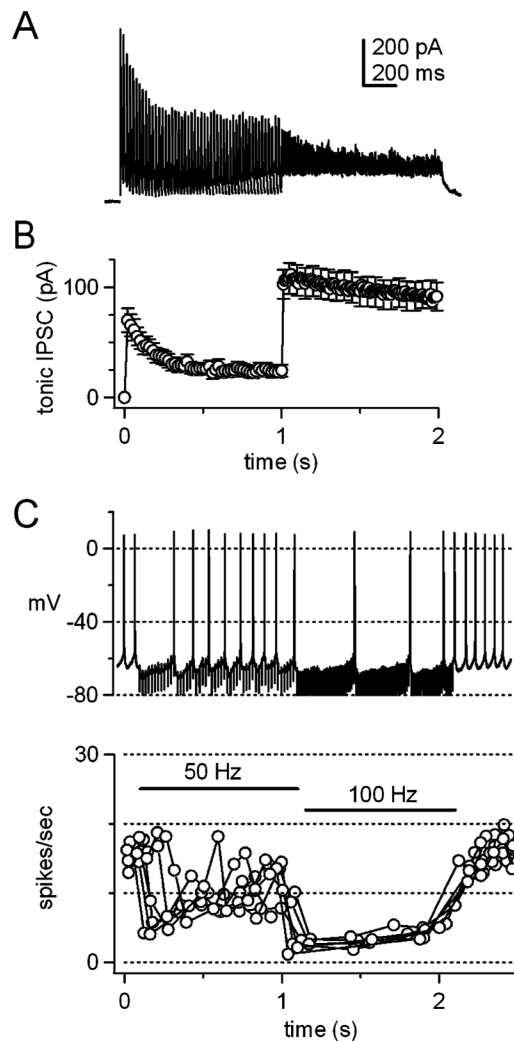


Fig. 2. Short-term synaptic depression and the efficacy of inhibition. **a** IPSCs in a cerebellar nuclear neuron in a cerebellar brain slice from a ~2-week-old mouse. Currents were evoked at -40 mV by stimulating Purkinje axons for 1-s at 50 Hz followed by a 1-s at 100 Hz; 31°C , $E_{\text{Cl}} = -70$ mV. Peak IPSC amplitudes depress by about 50% during the 50-Hz train and depression is slightly increased during the 100-Hz train. **b** The tonic IPSC, defined as the inhibitory synaptic current remaining just before the subsequent stimulation, during the 50-Hz and 100-Hz trains. Mean of 25 cells. **c** Spontaneous action potentials recorded in a cerebellar nuclear neuron with no holding current subjected to IPSPs evoked at 50 Hz (1 s) followed by 100 Hz (1 s). The firing rate is effectively decreased before the synaptic current has depressed (at the beginning of the 50-Hz train) or when the tonic IPSC is increased by rapid stimulation (during the 100-Hz train). **d** Instantaneous firing rate (spikes/s) for the cell in **c**, over six trials of identical stimuli. Data from [39], reproduced with permission

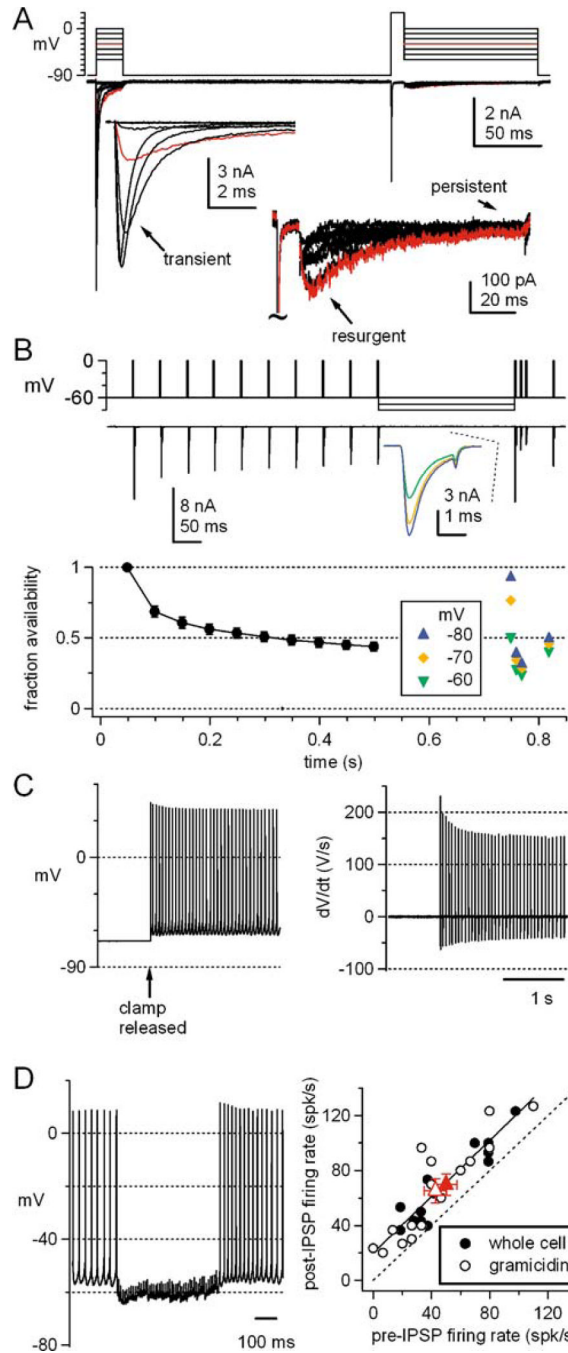


Fig. 3. Na currents, inactivation, and firing. **a** Transient, persistent, and resurgent tetrodotoxin-sensitive Na current evoked in a cerebellar nuclear cell acutely isolated from a ~2-week-old mouse. Currents were evoked by the voltage protocol shown. Responses to depolarizing and repolarizing steps are expanded in the insets. *Red trace* indicates steps to -30 mV. **b Top panel** Tetrodotoxin-sensitive Na currents evoked by step depolarizations from -60 to 0 mV applied at 20 Hz (10 stimuli), and interrupted by a 250 -ms step to -60 , -70 , or -80 mV. After this interval, three depolarizations are applied at 100 Hz to simulate a burst of action potentials. The first current evoked after the 250 -ms pause is expanded in the *inset*. **Bottom panel** Mean currents evoked by this protocol in five cells. During the 20 -Hz train, Na channels inactivate

by about 50%, and the availability of Na channels after the pause depends on the voltage during the interval. Data from [44], reproduced with permission. **c** Spontaneous action potentials with no holding current (*left*) and their derivatives (*right*) in an isolated cerebellar nuclear cell after a period of voltage clamp at -68 mV. Note the decrease in dV/dt over time, reflecting the decreasing availability of Na channels. Data from [10], reproduced with permission. **d** *Left* Action potentials and IPSPs evoked at 100 Hz in a cerebellar nuclear neuron in a slice (33°C). No holding current. *Right* Firing rate after the IPSPs plotted against the rate before the IPSPs, for 13 cells recording in the whole-cell configuration and 16 cells recorded in the perforated-patch configuration with gramicidin. *Red symbols* indicate the means of the two data sets. *Solid line* is a linear fit to the data, with a fitted slope of 1.0 and intercept of 20 Hz ($R^2=0.82$), indicating that after the period of inhibition, the firing rate increases by 20 Hz. Data from [45], reproduced with permission

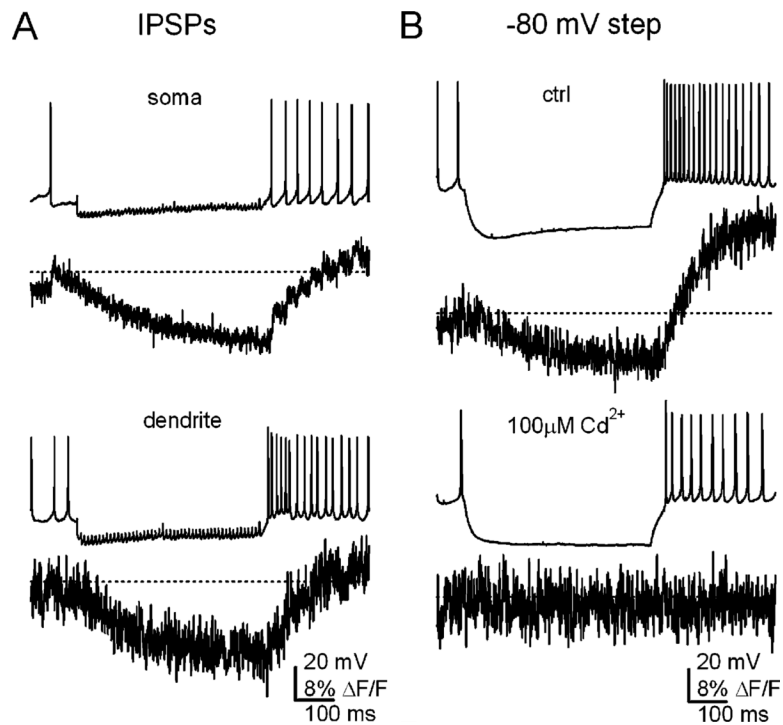


Fig. 4.

Ca signals during IPSPs and inhibition-like hyperpolarizations. **a** Spontaneous action potentials (no holding current) and IPSPs evoked at 100 Hz for 500 ms and somatic and dendritic Ca signals recorded with Fluo-4 ($K_d=345$ nM). $\Delta F/F$ values are calculated relative to the Ca signal when cells were held silent at -80 mV. Dotted lines indicate mean $\Delta F/F$ during spontaneous firing. Note that transients on the time scale of individual action potentials are evident. During IPSPs, Ca signals drop, indicating that firing neurons sustain a Ca load. After inhibition, Ca levels increase gradually at a rate proportional to the rate of firing, but do not rapidly exceed the value associated with spontaneous firing. **b** Experiments as in **a**, but with a current injection to -80 mV, a value about 5 mV negative to E_{Cl} , and about 10 mV hyperpolarized to the value reached during synaptic inhibition. With this hyperpolarization, the post-hyperpolarization dendritic Ca signal is large (*top*). The recording is repeated in the same cell (*bottom*) but with $100 \mu\text{M Cd}^{++}$ in the solution, which nearly fully blocks HVA channels but T-type channels by only 50%. The Ca changes are abolished, suggesting that they are mediated by HVA channels. Data from [45], reproduced with permission

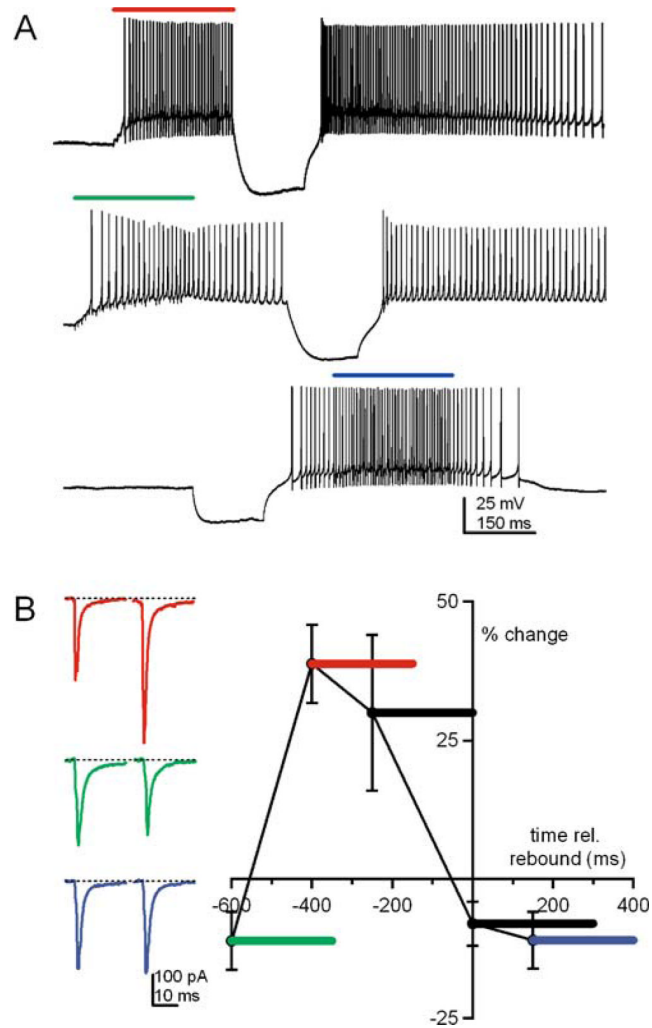


Fig. 5. Long-term potentiation of EPSCs. **a** Responses of cerebellar nuclear neurons to three induction protocols, consisting of a 250 ms train of EPSPs at 133 Hz, applied during the colored bar and a 150-ms hyperpolarization. **b** *Left* EPSCs evoked at -65 mV before and after 30 applications of the induction protocol. *Right* Efficacy of the various induction protocols in inducing long-term potentiation of EPSCs, plotted as the percent change in EPSC amplitude, against the onset of the excitatory synaptic stimulation relative to the time of disinhibition. *Bars* indicate the duration of the EPSPs. Data from [38], reproduced with permission