Lock-and-key mechanisms of cerebellar memory recall based on rebound currents

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Abstract (248 words)

A basic question for theories of learning and memory is whether neuronal plasticity suffices to guide proper memory recall. Alternatively, information processing that is additional to readout of stored memories might occur during recall. We formulate a ‘lock-and-key’ hypothesis regarding cerebellum-dependent motor memory in which successful learning shapes neural activity to match a temporal filter that prevents expression of stored but inappropriate motor responses. Thus, neuronal plasticity by itself is necessary but not sufficient to modify motor behavior.

We explored this idea through computational studies of two cerebellar behaviors and examined whether deep cerebellar and vestibular nuclei neurons can filter signals from Purkinje cells that would otherwise drive inappropriate motor responses. In eyelink conditioning, reflex acquisition requires the conditioned stimulus (CS) to precede the unconditioned stimulus (US) by >100 ms. In our biophysical models of cerebellar nuclei neurons this requirement arises through the phenomenon of post-inhibitory rebound depolarization and matches longstanding behavioral data on conditioned reflex timing and reliability. Although CS-US intervals <100 ms may induce Purkinje cell plasticity, cerebellar nuclei neurons only drive conditioned responses if the CS-US training interval was >100 ms. This bound reflects the minimum time for deinactivation of rebound currents such as T-type Ca²⁺. In vestibulo-ocular reflex adaptation, hyperpolarization-activated currents in vestibular nuclei neurons may underlie analogous dependence of adaptation magnitude on the timing of visual and vestibular stimuli. Thus, the proposed lock-and-key mechanisms link channel kinetics to recall performance and yield specific predictions of how perturbations to rebound depolarization affect motor expression.
Introduction

Research to date on the biological mechanisms of long-term memory has focused primarily on candidate mechanisms for memory formation, such as neuronal plasticity. But to what degree are the phenomenological properties of memory determined by biological mechanisms of memory recall? Studies on recall mechanisms have concerned re-consolidation processes that accompany retrieval (Debiec et al. 2006; Doyere et al. 2007), network attractor theories of associative memory (Hopfield 1982; Wills et al. 2005), and expression of learned reflexes (du Lac et al. 1995; Mauk and Donegan 1997; Medina et al. 2000; Medina and Mauk 2000). However, the electrophysiological dynamics that occur during recall might have an important role in shaping qualities such as memory reliability and generalization. Thus, a basic question is whether these dynamics function primarily as a readout mechanism for retrieving stored memories or also perform additional processing of the stored information. Pattern completion is one aspect of associative memory recall for which candidate biological mechanisms have been identified (Nakazawa et al. 2002). Nonetheless, the existing literature on recall has generally assumed that the electrophysiological dynamics of recall should facilitate effective readout, i.e. retrieving the appropriate memory in response to a stimulus. The possibility that some constraints on memory expression might also be enacted at recall has not been widely considered.

Recent work on cerebellar memory systems indicates there are multiple sites of neuronal plasticity and at least two memory storage sites with distinct induction kinetics (Boyd et al. 2004; De Zeeuw and Yeo 2005; Hansel et al. 2001; Lang et al. 1999; Ohyama and Mauk 2001; Ohyama et al. 2003a). According to two-stage models of cerebellar learning, the numerous synapses in cerebellar cortex support flexible and rapid acquisition of new associations, while subsequent plasticity in the deep cerebellar or vestibular nuclei allows long lasting memory storage (Boyd et al. 2004; Mauk 1997; Mauk and Donegan 1997; Miles and Lisberger 1981; du Lac et al. 1995). Purkinje cells in the cerebellar cortex receive inputs from \( \sim 10^5 \) parallel fibers, and project outputs to the deep cerebellar and vestibular nuclei in a highly convergent manner, with each nuclear cell influenced indirectly by \( 10^7 \text{-}10^8 \) parallel fibers (Napper and Harvey 1988; Mauk 1997). Given the vast number of potential network states in the cerebellar cortex, a rich set of training experiences might lead to network states that encode undesirable or inappropriate movements. The plausibility of this occurring is indicated by behavioral and computational studies that suggest the distribution of synaptic plasticity levels might evolve in a complex manner throughout learning experience, rather than purely reversing course during extinction or relearning (Kimpo et al. 2005; Mauk and Ohyama 2004). An example of an undesirable motor response is one executed in response to sensory cues that are reliably associated
with rewarding or aversive stimuli but that arrive too late to be predictive of an appropriate motor action. Are there memory recall mechanisms that selectively prevent the expression of inappropriate motor responses, despite significant induction of synaptic plasticity? Or does plasticity induction always suffice to modify cerebellar-mediated motor behavior? At least for some forms of associative motor learning mediated by non-cerebellar memory systems, it has been shown that associative memory storage by itself can be insufficient to modify behavior (Barnet et al. 1997).

To explore these issues we formulated a ‘lock-and-key’ hypothesis stating that the induction of plasticity is necessary but not sufficient for training to modify motor behavior. There is the additional requirement that plasticity must shape the dynamics of neural activity (the ‘key’) to match a temporal filter (the ‘lock’) that selectively precludes inappropriate motor responses to sensory stimuli. We then examined this hypothesis in the context of two cerebellum-dependent behaviors, classical eyeblink conditioning (Christian and Thompson 2003) and adaptation of the vestibulo-ocular reflex (VOR) (Ito 1982; Miles and Lisberger 1981), for which there exist longstanding, rich behavioral data sets (Gormezano et al. 1962; Raymond and Lisberger 1996). If our hypothesis is true, what biological mechanisms might serve as the lock for these two behaviors?

This paper focuses on rebound currents in the deep cerebellar nuclei (DCN) and medial vestibular nuclei (MVN) neurons as candidate lock mechanisms, because it is well established that these currents perform significant temporal transformations of hyperpolarizing inputs, such as those from cerebellar Purkinje cells believed to trigger learned movements. Rebound channels, such as low voltage-activated (T-type) and hyperpolarization-activated cation (h) channels, are expressed at sufficient density to generate robust post-inhibitory rebound depolarizations in DCN and MVN neurons, the output neurons of cerebellar circuits (Aizenman and Linden 1999; Aizenman et al. 1998; Jahnson 1986a; Llinas and Muhlethaler 1988; Sekirnjak and du Lac 2002). For both behaviors studied, rebound channel kinetics emerge as crucial determinants of the minimum allowable duration between a sensory cue and a trained motor response. If the delay between the cue and a well-timed response is less than the time needed to activate rebound channels fully from the neuronal resting potential, the magnitude of the learned response declines or vanishes, thereby enacting the lock mechanism. This proposal represents a direct link from channel kinetics to learning performance and yields specific predictions of how learning performance is affected by perturbations to the rebound process.

In eyeblink conditioning, key aspects of the behavior that remain poorly understood concern stimulus timing. Training of a reliable reflex requires the conditioned stimulus (CS), such as a tone, to start at least ~100 ms prior to the unconditioned stimulus (US), such as an air puff to the eye (Figure 1A) (Gormezano et al. 1962; Ohyama et al. 2003b). Even after averaging data over multiple subjects
there remains a steep dependence of reflex acquisition on the CS-US training interval (Figure 1B), with the expression of conditioned blinks falling sharply for intervals <100 ms (Ohyama et al. 2003b; Salafia et al. 1980; Schneiderman and Gormezano 1964; Smith 1968; Smith et al. 1969). What is the mechanistic basis for this effect? Analogous, unexplained dependencies on stimulus timing have been reported for VOR adaptation, in which the magnitude of learned eye movements depends on the timing between pulsed visual and vestibular training stimuli (Raymond and Lisberger 1996).

According to current thinking in the field an important mechanism of memory formation is the long-term depression (LTD) of cerebellar parallel fiber (PF) to Purkinje cell synapses induced by synchronous activation of PF and climbing fiber (CF) inputs to Purkinje cells (Albus 1971; Ito 1989; Ito and Kano 1982; Marr 1969). In eyeblink conditioning, it is thought that PF and CF inputs respectively convey signals regarding the CS and the US (Hesslow et al. 1999; Mauk et al. 1986; McCormick et al. 1985; Steinmetz et al. 1989; Steinmetz et al. 1986), and that LTD resulting from repeated CS-US pairings leads to a conditioned reflex to the CS alone. This is proposed to occur since LTD should diminish the efficacy of CS-driven input to Purkinje cells, allowing disinhibition of deep cerebellar nuclei (DCN) neurons that receive GABAergic Purkinje cell inputs and drive conditioned reflexes (Albus 1971). In VOR adaptation, CFs and PFs respectively convey visual and vestibular information, and LTD is proposed to allow reflex adaptation by reducing the strength of PF inputs signaling ipsiversive head rotation (Ito 1989). Although other cerebellar plasticity mechanisms exist (Boyden et al. 2004; De Zeeuw and Yeo 2005; Hansel et al. 2001), multiple strains of mice with disrupted LTD show deficits in eyeblink conditioning and VOR adaptation (Feil et al. 2003; Kishimoto et al. 2001; Koekkoek et al. 2003; Koekkoek et al. 2005; Miyata et al. 2001; Shibuki et al. 1996). Nonetheless, accounts of cerebellar-mediated learning based solely on LTD do not easily explain the full range of behavioral data (Boyden et al. 2006; Boyden and Raymond 2003; Kimpo et al. 2005; Medina and Mauk 1999; Ohyama and Mauk 2001; Ohyama et al. 2003a).

One issue concerns whether Purkinje cells purely inhibit motor responses. Purkinje cells might be partly excitatory in their net effect, due to post-inhibitory depolarization in their target DCN and MVN neurons (Aizenman et al. 1998; Jahnsen 1986a; b; Llinas and Muhlethaler 1988; Sekirnjak and du Lac 2002). Another issue concerns the possible role in learning of long-term potentiation (LTP) at the PF-Purkinje cell synapse. LTP and LTD induction at this synapse are spike-timing dependent (Abbott and Nelson 2000), with LTP induced by unpaired PF or asynchronous PF-CF input (Coesmans et al. 2004; Wang et al. 2000). Maximal LTD induction seems to occur for PF activity that slightly precedes CF activity, by a time \( t_{LTD} \) of 50-100 ms that likely reflects the kinetics of postsynaptic \( \text{Ca}^{2+} \) dynamics (Doi et al. 2005). However, LTD induction can occur with either PF or CF activity occurring first, but delays of more than \(~200\) ms are ineffective with either ordering (Wang et al.
2000). It has been suggested that disinhibition of cerebellar nuclei neurons and spike-timing dependent plasticity suffice to explain the requirement in eyeblink conditioning for the CS-US interval to be >100 ms (Wang et al. 2000). However, this has never been demonstrated explicitly using either computational modeling or experimental manipulation of behavior. A main difficulty is that the empirically determined rules for LTD induction suggest LTD should occur at short CS-US intervals that do not lead to acquisition of conditioned reflexes in behavioral experiments (Wang et al. 2000). Furthermore, dependence of learning performance on the CS-US interval appears much steeper than that of spike-timing dependent plasticity at the PF-Purkinje cell synapse (Salafia et al. 1980; Schneiderman and Gormezano 1964; Smith 1968; Smith et al. 1969; Wang et al. 2000). Thus, the degree to which conditioned reflex acquisition is shaped by physiological mechanisms other than spike-timing dependent plasticity remains an important issue for experimental investigation.

Here, we consider the novel possibility that significant shaping of learned motor expression might occur through the electrophysiological mechanisms of memory recall. In our work LTP and LTD emerge as complementary processes, both of which are important for memory formation as well as for memory clearance. This contrasts with the common view of LTD and LTP as opposing processes, one allowing memory storage and the other clearance (Boyd and Raymond 2003; Coesmans et al. 2004; Lev-Ram et al. 2003). Because plasticity induction is spike-timing dependent, we begin by considering the timing of sensory driven activity in the PF axons of cerebellar granule cells. Using a series of electrical compartmental models of increasing complexity, we simulate responses of DCN and MVN cells to learned sensory cues. This allows us to validate quantitatively the data from our DCN cell simulations against the classic behavioral data on eyeblink conditioning (Salafia et al. 1980; Schneiderman and Gormezano 1964; Smith 1968; Smith et al. 1969), by comparing the percentage of trials with successful responses as found experimentally to data generated by our models.

Comparison of the VOR adaptation magnitude in our modeling to that in behavioral studies suggests post-inhibitory rebounds might play a role in multiple cerebellum-dependent behaviors. Based on the results of our biophysical models we provide an algorithmic description of the 'lock-and-key' mechanism as a temporal filter. Learning experience that successfully modifies motor behavior shapes neural activity to match this temporal filter. Unsuccessful training can yield comparable magnitudes of synaptic plasticity, but the resulting patterns of Purkinje cell activity do not trigger learned motor responses. We have organized the following sections so that readers who wish to omit the computational details may skip Materials & Methods without loss of logical continuity.
Materials and Methods

General simulation procedures

We created compartmental models of DCN and MVN cells in the NEURON (Hines and Carnevale 1997) and MATLAB software environments and set model parameters using empirically determined values whenever possible. Fortunately, much is known about DCN cells from in vitro studies. We found that values determined from measurements in DCN and MVN cells, rather than other cell types, facilitated consistency with behavioral data. The current balance equation describing the balance of capacitive and ionic currents, $C_m (dV / dt) = - \sum I_{ionic}$, was integrated over time using Euler’s method (single-compartment simulations) or a variable time-step method (two-compartment simulations). Time-steps in all cases were less than 0.1 ms. For channel time constants that were empirically determined at temperatures other than 37°C, values were adjusted to 37°C using measured $Q_{10}$ values (Tables 1 and 2).

Voltage-dependent currents obeyed equations of the form $I = \bar{g} x^r y(V - V_{rev})$, where $\bar{g}$ is the maximum conductance and $V_{rev}$ is the reversal potential. Activation variables, $x$, followed first-order kinetics as defined by $dx/dt = \phi_x [\alpha_x (V)(1 - x) - \beta_x (V)(x)]$, where $\alpha_x$ and $\beta_x$ are forward and backward rates and the temperature factor, $\phi_x = 2.3$, was the same value for all conductances. Inactivation variables, $y$, obeyed analogous expressions. Steady state and relaxation time constants are given in terms of $\alpha_x$ and $\beta_x$: $x_s = \alpha_x / (\alpha_x + \beta_x)$ and $\tau_x = 1 / (\alpha_x + \beta_x)$.

The firing rates of Purkinje cells were modeled to be from a cerebellar network after behavioral training. Electrophysiological data from in vivo recordings were used to constrain background firing rates, $r_{pki,b} = 40$ Hz (Berthier and Moore 1986), and the modulation of Purkinje cell firing rates due to learning-related cerebellar plasticity (low and high Purkinje cell spikes rates were $r_{pki,d} = 20$ Hz and $r_{pki,p} = 100$ Hz (Berthier and Moore 1986)).

Given these basic constraints the average Purkinje cell spike rate, $R_{pki}(t)$, was determined by first convolving a smooth plasticity function, $S(\Delta t)$, whose argument is the relative delay between activity in parallel fibers and climbing fibers (shown in Figure 2B), with a boxcar function, $U(t)$,
representing a US of 10 ms duration. The result of this convolution was then multiplied by a smooth function, \( C(t) \), representing a CS of duration no less than a minimum interval, \( t_{CS\text{min}} = 50 \) ms:

\[
S(\Delta t) = N \left( T \left[ \frac{(\Delta t - t_{LTD-})}{\tau} \right] - T \left[ \frac{(\Delta t - t_{LTD+})}{\tau} \right] \right)
\]

\[U(t) = 0, \ t < t_{US} \quad \text{;} \quad U(t) = 1, \ t_{US} \leq t \leq t_{US} + 10 \text{ms} \quad \text{;} \quad U(t) = 0, \ t > t_{US} + 10 \text{ms}
\]

\[C(t) = T \left[ \frac{(t - \Delta t)}{\tau} \right] - T \left[ \frac{(t - \max(t_{CS\text{min}}, t_{ISI} + 10 \text{ms})}{\tau} \right]
\]

\[R_{pj}(t) = r_{pj,b} + C(t) \int_{-\infty}^{\infty} S(t - t') U(t') \ dt'
\]

where \( t_{US} \) is the time of US onset and \( t_{ISI} \) is the interstimulus interval between the CS and US onset times. \( t_{LTD-} = -10 \) ms and \( t_{LTD+} = 75 \) ms are, respectively, the minimum and maximum allowable delay between CS and US activity for induction of LTD, and \( \tau = 10 \) ms is a characteristic transition time describing the smooth temporal evolution of neural dynamics. We used the smoothing function, \( T[x] \), to ensure gradual changes in spike rates:

\[T[x] = 0, \ x \leq -1 \quad \text{;} \quad T[x] = \frac{(1 + \cos(\pi x))}{2}, \ -1 \leq x \leq 0 \quad \text{;} \quad T[x] = 1, \ x \geq 0.
\]

The constant, \( N \), was set such that the minimum and maximum Purkinje cell firing rates for a long ISI were \( r_{pj,d} = 20 \) Hz and \( r_{pj,p} = 100 \) Hz respectively (Table 1). These plasticity rules led to gradual transitions between distinct average firing rate values over ~20 ms intervals, approximating the observed intervals over which Purkinje cells modulate their spiking rates during expression of motor learning (Berthier and Moore 1986; King et al. 2001).

CS-driven modulation of the rate of mossy fiber spiking was also constrained by data from in vivo electrophysiological recordings and was expressed as:

\[R_{MF}(t) = r_{MF,b} + (r_{MF,CS} - r_{MF,b}) C(t)
\]

where \( r_{MF,b} = 10 \) Hz is the background firing rate for mossy fibers and \( r_{MF,CS} = 50 \) Hz is the spiking rate of mossy fibers during presentation of the conditioning stimulus (Freeman and Nicholson 1999; Nicholson and Freeman 2002).
Model 1 – A single-compartment model of DCN neurons

We modeled a DCN cell with a single electrical compartment that included leak ($I_L$) and T-type Ca$^{2+}$ ($I_T$) currents, as well as synaptic currents due to inputs from Purkinje cells ($I_{syn,Pl Dj}$) and mossy fibers ($I_{syn,MF}$). Membrane voltage dynamics were determined by time integration of the current balance equation: $C_m(dV/dt) = -I_T - I_L - I_{syn,Pl Dj} - I_{syn,MF}$. The passive current, $I_L = g_L(V - V_L)$, was an admixture of two components: a tonic mixed-cation current that is characteristic of DCN cells and has a ~30 mV reversal potential (Raman et al. 2000), and a standard leak current with ~75 mV reversal potential (Jahnsen 1986b; Llinas and Muhlethaler 1988). Total leak conductance, $g_L$, and leak reversal potential, $V_L$, were determined by the DCN cell’s resting potential of $V_{rest} = -58$ mV (Aizenman and Linden 1999; Llinas and Muhlethaler 1988), and the observed membrane time constant of ~12 ms (Jahnsen 1986a; Llinas and Muhlethaler 1988).

Parameter values for Purkinje and mossy fiber synaptic inputs were constrained by physiological measurements: $V_{syn,Pl Dj} = -75$ mV (Jahnsen 1986b; Llinas and Muhlethaler 1988), $\tau_{syn,Pl Dj} = 14$ ms (Anchisi et al. 2001), and $V_{syn,MF} = 55$ mV (Hille 2001) (Table 1). Glutamatergic synapses in the DCN have significant AMPA and NMDA components (Anchisi et al. 2001). As a simplification, $\tau_{syn,MF}$ for mossy fibers was chosen to be 31 ms by weighting the AMPA and NMDA decay time constants at ~60 mV by the measured relative amplitudes of AMPA and NMDA glutamatergic input (Anchisi et al. 2001). T-current was the sole voltage-dependent current: $I_T = g_T n_l(V - V_{Ca})$, with $V_{Ca} = 140$ mV (Hille 2001). T-type kinetics were adapted from a model of the $\alpha 1$G T-type channel (McRory et al. 2001), which is highly expressed in the DCN (Talley et al. 1999). Steady-state values of the gating variables, $n_x$, and $I_x$, were modified to fit measurements of T-type currents in DCN cells (Gauck et al. 2001). $\tau_n$ and $\tau_I$ were increased twofold in order to produce rebound depolarizations at 37°C (Jahnsen 1986b). In our model these expressions were:

$$\tau_n = 1 + 0.24 \cdot \exp\left(-V/15.8\right) \text{ms}$$

$$\tau_I = 20.8 + 0.24 \cdot \exp\left(-V/7.9\right) \text{ms}$$

$$n_x = \left(1 + \exp\left((V + 42)/-4.25\right)\right)^{-1}$$
\[ I_\alpha = \left( 1 + \exp \left( \frac{V + 63}{3.5} \right) \right)^{-1}. \]

Unfortunately, several DCN cellular parameters could not be tightly constrained by biophysical data. T-type channels appear to be most dense in DCN cell distal dendrites (Gauck et al. 2001). Thus, estimation of total T-type conductance from somatic recordings is difficult. However, in initial simulations we identified a broad range of T-type conductance values over which rebound depolarizations occurred, indicating that occurrence of post-inhibitory rebound is not highly sensitive to the value of the T-conductance. In all subsequent single-compartment simulations (Models 1 and 2) we chose \( g_T = 0.9 \text{ mS/cm}^2 \) within the middle of this identified range.

The weights of synaptic inputs, \( W_{\text{syn,Pkj}} = 0.1 \text{ mS/cm}^2 \) and \( W_{\text{syn,MF}} = 1 \text{ uS/cm}^2 \), were chosen to be near the middle of a range of values capable of inducing physiological 10-15 mV changes in membrane voltage when input firing rates were modulated. These synaptic conductance values were also chosen such that mossy fiber input alone was insufficient to drive the cell to rebound. Without this stipulation there would be little dependence of learned responses on Purkinje cell input, contrary to experimental findings. In Model 1 all synaptic inputs were simulated in a deterministic fashion. The synaptic conductance, \( g_{\text{syn}}(t) \), was determined by convolving the input spike rate with an exponential function of time constant \( \tau_{\text{syn}} \) and amplitude \( W_{\text{syn}} \), which represented the conductance response (Table 1).

**Model 2 – A single-compartment model of dendritic Ca\(^{2+}\) spiking**

Model 2 was the same as Model 1 but with two additions. First, we added a high-voltage activated (HVA) Ca\(^{2+}\) current, so we could study how graded T-current mediated rebound depolarizations led to the initiation of HVA Ca\(^{2+}\) spikes. The conductance model for \( I_{\text{HVA}} \) was identical to that used by Mainen and Sejnowski (Mainen and Sejnowski 1996). Second, membrane voltage dynamics were no longer deterministic, because synaptic inputs arrived stochastically at a time-varying mean rate. The number of Purkinje cell (\( N_{\text{Syn,Pkj}} = 50 \)) and mossy fiber (\( N_{\text{Syn,MF}} = 10 \)) inputs roughly matched the ratio, \( R \), of GABAergic to glutamatergic synapses found in the DCN (Chan-Palay 1973) (Table 1). The occurrence of a pre-synaptic spike on any of these independent individual inputs
led to an instantaneous jump in synaptic conductance of amplitude $W_{\text{syn},ij} N_{\text{syn},ij}$ or $W_{\text{syn, MF}} N_{\text{syn, MF}}$, which then declined exponentially with time constant $\tau_{\text{syn, ij}}$ or $\tau_{\text{syn, MF}}$, respectively (Table 1).

**Model 3 – A two-compartment model of DCN neurons**

A DCN cell model with dendritic and somatic compartments was used to test the effect of rebound conductances on $\text{Na}^+$ spiking output under slightly more realistic conditions. The potentials of dendritic ($V_d$) and somatic ($V_s$) membranes were determined by the currents flowing in each compartment (Pinsky and Rinzel 1994):

\[
C_m \frac{dV_d}{dt} = -I_T - I_L - I_{\text{HVA}} - I_{\text{SK}} - I_{\text{syn, ij}} - I_{\text{sym, MF}} - \frac{g_c(V_d-V_s)}{1-\rho}
\]

\[
C_m \frac{dV_s}{dt} = -I_T - I_L - I_{\text{HVA}} - I_{\text{SK}} - I_{\text{Kv}} - I_{\text{Na}} - \frac{g_c(V_s-V_d)}{\rho}
\]

where the coupling between the two compartments was specified by the conductance between compartments, $g_c$, and the ratio of somatic area to total cell area, $\rho$. As in Models 1 and 2, the two-component voltage-independent current, $I_L$, determined both $V_{\text{rest}} = -58$ mV and the membrane time constant via $\tau_m = 12$ ms. Stochastically arriving synaptic currents entered the dendritic compartment and were modeled as in Model 2.

Active somatic currents were T-type $\text{Ca}^{2+}$, high-voltage activated $\text{Ca}^{2+}$ ($I_{\text{HVA}}$; Gauck et al. 2001), $\text{Ca}^{2+}$-activated $\text{K}^+$ ($I_{\text{SK}}$; Raman et al. 2000), fast $\text{Na}^+$ ($I_{\text{Na}}$), and delayed rectifying $\text{K}^+$ ($I_{\text{Kv}}$). The simulation used the total $\text{Ca}^{2+}$ current, $I_{\text{Ca}}$, to determine the internal $\text{Ca}^{2+}$ concentration, which controlled the gating of $I_{\text{SK}}$. In addition to $I_L$, the dendrite had T-type currents (Gauck et al. 2001), $I_{\text{HVA}}$ (at half the density as in the soma; see (Gauck et al. 2001)), $I_{\text{SK}}$, and synaptic input.

Conductance models for $I_{\text{HVA}}$, $I_{\text{SK}}$, $I_{\text{Na}}$, $I_{\text{Kv}}$, and $I_{\text{Ca}}$ were identical to those used by Mainen and Sejnowski (Mainen and Sejnowski 1996), including parameter values, except for $\tau_{\text{Na},L}$ and $\tau_{\text{Kv},n}$, which were increased fourfold to reproduce the observed tonic DCN cell firing rates of $\sim25$ Hz; (Aksenov et al. 2005; Jahnsen 1986a; Raman et al. 2000) and spike width of $\sim1.5$ ms (Aizenman and
Linden 1999; Llinas and Muhlethaler 1988). Reversal voltages were $V_{Na} = 55$ mV, $V_K = -90$ mV according to (Jahnsen 1986b; Spain et al. 1987), and $V_{Ca} = 140$ mV.

Coupling parameters, $g_c = 0.05$ nS and $\rho = 0.05$, were chosen to ensure each compartment had relatively independent dynamics while still permitting dendritic voltage deflections to affect somatic spiking. Synaptic weights were set by the same criteria as for the one-compartment model, with $W_{syn,PIl} = 0.1$ mS/cm² and $W_{syn,MP} = 1$ μS/cm². As in Models 1 and 2, $\bar{g}_{T} = 0.6$ mS/cm² was set near the middle of a broad range of values that allowed rebound depolarization. The Ca²⁺-activated K⁺ conductance, $g_{SK} = 0.1$ mS/cm², and the high-voltage activated Ca²⁺ current, $g_{HVA} = 0.05$ mS/cm², had little to no effect on the probability of HVA Ca²⁺ spiking in response to synaptic inputs across a broad range of conductance densities and were chosen to reduce the duration of the Ca²⁺ spike to physiologically realistic values (Jahnsen 1986b; Llinas and Muhlethaler 1988). Hodgkin-Huxley conductances, $g_{Na} = 45$ mS/cm² and $g_{Kv} = 25$ mS/cm², were chosen to reproduce the experimental observation of spontaneous spiking in the soma at $V_{rest} = -58$ mV.

**Phase plane analysis of rebound depolarizations**

To study whether a memory recall mechanism based on rebound depolarization would be robust, we reduced Model 1 to a system of two dynamical degrees of freedom amenable to graphical phase plane analysis. This involved an approximation in which the T-type activation variable was set equal to its asymptotic value, $n = n_A(V)$, reducing the dynamical variables to only the T-type inactivation variable, $(I)$, and the membrane voltage, $(V)$. Because this approximation increased the membrane excitability, resulting in larger magnitude rebounds, we decreased the density of T-type Ca²⁺ channels to $\bar{g}_T = 0.6$ mS/cm² as a compensatory measure. The system’s dynamical trajectories within the $(V, I)$ phase plane can then be fruitfully studied by determination of the two nullcline curves, on which the partial time derivatives vanish:

$$\frac{\partial I}{\partial t} = 0 \quad \Rightarrow \quad I = I_A(V)$$

$$\frac{\partial V}{\partial t} = 0 \quad \Rightarrow \quad I = \frac{g_L(V - V_L) + I_{syn,PIl}(V) + I_{syn,MP}(V)}{\bar{g}_T \cdot n_A(V) \cdot (V - V_{Ca})}$$

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where the Purkinje cell and mossy fiber synaptic input currents $\bar{T}_{\text{syn}}(V) = g_{\text{syn}}(V - V_{\text{syn}})$ represent the mean synaptic currents as determined from the synaptic weights and background firing rates. Both partial time derivatives vanish at the intersection point of the two nullclines, so this is a fixed-point of the dynamics. Fixed-points during the neuronal resting state (stage 1), the CS-US interstimulus interval up until $t_{LTD}$ before the expected US onset (stage 2), and the remaining portion of the CS (stage 3), were found using the MATLAB function fzero to solve for the intersection of the nullclines. Linear stability analysis within a neighborhood of the resting (stage 1) fixed-point at $V = -58$ mV revealed that this fixed-point is stable for $\bar{g}_T < 1.1$ mS/cm$^2$. The dynamical trajectories near this fixed-point exhibit damped oscillations for $\bar{g}_T \geq 0.05$ mS/cm$^2$. In the regime relevant for our studies, the low density of T-type channels (Table 1) results in a stable spiral fixed-point.

A map of rebound magnitudes in the phase plane (Figure 6C) was built by numerically integrating the equations of motion using MATLAB’s 4$^{\text{th}}$ order Runge-Kutta initial-value differential equation solver, ode45. A series of evenly spaced initial points was chosen along the boundary lines of the phase plane, defined by $V = -72$ mV and $l = 0$, and trajectories were integrated forward in time using the current balance equation and the constant synaptic input values of stage 3. Integration proceeded until the trajectories reached the stage 3 fixed point or left the region of interest, $0 < l < 1$. Each trajectory formed a contour (level-curve) on the phase plane map with the contour amplitude given by the maximum depolarization achieved on that trajectory.

To determine the voltage threshold curve for firing all-or-none Ca$^{2+}$ spikes (Figure 6D), we added a high-voltage activated (HVA) Ca$^{2+}$ conductance (Mainen and Sejnowski 1996) to the phase plane treatment of Model 1 in which $n$ relaxes instantaneously to its asymptotic value, $n = n_{\infty}(V)$ (Figure 6 panels A-C). This yielded a deterministic version of Model 2 that produced virtually the same trajectories as Model 1 over the voltage range, $V < -35$ mV, in which the HVA channels are largely closed (Figure 6D). As before, the instantaneous activation of T-type currents led to increased membrane excitability, for which we compensated by decreasing the density of T-type Ca$^{2+}$ channels to $\bar{g}_T = 0.6$ mS/cm$^2$ and the density of HVA Ca$^{2+}$ channels to $\bar{g}_{\text{HVA}} = 0.027$ mS/cm$^2$. We solved for the
dynamical trajectories by integrating the equations of motion forward in time starting at a series of initial conditions spaced evenly along the boundary lines of the phase plane, defined by \( V = -72 \) mV and \( I = 0 \). The trajectories fell into two classes depending on whether the T-current mediated rebound was of sufficient magnitude to cross the threshold for generation of a HVA Ca\(^{2+}\) spike.

**Phase plane movies showing model trajectories**

Movies of deterministic (Movies 1, 2 using Model 1) and stochastic (Movie 3 using Model 2) voltage trajectories were created in MATLAB. As in the phase plane analysis of Figure 6, Movies 1 and 2 relied on the mathematical approximation of instantaneous relaxation of the T-channel activation variable to its asymptotic value. The motion of the \( V \) nullcline was determined by solving the equation \( \frac{\partial V}{\partial t} = 0 \) for \( V \), using the steady state values of the synaptic conductances, \( g_{syn} \), that would be attained given constant Purkinje cell and mossy fiber spiking at rates equal to their instantaneous values. Numerical integration of the current balance equation used the Euler method with a timestep of 0.1 ms. In Movie 3 the synaptic conductances were modulated by the independent but stochastic arrivals of spikes from 50 Purkinje cells and 10 mossy fibers.

**Single-compartment model of MVN neurons**

We created a simple one-compartment model of MVN cells (Table 2) obeying the current balance equation, \( C_m (dV/dt) = -I_L - I_h - I_{syn,PK} - I_{syn,MF} \), in which the reversal potential and conductance values for the leak current, \( I_L = g_L (V - V_L) \), were determined by the membrane time constant (12 ms) and resting potential (−58 mV). Synaptic inputs were modeled in a deterministic fashion, as in Model 1 above.

Electrophysiological studies have repeatedly shown that the h-current exhibits bi-exponential kinetics, which is consistent with the current being an admixture of fast and slow components (Dickson et al. 2000; Pape 1996; Santoro et al. 2000). Thus, in our model these two components, \( f \) (“fast”) and \( s \) (“slow”), obeyed \( I_h = \bar{g}_h (f + s) (V - V_h) \), where \( V_h = -20 \) mV is the mixed-cation reversal potential (Pape 1996; Santoro et al. 2000).
The kinetic behavior of the first-order activation variables \( f \) and \( s \) was based on two sets of measurements. Isoform HCN2 appears to be the predominant subtype of h-channel in the vestibular nuclei, so we relied heavily on studies of HCN2 kinetics in a xenopus oocyte expression system (Santoro et al. 2000). By fitting measurements of the ratio of the fast and slow current components (Fig. 9B in Ref. (Santoro et al. 2000)) and of the total h-current (Fig. 9C in Ref (Santoro et al. 2000)) to sigmoidal curves, we found expressions for the steady-state voltage dependencies:

\[
f_{ss} = \left( \frac{1}{1 + \exp \left( \frac{V + 75}{5.53} \right)} \right) \left( \frac{1}{1 + \exp \left( \frac{V + 86.4}{8.7} \right)} \right)^{-1}
\]

\[
s_{ss} = \left( \frac{1}{1 + \exp \left( \frac{V + 75}{5.53} \right)} \right) \left( \frac{1}{1 + \exp \left( \frac{V + 86.4}{-8.7} \right)} \right)^{-1}
\]

Activation time constants for both \( f \) and \( s \) were modeled using the functional form \( \tau_{f,s} = 1 / (a_{f,s} + b) \), where \( a_{f,s}(V) \) and \( b(V) \) dominate the behavior of the time constants at hyperpolarized and depolarized voltages, respectively. Both the \( f \) and \( s \) time constants rise exponentially with voltage for hyperpolarizations of more than about \(-65 \) mV (Santoro et al. 2000). Thus, we used exponential fits to the HCN2 activation data (Fig. 9A in Ref. (Santoro et al. 2000)) to describe \( a_f \) and \( a_s \):

\[
a_f = \exp \left( \frac{-(V + 178)}{16.4} \right) \text{ ms}^{-1}
\]

\[
a_s = \exp \left( \frac{-(V + 285)}{27.5} \right) \text{ ms}^{-1}
\]

At more depolarized potentials the time constants for both the fast and slow current components followed the expression used by Dickson et al. (Dickson et al. 2000) to describe slow deactivation:

\[
b = \left( \frac{0.0027 \cdot (V - 38)}{1 - \exp \left( \frac{38 - V}{17.4} \right)} \right) \text{ ms}^{-1}
\]
\( g_b = 0.5 \text{ mS/cm}^2 \) was chosen to be within a range of values that produced rebound depolarizations.

**Linear-Nonlinear (LN) model of lock-and-key mechanism**

For our algorithmic description of memory retrieval we generated a set of ‘key’ activity patterns, \( K(t) \), using the CS-driven waveforms for the instantaneous Purkinje cell spike rates arising for ISI values ranging from 0 to 200 ms. These Purkinje cell spiking rates were the same as for the biophysical Models 1, 2 and 3. We created a linear filter,

\[
F(t) = (r_{p\hat{k},d} - r_{p\hat{k},p}) \left( T\left[ \frac{t}{\tau} \right] - T\left[ \frac{t-t_p}{\tau} \right] + T\left[ \frac{t-2t_p}{\tau} \right] \right),
\]

where \( r_{p\hat{k},d} \) and \( r_{p\hat{k},p} \) are the minimum and maximum Purkinje cell spiking rates (Table 1), \( T[t] \) is the smooth transition function (see General Stimulation Procedures), \( t_p = 20 \text{ ms} \) and \( \tau = 10 \text{ ms} \). The filtered key activity was determined by the convolution:

\[
x(t) = \int K(\tau) \cdot F(t - \tau) \ d\tau.
\]

Finally, this signal was passed through an exponential nonlinearity, \( M(t) = G[x(t)] = \exp[10x(t)] \).

The response amplitude for a given ISI value (Figure 8E) was determined by the peak value of \( M(t) \) attained during Purkinje cell input. We normalized the response amplitudes relative to the value attained for a long ISI of 200 ms.
Results

A theoretical framework for cerebellum-dependent learning and memory

Cerebellar granule cells number in the ten billions but individually appear to be rarely active, producing only a few spikes at a time (Chadderton et al. 2004). Such transient activation implies that after behavioral training and plasticity induction at PF-Purkinje cell synapses, presentation of a learned sensory cue should drive a biphasic modulation of population Purkinje cell activity (Figure 2). For example, in classical conditioning transient CS-driven granule cell activity that is concurrent with US-driven CF activity will lead to LTD at PF-Purkinje cell synapses (Figure 2A). CS-driven granule cell activity that is asynchronous with CF activity will lead to LTP. During subsequent CS input, the net effect of LTD and LTP induction at distinct PF-Purkinje cell synapses will be biphasic modulation of the aggregate Purkinje cell activity received by a DCN neuron (Figures 2B and 2C). Similarly, overlapping pulses of vestibular and visual input in VOR adaptation will also lead to biphasic modulation of Purkinje cell activity. This general pattern of modulation does not hinge on the details of granule cell coding but is contingent on there being spike-timing dependent bi-directional plasticity and subsets of granule cells in which sensory driven activity lasts for only portions of the sensory cue duration (Buonomano 1994; Mauk and Donegan 1997; Medina et al. 2000).

We explored the conditions under which biphasic activation of Purkinje cells leads to reliable post-inhibitory rebound depolarization of their target neurons that drive learned motor responses. In classical conditioning, whether Purkinje cell spiking first rises and then falls in response to a learned CS, or vice versa, depends on whether the CS and US were paired with a ‘forward’ (CS-US) or ‘backward’ (US-CS) ordering. The two patterns of aggregate Purkinje cell activity should be quite distinct in their propensity to induce DCN cell rebounds. A rise and then fall of Purkinje cell spiking appears well suited to induce rebounds by causing a hyperpolarization and then a depolarization in DCN target cells. The DCN cell resting potential is about –58 mV (Aizenman and Linden 1999), at which T-channels are largely inactivated (Figure 3). The initial hyperpolarization allows T-channels to deinactivate and the ensuing depolarization allows them to activate. The opposite pattern of Purkinje cell spiking resulting from backward training should be a poor initiator of DCN cell rebounds, because the initial depolarization will heighten T-channel inactivation and should largely preclude rebounds. To test these ideas, we performed compartmental modeling of DCN cells to explore if such a disparity in rebound generation could account for the observed differences in behavioral responses following backward versus forward classical conditioning. For our modeling, we described the timing dependence of LTP and LTD induction on the interval between paired activation of PF and CF
afferents as a smooth function that permits LTD for PF activity anticipating CF activity by up to a time $t_{\text{LTD}} \sim 75$ ms (Figure 2A) (see Materials & Methods). This timing dependence mimics that of the experimental data (Wang et al. 2000). The maximal levels of LTD and LTP induction in our models did not depend on the CS-US training interval, and thus the full range of Purkinje cell spike rates was the same for all but the briefest (<14 ms) delays between the CS and US, reflecting a small finite time for changes in Purkinje cell spiking frequency. By comparison, the durations of each phase of the biphasic Purkinje cell activity did vary with the CS-US interval. This distinction allowed us to focus initially on the signal processing performed by the DCN cells rather than on effects that depend on plasticity amplitude. We subsequently explored how changes in plasticity amplitude, as quantified through the resulting changes in Purkinje cell spike rates, affect a rebound-based mechanism for memory recall in the DCN cells.

**Memory recall in a one-compartment model DCN neuron**

We studied whether forward and backward patterns of biphasic Purkinje cell spiking could lead to distinct patterns of rebound activity in DCN cells after presentation of a classically conditioned stimulus. We created a series of compartmental DCN cell models that received inputs from both Purkinje cells and mossy fibers, and we interpreted the resulting rebounds as the initiators of conditioned motor responses. The simplest model (Model 1) had one electrical compartment, lacked fast spiking capability, and had only leak, T-type, and synaptic conductances (Figure 3A). This allowed us to focus initially on rebound generation, apart from issues studied later concerning membrane potential noise and downstream readout. Kinetic parameters for T-currents were obtained from *in vitro* measurements in DCN cells (Gauck et al. 2001; McRory et al. 2001). Deinactivation can occur within ~20-100 ms of hyperpolarization from the resting potential, and activation can then occur within a few milliseconds during subsequent depolarization (Figures 3D and 3E). Conductance densities were set to reproduce the observed resting potential of -58 mV and membrane time constant of ~12 ms (Aizenman and Linden 1999; Jahnsen 1986a; Llinas and Muhlethaler 1988).

We compared the model’s responses to forward and backward patterns of biphasic Purkinje cell input. In our initial studies, the forward CS-US interstimulus interval (ISI) was at least 200 ms, more than sufficient delay for reliable conditioning in rabbits (Figure 1B) (Ohyama et al. 2003b). Mossy fiber excitation rose during the entire CS but was insufficient to drive a rebound during baseline or elevated Purkinje cell spiking. This is consistent with data supporting a key role for Purkinje cells in generating properly timed reflexes via the suppression of early, mossy fiber driven responses to the CS, which can be unveiled by blocking Purkinje cell inputs to the DCN (Ohyama and Mauk 2001; Perrett et al. 1993). We found that biphasic Purkinje cell input shaped by forward training led to
rebounds that initiated as Purkinje cell spiking transitioned from an elevated to a diminished rate, about $t_{LTD}$ prior to the expected US onset (Figure 4A, red traces). Hence, rebounds could drive blinks that anticipate the US. We then tested the effect of varying the ISI value. With backward training there was insufficient deinactivation of T-currents to generate rebounds (Figure 4A, blue traces). With positive ISI values $<100$ ms, rebounds occurred but with diminished amplitude, since there was insufficient time for T-channel deinactivation during the brief increase in Purkinje cell spiking (Figure 4A, orange trace). Thus, rebound generation occurred selectively for sufficiently positive ISI values and anticipated US arrival.

We also explored the dependence of rebound generation on the graded magnitude of LTP and LTD at the PF-Purkinje cell synapse, as quantified through the resulting elevation and diminution in Purkinje cell spike rates, respectively (Figures 4B and 4C). Rebound generation in the DCN cell required biphasic Purkinje cell spiking, with both a sufficient elevation and subsequent decline in spiking needed for large amplitude rebounds (~50 mV). Ample levels of both LTP and LTD would thus be needed to induce sufficient biphasic variation in Purkinje cell spiking. These findings held across a broad range of T-channel densities and open the possibility that DCN cell T-currents help shape the differences in conditioned reflex expression following backward and forward training (Ohyama et al. 2003b).

Readout mechanisms of rebound depolarization and correspondence to conditioned behavior

If rebounds induce learned motor action, how do DCN cells convey rebound magnitudes via the rate of Na$^+$ spikes sent to pre-motor areas? The graded amplitude of pure T-current mediated rebounds indicates these low-voltage activated events are not stereotyped Ca$^{2+}$ spikes. Real DCN neurons do exhibit Ca$^{2+}$ spikes, mediated by high-voltage activated Ca$^{2+}$-channels, and as in other cell types dendritic Ca$^{2+}$ spikes may be good triggers of somatic Na$^+$ spike bursts (Jahnsen 1986b; Linas and Muhlethaler 1988). We reasoned that the amplitude of T-current mediated rebounds should set the likelihood of crossing the voltage threshold for Ca$^{2+}$ spike generation, with membrane potential fluctuations influencing the degree of variability. Smaller amplitude rebounds that occur with shorter ISI values would be less likely to cross the Ca$^{2+}$ spike threshold. Within this framework we interpret a Ca$^{2+}$ spike as the initiator of signals sent downstream to drive a conditioned motor response.

To test whether this readout mechanism would be able to convert the amplitude of rebound depolarization into the probability of Ca$^{2+}$ spike generation, we examined an enhanced one-compartment model that included high-voltage activated Ca$^{2+}$-channels (Model 2, Figure 3B) and membrane potential fluctuations due to stochastic arrival of synaptic inputs (Materials & Methods). This contrasts with Model 1, in which both synaptic inputs and membrane voltage followed
deterministic time courses. In Model 2 a biphasic pattern of Purkinje cell input resulting from forward training with a long ISI value led reliably to a T-current mediated rebound of sufficient magnitude to trigger a Ca^{2+} spike. These Ca^{2+} spikes were properly timed, prior to the expected US. Backward training led to small rebounds and virtually no Ca^{2+} spiking. Forward training with a short ISI value led to unreliable Ca^{2+} spiking, with the amplitude of the T-current mediated rebound being sufficient to trigger a Ca^{2+} spike on some trials but not others (Figure 5A). Thus, as the ISI value varied, the amplitude of T-current driven rebounds set the probability of crossing the Ca^{2+} spike threshold (Figure 5B, closed green triangles). Of prime interest, the shape of the curve describing this response probability as a function of the ISI closely resembles that obtained in rabbit eyeblink conditioning studies (Figure 5B, open red squares, diamonds, and downward-facing triangles), validating the plausibility of a rebound based recall mechanism. The sum of t_{LTD} and the T-channel inactivation time constant determine the temporal offset of the curve from the origin. It follows that experimental manipulations lengthening the time needed for T-channel deinactivation during the ISI are predicted by our theory to cause a rightward shift of the behavioral data curve (Discussion).

We examined readout issues in greater depth using a two-compartment model DCN cell (Model 3, Figure 3C) that included a dendrite and soma, as well as channels mediating dendritic Ca^{2+} and somatic Na^{+} spikes (Materials & Methods). The somatic and dendritic compartments were only weakly coupled, which was intended to mimic the electrotonic isolation between the cell body and the long distal dendrites of DCN cells where T-channels appear to be most dense, >100 μm from the cell body (Gauk et al. 2001). This is consistent with the observation that Purkinje input triggers DCN cell rebounds much more effectively than somatic hyperpolarization of comparable magnitude (Aizenman and Linden 1999). Synaptic inputs in Model 3 arrived stochastically, inducing membrane potential fluctuations. As in real DCN cells, a tonic cation current induced a basal rate of somatic spiking at ~25 Hz (Aksenov et al. 2005; Jahnsen 1986a; Raman et al. 2000). Simulations revealed that a dendritic rebound induces a Ca^{2+} spike, which in turn drives a corresponding increase in the rate of somatic Na^{+} spikes (Figures 5C-E). This increase represents a plausible signal from the DCN cell to downstream pathways for driving learned motor output (Figure 1A). Forward training with an ISI >100 ms virtually always led to such a spike burst. Na^{+} spike bursts occurred with lower probability under the same conditions that failed to produce large amplitude rebounds in Model 1, such as backward training or forward training with a short ISI. Across ISI values the probability of a Na^{+} spike burst closely matched the behavioral dependence of conditioned blinking on the ISI value as observed in rabbits (Figure 5B, closed blue circles).
Phase plane analysis of rebound generation as a robust mechanism for recall
To explore further the basic dynamics and robustness of rebound mechanisms, we studied DCN neuronal dynamics using a phase plane analysis of Model 1. Prior applications of such analysis to other neuron types have provided considerable insight into Ca\(^{2+}\) spike generation, spike bursting, and transitions between ‘up’ and ‘down’ activity states (Fitzhugh 1960; Loewenstein et al. 2005; Rinzel and Ermentrout 2001; Rush and Rinzel 1994). As is common in phase plane analysis, we focused on the slow dynamical variables that set the relevant timescale. Here, these variables are membrane voltage, \(V\), and the T-type channel inactivation variable, \(I\). The latter has a voltage-dependent time constant of ~50-100 ms (Figure 3E), close to the minimum ISI for reliable memory retrieval (Figure 1B). By comparison, the time constant for T-current activation is ~1-10 ms, considerably faster than motor memory recall and rebound depolarization. Because of this separation of timescales we approximated T-type activation as occurring instantaneously and thus restricted to the \((V, I)\) plane. Rebounds may then be viewed as trajectories in this two-dimensional (2-D) phase plane (Figure 6).

Phase plane analysis of Model 1 revealed the key ingredients for rebounds. The analysis can best be understood by breaking a CS presentation into three stages: the initial resting condition, the ISI, and the remainder of the CS following the ISI (Figure 6A). During each stage, the system has a unique attractive fixed-point at the intersection of the \(V\) and \(I\) nullclines, the curves on which the partial time derivatives \(\partial V/\partial t\) and \(\partial I/\partial t\) respectively vanish (Figure 6B). The three fixed-points and the ISI value are the chief determinants of the dynamics. At rest (stage 1), the system resides at a fixed-point location at which the T-current is mainly inactivated (Figure 6B, open black circle). At CS onset and during the ISI (stage 2), mossy fiber and Purkinje cell input to the DCN cell shift the fixed-point location to a potential at which T deactivates (open green triangle in Figure 6B and Movie 1). The system approaches the stage 2 fixed-point during the ISI, starting from the resting position (Figure 6B; Movie 1). The ISI value determines the duration and proximity of the system’s approach. At \(\approx t_{\text{LTD}}\) prior to the moment of the expected US (stage 3), Purkinje cell activity declines and the fixed-point shifts to a third location that is depolarized relative to rest (open red square in Figure 6B and Movie 1). This initiates a rebound that is well timed for driving an anticipatory reflex. More precisely, there is a family of trajectories that undergo rebound depolarization during stage 3, with rebound amplitude a strict function of the \((V, I)\) values attained by the end of stage 2. A 2-D color map of rebound amplitude as a function of \((V, I)\) reveals the basis for the sharp dependence on the ISI value and the stage 2 and 3 fixed-point locations (Figure 6C). In turn, these fixed-point locations depend critically on the degree of biphasic Purkinje cell spiking and thus on the levels of LTP and LTD attained during training.

Stage 3 rebound trajectories with the greatest depolarization initiate in a neighborhood of the
The $(V, I)$ plane that may be viewed as a memory recall ‘reliability zone’ from which a large rebound will occur without fail (Figure 6C, red shaded region). The level of LTP and the peak Purkinje cell spiking rate are important, because they determine the proximity of the stage 2 fixed-point to the reliability zone. However, even with sufficient LTP if the ISI is too brief the system does not have time to reach the reliability zone during stage 2, leading to a small or no rebound (Figure 6C, blue shaded region; Movie 2). The rebound amplitude also hinges on the location of the stage 3 fixed-point, due to the dependence of T-channel activation on the reduction in Purkinje spike rate and the level of LTD.

To understand the implications of these observations for a readout mechanism based on Ca$^{2+}$ spike generation (Figure 5), using Model 2 we determined the set of stage 3 trajectories in the $(V, I)$ plane that lead to a Ca$^{2+}$ spike (see Materials & Methods). Large amplitude rebounds that initiated within the reliability zone passed furthest above the spiking threshold (Figure 6D). Rebounds that initiated elsewhere either failed to reach or just crossed threshold. In the presence of membrane potential noise, this implies that if the system reaches the reliability zone the probability of Ca$^{2+}$ spike generation is high. Much as in our two-compartment simulations, this probability falls markedly as the ISI is shortened (Movie 3 and Figure 5B). The Ca$^{2+}$ spike voltage threshold does not vary much across a wide range of HVA Ca$^{2+}$ channel density (data not shown), indicating Ca$^{2+}$ spiking is a robust readout of whether the system has entered the recall reliability zone. Thus, the phase plane analysis illuminates key features of a rebound-based memory recall mechanism, including conditions for reliable recall.

**Role of post-inhibitory rebounds in VOR gain adaptation.**

Because cerebellar circuitry is highly conserved, rebound depolarization might serve multiple forms of cerebellar memory recall. For example, floccular Purkinje cells involved in horizontal VOR adaptation project to target cells in the vestibular nuclei that also exhibit significant rebound depolarization in vitro mediated by hyperpolarization-activated currents (Sekirnjak et al. 2003; Serafin et al. 1991). These currents require further characterization and are expressed to varying degrees across MVN cell types, but as a group the MVN neurons receiving input from the floccular Purkinje cells exhibit exceptionally pronounced rebound burst spiking (Sekirnjak et al. 2003). The currents involved seem to include the h-type cation current and probably some amount of Na$^+$ and T-type Ca$^{2+}$ currents (Sekirnjak and du Lac 2002; Serafin et al. 1991; Smith et al. 2002). Regardless of the current identities, the empirically determined time constant (~620 ms) describing the duration of hyperpolarization needed for maximal rebound burst firing is considerably longer than that for DCN cells (Figure 2E) (Sekirnjak and du Lac 2002). Might rebound depolarization and the need for a long period of hyperpolarization underlie some of the temporal asymmetries seen in behavioral studies of VOR adaptation?
Well known primate behavioral studies have shown that the amplitude of learned VOR responses depends on the relative timing of vestibular and visual stimuli in a manner resembling the dependence on CS-US timing in classical conditioning. Raymond and Lisberger repeatedly paired a vestibular stimulus, a 600 ms pulse of head rotation, with a brief visual stimulus consisting of moving dots (Raymond and Lisberger 1996). The visual motion stimulus was presented at one of three different ISI values, a zero ISI condition analogous to backward conditioning (Figure 7C, left panel), a short forward ISI of 225 ms (Figure 7C, middle panel), and a long forward ISI of 450 ms (Figure 7C, right panel). A learned VOR response developed in all cases, but the response amplitude grew as the ISI lengthened. Such dependence on the ISI may be analogous to that seen in eyeblink conditioning. Could rebounds underlie this effect? The timescale of the behavioral effect is similar to that of h-current activation.

To study the issue we created a simple, one-compartment model of an MVN cell in which h-currents mediated rebounds (Figure 7B). The model is analogous to Model 1 of a DCN cell in that it has only one compartment and lacks the channels responsible for the fast spontaneous spiking that these cells exhibit. The single compartment thus better mimics a dendrite than a soma. As before, we interpreted the rebounds as signals driving learned motor responses. We used a stimulus protocol based on the Raymond-Lisberger experiments and found the largest rebounds arise when the visual stimulus occurs during the latter portion of the vestibular impulse (Figures 7D and 7E). Longer ISI values allow more time for h-currents to activate at hyperpolarized voltages, heightening rebound depolarization. Rebound amplitude also depends on biphasic Purkinje cell spiking and thus on the levels of LTP and LTD induced during training (data not shown). Thus, the amplitude dependence of learned eye movements on the ISI value might stem from variable levels of current flow through hyperpolarization-activated conductances such as h. However, the component of the learned response that is independent of the ISI value is unlikely to be driven by rebounds and is beyond the scope of our present model, which seeks only to account for the ISI-dependent component.

Discussion
We have presented a lock-and-key hypothesis on how the expression of memory responses may undergo filtering via neurophysiological mechanisms active during memory retrieval. This hypothesis and our computational work exploring a candidate rebound-based lock-and-key mechanism were prompted by data suggesting that backward-ordered classical eyeblink conditioning as well as non-cerebellar forms of aversion conditioning can lead to latent memory storage or changes in neural activity, despite a lack of conditioned responses (Barnet et al. 1997; Gould and Steinmetz 1996). The complex manner in which plasticity might evolve across a large network of synaptic connections
throughout learning experience also suggests some constraints on motor memory expression might be implemented via neurophysiological mechanisms of recall (Mauk and Ohyama 2004).

We explored these ideas through computational studies of two cerebellar behaviors by examining whether DCN and MVN cells can filter signals from Purkinje cells to influence response timing and prevent certain motor responses. Biophysical models of these two cell types that incorporate rebound channels lead to consistent explanations for behavioral data on cerebellar motor learning. These models make direct links between ion channel kinetics and memory expression, and particularly for eyeblink conditioning yield specific predictions of how learning performance varies as a function of the relative timing of paired training stimuli. Within our lock-and-key framework for these models, subjects undergo both cerebellar LTP and LTD regardless of whether the training stimuli were presented in forward or backward order. As a result, the learned sensory cue drives biphasic Purkinje cell activity. Yet, this biphasic activity triggers rebound depolarization in the DCN cells and drives well-timed classically conditioned reflexes only if the training ISI was sufficiently positive. In this way inappropriate motor responses to conditioned stimuli that do not precede the US sufficiently are avoided. Phase plane analysis reveals the basic ingredients for reliable reflex expression, including ample levels of both LTD and LTP. In MVN cells, rebound currents may underlie the variation of VOR adaptation magnitude with the relative timing of visual and vestibular training stimuli. Such effects hinge on the observed capabilities of both DCN and MVN cells for rebound depolarization.

Electrophysiological properties of DCN neurons are consistent with the rebound theory

In vitro studies of DCN cells have found that rebounds occur in both cerebellar slice and isolated cerebellum-brain stem preparations (Aizenman and Linden 1999; Jahnsen 1986a; b; Llinas and Muhlethaler 1988). MVN neurons also undergo rebounds in vitro, but there is more uncertainty about the channels involved (Sekirmjak and du Lac 2002; 2006). There is also indirect physiological and pharmacological evidence DCN cells rebound in vivo (Aksenov et al. 2005; Hesslow 1994a), including for a class of neurons with blink-related activity (Chen and Evinger 2006). Input from a single Purkinje cell induces a large conductance change in the DCN cell (Pedroarena and Schwarz 2003), which is sufficient to allow a modest post-inhibitory rebound and increase in Na\(^+\) spike rate (M. Molineux, personal communication). Multiple Purkinje cells might drive larger rebounds and spike bursts in concert, such as through coordinated Purkinje cell spiking (Heck et al. 2002; Thier et al. 2000). The anatomical convergence of many hundreds of Purkinje cells onto each DCN neuron implies that the aggregate activity of a population of Purkinje cells influences DCN cell activity.

The in vivo extracellular recordings performed to date of DCN neurons during classical conditioning do not provide strong evidence either for or against our rebound theory. Single-unit and
multi-unit recordings both reveal an increase in DCN spiking rate that precedes motor output (Berthier and Moore 1990; Choi and Moore 2003; McCormick et al. 1982; McCormick and Thompson 1984a; b). By comparison, evidence for a pause in spiking during early portions of the ISI is limited. Berthier and Moore reported some cells with reduced spiking at the beginning of the CS, although this pattern is not apparent in all single unit recordings (Berthier and Moore 1990; Choi and Moore 2003). Multi-unit recordings of DCN cell activity do not exhibit a pause, but these recordings may not provide sufficient sensitivity to reveal a partial reduction in spiking within a sub-population of recorded neurons (McCormick et al. 1982; McCormick and Thompson 1984a; b). Irrespective of these results, the rebound model does not make a strong prediction concerning DCN firing during early portions of the CS. During the early portion of the ISI, DCN neuron spiking might remain virtually unchanged despite increased Purkinje input, due to the dendritic location of most Purkinje synapses and T-type channels (Figure 5). Technically difficult in vivo intracellular recordings would be required to determine how sub-threshold responses in DCN cells develop during conditioning.

**Experimental predictions for studies of cerebellum-dependent motor learning**

The lock-and-key hypothesis leads to a clear prediction that is testable independent of whether rebound depolarization provides a lock mechanism: Classical conditioning with a short (<100 ms) or backward CS-US interval should lead to plasticity in the cerebellar cortex despite the lack of reflex acquisition. Several experimental tests of this prediction are possible. Second-order classical conditioning, which can induce expression of previously latent first-order conditioning, might be useful for demonstrating explicitly that a memory of backward conditioning is formed (Barnet et al. 1997). Alternatively, studies of backward eyeblink conditioning using human brain imaging techniques might be capable of revealing plasticity related effects (Cheng et al., 2006, Soc. Neurosci. abstract).

Electrophysiological recordings of Purkinje cell spiking during reflex conditioning in decerebrate ferrets and guinea pigs have found changes in spiking patterns over forward training (Jirenheid et al. 2007; Kotani et al. 2003), and thus might be used to examine the effects of backward or short interval conditioning. Recordings from rabbit Purkinje cells, but not DCN cells, were reported to show changes in activity patterns following backward US-CS pairings (Gould and Steinmetz 1996). This lends support to the lock-and-key hypothesis, but more experimental data is still needed.

Several other testable predictions emerge from our modeling of rebound dynamics during memory recall. Removal or blockade of DCN cell rebound conductances should hinder expression of conditioned blinks. To test this idea, T-channel blockers might be used to prevent rebounds in trained subjects (McDonough and Bean 1998; Porcello et al. 2003). Failure to impair conditioned blinks would cast serious doubt on our proposed rebound mechanism. A related test might be performed in trained
animals during recordings of DCN cells that drive conditioned responses. Transient depolarization of these cells during the ISI in trained animals should prevent or diminish blink-related spiking activity by thwarting T-channel deactivation. Although perturbation of one cell seems unlikely to disrupt the blink itself, stimulation of many DCN cells might have such an effect.

A corollary to this logic concerns inhibition of olivary cells by projections from GABAergic DCN cells that also receive Purkinje cell inputs and exhibit rebounds (De Zeeuw and Berrebi 1995; Teune et al. 1998). These connections appear critical for extinction of conditioned reflexes following unpaired CS presentations (Medina et al. 2002). Thus, blockade of rebound channels should hinder both expression and extinction of conditioned reflexes. A caveat is that rebound channel blockers or electrical stimulation applied to the DCN might alter climbing fiber input to the cerebellum through nucleo-olivary inhibitory feedback (Bengtsson et al. 2004; Hesslow and Ivarsson 1996). To dissociate the role of rebounds in excitatory versus inhibitory DCN neurons might require genetic tools for cell-type specific manipulation, such as optogenetic techniques (Zhang et al. 2007).

Another prediction of the rebound theory is that blink-related Purkinje cells should exhibit, in the aggregate, biphasic patterns of CS-driven activity in conditioned subjects. This contrasts with Albus’s proposal that GABAergic Purkinje cells should drive DCN cells through disinhibition (Albus 1971). The recordings made to date of the spiking activity of individual Purkinje neurons in trained animals have revealed a diversity of spiking patterns in response to the CS (Berthier and Moore 1986; Gould and Steinmetz 1996; Green and Steinmetz 2005; Jirenhed et al. 2007; Kotani et al. 2006; 2003; Tracy and Steinmetz 1998). Although multiple regions of cerebellar cortex have been implicated in eyeblink conditioning, most studies of Purkinje cell activity in classical conditioning have focused on a subset of these areas.

Recordings of Purkinje cell activity in trained rabbits from the cerebellar anterior lobe (Green and Steinmetz 2005) and cerebellar lobule HVI (Berthier and Moore 1986; Gould and Steinmetz 1996), both areas implicated in eyeblink conditioning, have revealed a mixture of activity increases and decreases in response to the CS. Excitatory responses might occur earlier in the ISI, with activity decreases occurring later (Green and Steinmetz 2005). Recordings from lobule HVI in decerebrate guinea pigs and ferrets have also revealed a mixture of responses (Jirenhed et al. 2007; Kotani et al. 2006). One particularly careful study, in which Purkinje cells were first identified as being responsive to the US prior to training, reported mainly decreases in Purkinje cell spiking in response to the CS after training (Jirenhed et al. 2007). However, this study also mentions that after training some cells undergo an increase in firing during the first 50-100 ms of the CS followed by an abrupt drop in spiking. Overall, Purkinje cell recordings in trained animals are mainly consistent with the rebound theory but do not provide sufficient evidence to validate the prediction that aggregate Purkinje cell
activity should be biphasic. Given the central role in the theory of such biphasic aggregate responses, it seems important that future studies testing this prediction should record from multiple Purkinje cells concurrently using multi-electrode techniques.

If rebounds are instrumental in driving classically conditioned responses, the timing of training stimuli needed for successful conditioning should depend on rebound channel kinetics. Because the minimum ISI that leads to rebounds is set by the sum of the inactivation time constant and $t_{LTD}$, an increase in the inactivation time constant should increase the minimum ISI for successful training. Likewise, slowing the kinetics of hyperpolarization-activated currents in MVN cells should increase the interval required between vestibular and visual inputs needed to generate the greatest changes in VOR gain.

Further predictions of the rebound theory concern classically conditioned subjects in which cerebellar LTD or LTP is impaired. Elimination of both LTD and LTP would prevent biphasic patterns of Purkinje cell spiking, precluding rebounds. Our simulations also suggest that if either LTP or LTD is partially impaired, rebound amplitude decreases and sporadically triggers readout by $Ca^{2+}$ or $Na^+$ spikes (data not shown). However, rebound driven spiking remains well timed when it occurs. Thus, some animals partially deficient in LTD or LTP might exhibit conditioned responses sporadically or of diminished amplitude, but with proper timing. Greater disruptions of cerebellar plasticity would disrupt response timing due to insufficient biphasic modulation of Purkinje cell spiking.

To date, several groups have studied eyelink conditioning in mice with disrupted cerebellar LTD. Four strains of such mice exhibited blinks that occurred sporadically or with altered amplitude, but with the same distribution of time courses as wild-type mice (Kishimoto et al. 2001; Koekkoek et al. 2005; Miyata et al. 2001; Shibuki et al. 1996). It was reported that mice expressing a protein kinase C inhibitor that impaired LTD had ill-timed residual blinks (Koekkoek et al. 2003). However, questions have been raised about this study regarding the degree to which motor learning was cerebellar in origin (Christian et al. 2004; Jirenhe et al. 2007; Koekkoek et al. 2003). It is possible PKC inhibition disrupts more than just LTD. Although further study is needed, the former four strains seem consistent with the rebound theory and suggest normal LTD levels might be unnecessary for normally timed responses.

Comparison to other work on the role of rebounds in cerebellar function
Several other authors have considered a potential role for DCN cell rebounds in cerebellar network function. Inspired by the observation that Purkinje cell activity effectively triggers rebounds in vitro (Aizenman and Linden 1999), Kistler and de Zeeuw created computational models emphasizing a potential role for rebounds in reverberatory olivo-cerebellar network activity through which Purkinje
cells can affect their own subsequent activity and timed motor responses (Kistler and De Zeeuw 2003). In experiments on classically conditioned decerebrate cats, Hesslow noted that brief electrical stimulation of the cerebellar cortex leads to a delayed activation of muscle fibers as assessed by electromyography (Hesslow 1994a; b). This finding was explained as arising from post-inhibitory rebound depolarization in the DCN and supports a basic assumption underlying our models, namely that rebound driven DCN cell activity can trigger motor action.

Aizenman and Linden have found that DCN cell rebound depolarization and the associated spike burst are important for determining the polarity of gain changes at the Purkinje cell to DCN cell synapse (Aizenman and Linden, 1998). Mauk and collaborators have performed extensive network modeling studies of cerebellar learning and have suggested that the rise in intracellular Ca$^{2+}$ due to DCN cell rebounds might be a potent trigger for plasticity at the mossy fiber to DCN cell synapse (Mauk and Donegan 1997; Medina and Mauk 1999). A recent experimental study has demonstrated this effect, confirming the importance of post-inhibitory rebound current for potentiation of mossy fiber to DCN cell synapses (Pugh and Raman 2006). However, in the original simulations the inefficacy of brief ISI values for classical conditioning arises from a minimum time interval for synchronization of granule cell activity following the CS, rather than from a rebound based mechanism (Mauk and Donegan 1997). For ISI values shorter than the minimum for granule cell synchronization, plasticity in the cerebellar cortex is precluded. By comparison, our lock-and-key hypothesis is based on the supposition of at least some plasticity occurring with brief forward or backward ISI values.

A non-synaptic form of plasticity capable of increasing the intrinsic excitability of DCN and MVN neurons appears suited to raise the propensity for rebounds over the course of training. Bursts of synaptic input can induce DCN and MVN cells to display long-lasting gains in excitability due to increases in the maximum rebound current (Aizenman and Linden 2000; Smith et al. 2002; Zhang et al. 2004). A biphasic burst-and-pause pattern of Purkinje cell activity may be optimally suited to induce this increase (Aizenman and Linden 2000; Medina and Mauk 1999; Pugh and Raman 2006). Thus, rebound generation might be facilitated during training, perhaps reducing the number of Purkinje inputs needed to drive motor action. Overall, multiple cerebellar plasticity mechanisms in addition to those invoked here at the PF-Purkinje synapse are likely to occur during motor training and to shape both cerebellar dynamics and motor learning in a cooperative fashion (Boyden et al. 2004; D'Angelo et al. 1999; De Zeeuw and Yeo 2005; Hansel et al. 2001; Mauk and Donegan 1997; Medina and Mauk 1999; Soler-Llavina and Sabatini 2006). Collectively, the existing experimental data are not only consistent with our computational models but also reach well beyond, suggesting that rebound currents have multiple functions additional to the temporal filtering role explored here.
A role for rebounds in temporal shaping of learned responses

A longstanding debate has concerned the issue of whether cerebellar-mediated memory storage occurs within the cerebellar cortex, or within the deep cerebellar or vestibular nuclei (du Lac et al 1995; Ito 1982; McCormick and Thompson 1984a; Miles and Lisberger 1981). Consistent with the identification of multiple plasticity mechanisms in vitro, recent in vivo studies indicate both the cortex and the nuclei have a role in memory storage, but with distinct kinetics for plasticity induction and with distinct roles in setting the timing of motor expression (Kassardjian et al. 2005; Medina and Mauk 1999; Ohyama and Mauk 2001; Perrett et al. 1993; Shutoh et al. 2006). In both eyeblink conditioning and ocular reflex adaptation, shortly after the start of training motor memory is susceptible to extinction and is impaired by lesion or pharmacological disconnection of the cerebellar cortex. After a few days, motor memories are more resistant to extinction and can persist without Purkinje cell involvement (Kassardjian et al. 2005; Ohyama and Mauk 2001; Perrett et al. 1993; Shutoh et al. 2006).

However, even long after training, the Purkinje cells appear important for setting the proper timing of learned responses. In eyeblink conditioning, disconnection of Purkinje cells’ projections to the DCN leads to ill-timed, short-latency blink responses to the CS (Ohyama and Mauk 2001; Perrett et al. 1993). These short-latency blinks probably arise because without inhibitory input from Purkinje cells, DCN cells may be driven strongly by mossy fiber inputs starting from the onset of the CS. For VOR adaptation, the relative contributions of the floccular Purkinje cells and the MVN neurons in shaping the temporal character of the learned motor response remain less clear. Our interpretation of the available data is that for VOR adaptation, a baseline component of the learned motor response that is not as sensitive to the relative timing of vestibular and visual training stimuli arises from mossy fiber driven activity in the MVN (Fig. 7C). However, there also appears to be another component that is sensitive to the relative timing of the training stimuli (Raymond and Lisberger 1996), and it is this second component that we propose has the proper form and kinetics to be explained by a rebound based mechanism using a slow, hyperpolarization-activated current (Fig. 7E), such as that identified by (Sekirnjak and du Lac 2002). Unlike eyeblink conditioning, for which there is no learned response with short ISI values, there is VOR adaptation following short interval training, but at reduced amplitude relative to adaptation at longer ISI values (Raymond and Lisberger 1996). Thus, rebound mechanisms cannot account quantitatively for the full magnitude of VOR adaptation.

Generalizations of the proposed rebound mechanism

Our simulations involved specific choices of cellular parameters, but the ideas presented here on rebounds may have explanatory power surpassing that of our detailed models. One aspect of
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generality concerns the channels that mediate rebounds. It was first thought mainly T-type channels drive DCN cell rebounds, but new evidence also suggests a role for Na\(^+\) currents (Sangrey & Jaeger, 2005, Soc. Neurosci., abstract). Further, rebound amplitude or kinetics may vary between individual DCN neurons due to differential expression of T-channel isoforms (Molineux et al. 2006). Identities of the rebound channels in MVN cells are still in question, but in addition to the h-type current there is evidence Na\(^+\) and low-voltage activated Ca\(^{2+}\) currents play a role (Sekirnjak and du Lac 2002; Serafin et al. 1991). Further characterization of rebound channels may be important for understanding the precise timing relations seen in behavioral studies. However, the basic idea that rebounds shape temporally asymmetric behavioral responses is general and may apply to other cerebellum-dependent learning paradigms. For example, learned aspects of smooth visual pursuit exhibit temporal dependencies on the ~200 ms scale that might reflect requirements for rebound generation in cells of the cerebellar caudal fastigial nucleus (Medina et al. 2005).

Role of synaptic plasticity in memory formation and recall.

A prevalent view holds that LTD and LTP are opposing mechanisms, with one encoding memories and the other erasing (Boyden and Raymond 2003; Coesmans et al. 2004; Lev-Ram et al. 2003). We are proposing a different view, in which both cerebellar LTD and LTP are needed for reliable recall. The lock mechanism based on rebound depolarization enforces the requirement for forward training, but nevertheless both LTD and LTP are induced during forward and backward training.

In our lock-and-key framework, backward conditioning and forward training at short intervals induces synaptic plasticity at parallel fiber to Purkinje cell synapses despite the absence of behavioral output. In this respect, our work follows several prior models of cerebellar-mediated learning in which neuronal plasticity can occur without any change in motor behavior. Models of learning with two or more stages have been proposed for eyeblink conditioning (Mauk 1997; Mauk and Donegan 1997), VOR adaptation (Boyden et al 2004; du Lac et al. 1995) and other motor behaviors (Smith et al. 2006). These models involve an intermediate stage of learning during which plasticity has occurred but learning is not yet expressed. More generally, any model of learning with two or more serial stages of plasticity inherently implies the possible existence of plasticity without expression of learning. We also present a two-stage model, but here the second stage of processing is implemented as temporal filtering occurring at memory recall rather than as plasticity occurring during memory formation. As with prior two-stage models of plasticity, our model also predicts the possibility of plasticity without behavior modification.

The idea of rebound generation as a temporal filter pertains to the ongoing debate about mechanisms that shape the dependence of classically conditioned responses on the CS-US ISI.
(Figure 1B). Ideas from recent studies on synaptic plasticity and animal behavior have suggested conflicting explanations. Some authors have suggested spike-timing dependent plasticity can account for the variation in performance with the ISI value (Wang et al. 2000). A timing-dependent plasticity rule in which PF activity preceding CF activity is optimal for LTD induction does create a distinction between backward and forward training (Figure 2). However, the recent and older experimental data show that LTD can occur with forward or backward timing protocols (Figure 2A) (Chen and Thompson 1995; Ito and Kano 1982; Wang et al. 2000), so another mechanism auxiliary to LTD seems needed to create conditioned reflexes selectively following forward but not backward training. Our model incorporates spike-timing dependent plasticity, and \( t_{\text{TD}} \) is a key factor that causes the conditioned response to precede the expected US. However, it is rebound generation that precludes learned responses following backward training. In a sense, a memory of backward training is formed in the resulting patterns of LTD and LTP, but this memory is not retrieved in response to the CS.

This statement of the lock-and-key hypothesis fits well with studies of classical aversion conditioning, which have shown backward training leads to an associative memory of the US-CS pairing (Gallistel and Gibbon 2000). CS presentation alone does not yield conditioned motor responses, but the associative memory can be demonstrated through second-order conditioning (Barnet et al. 1997). Thus, at least for this form of aversion conditioning, mediated outside the cerebellum, the lock-and-key hypothesis appears to be correct. What mechanism prevents the stored memory from yielding a response to the CS? For aversion conditioning the answer remains unknown, but our study points to rebound depolarization as a candidate mechanism by which such behavioral filtering might occur for cerebellum-dependent behaviors.

**An algorithmic lock-and-key description of memory retrieval**

In sensory neuroscience, filters have been fruitfully used to describe receptive fields and spiking behavior in an algorithmic manner, apart from physiological details. Our study suggests an algorithmic lock-and-key description of cerebellar memory recall (Figure 8). The ‘lock’ resides in the DCN or MVN as a temporal filter that requires specific ‘key’ input activity to drive rebound spiking and a motor response. The key is the biphasic pattern of Purkinje cell activity that is shaped by training and driven by a learned sensory cue. Synaptic plasticity does not lead to learned responses unless key activity is shaped to match the temporal filter of the lock (Figures 8A and 8B). Backward training induces plasticity, but the resulting key activity does not fit the lock, precluding conditioned responses.

We found that a simple linear-nonlinear (L-N) filter model, inspired by those used to describe visual receptive fields (Baccus and Meister 2002; Korenberg and Hunter 1986), can accurately predict the responses of our biophysical models and the behavioral dependence of learned responses on ISI.
(Figures 8C-E). Thus, from an algorithmic standpoint memory retrieval may involve a temporal filter that excludes certain behaviors and allows others.

The lock-and-key model of recall might be adaptable to other non-declarative forms of memory involving feed-forward networks. Striatal memory for motor sequences shows promising similarities, since both Purkinje cells and striatal medium spiny neurons receive diverse sensory information, undergo bi-directional plasticity, and are GABAergic projection neurons that trigger motor sequences (Fino et al. 2005). The similarity between these two GABAergic cell types extends also to their respective targets, for there is one class of striatal target neuron in the globus pallidum that is spontaneously active, has a baseline membrane potential of about -60 mV, and reliably undergoes post-inhibitory rebound depolarization in response to electrical stimulation of striatal input fibers (Nambu and Llinas 1994). By analogy, cortico-striatal plasticity alone may not represent a memory, since select patterns of striatal input activity to the globus pallidum may be needed to unlock motor expression. These ideas challenge classical theories of memory storage that focus almost exclusively on neuronal plasticity but avoid mention of how retrieval dynamics may help shape memory expression.

Neuroscientists have long sought the location and substance of memories. This quest led to the notion of the engram, the physical unit of memory, and to the later idea that changes in synaptic strength are a candidate substrate for memory (Bliss and Lomo 1973; Lashley 1950). Cognitive scientists have countered that synaptic plasticity alone does not account for the complex phenomenology of memory recall (Gallistel 1993; Martin et al. 2000). In this opposing view, a memory is a physical dynamic that occurs exclusively at recall. Plasticity may help shape this dynamic, but is not by itself a memory. Our computational models provide concrete examples in which it is difficult to identify any physically localized engram. Would the engram include the synapses that have undergone plasticity, the key activity that triggers a memory, the activity unlocked at a rebound, or all of the above? By itself plasticity seems a poor candidate for a complete engram, since it is insufficient to allow recall. Questions regarding the physical substance of memory may be misleading, neglecting that recall occurs due to a sequence of events culminating in a specific form of neural dynamics. This caveat regarding the substance of memory in our models might also apply broadly to multiple memory systems.

Acknowledgments

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Figure Legends

Figure 1. Neural pathways and stimulus timing requirements for eyeblink conditioning.

(A) Neural pathways involved in delay eyeblink conditioning. Cerebellar climbing fibers (CFs) originate in the inferior olive (IO) and convey activity driven by the unconditioned stimulus (US). Mossy fibers (MF) originate in the pons and convey activity driven by the conditioned stimulus (CS). The Golgi (Go) and granule (Gr) cell network processes the CS-driven signals. Purkinje (PkJ) cells receive synaptic inputs from parallel fiber (PF) axons of Gr cells. PkJ cells send GABAergic projections to neurons in the deep cerebellar nuclei (DCN) that drive conditioned motor responses via the red nucleus (RN).

(B) The reliability of conditioned responses to a CS in trained rabbits, as a function of the CS-US interstimulus interval used in training. Data were collected from classic studies of (Smith et al. 1969; solid black line and black squares), (Salafia et al. 1980; dotted blue line and blue diamonds), (Smith 1968; solid red line and red triangles), and (Schneiderman and Gormezano 1964; dotted green line and green circles).

Figure 2. Cerebellar memory formation based on temporally sparse granule cell coding and bidirectional plasticity at the PF-Purkinje cell synapse.

(A) The relative timing of PF and CF activation sets the propensity towards LTD or LTP. Maximal LTD induction arises when PF activity precedes CF activity by up to a time, \( t_{\text{LTD}} \), of \(~75 \text{ ms} \), but LTD can also occur when CF activity slightly precedes PF activity (Coesmans et al. 2004; Wang et al. 2000).

(B) In classical eyeblink conditioning, individual PFs exhibit elevated activity during only a brief portion of the CS. By the plasticity rule in A, some PF inputs will be strengthened and others depressed, depending on the relative timing of PF and US-driven CF activity. DCN cells receive input from populations of Purkinje cells whose activity reflects aggregate input from CS-activated PFs.

(C) Repeated CS-US training (upper panels) leads to biphasic CS-driven Purkinje cell spiking due to the bi-directional plasticity shown in B. In subjects that received forward training (lower left), spiking rises and then falls relative to baseline (red curve). In subjects that received backward training (lower right), spiking falls and then rises (blue curve).
Figure 3. Compartmental modeling of T-type Ca\(^{2+}\) current rebounds in DCN cells.

Compartmental simulations of a Purkinje target neuron in the DCN involved three models of increasing complexity.

(A) Model 1 has one electrical compartment, contains T (\(g_T\)) and leak (\(g_L\)) conductances, and receives glutamatergic mossy fiber and GABAergic Purkinje cell inputs. Membrane voltage follows a deterministic time course.

(B) Model 2 adds high-voltage activated Ca\(^{2+}\) (\(g_{\text{HVA}}\)) channels. Synaptic inputs arrive stochastically, leading to membrane potential fluctuations and non-deterministic dynamics.

(C) Model 3 has dendritic and somatic compartments, coupled by a conductance, \(g_c\). Synaptic inputs are localized to the dendrite, approximating empirical findings. The soma has fast Na\(^+\) (\(g_{\text{Na}}\)) and delayed rectifier K\(^+\) (\(g_{\text{K}}\)) conductances. Both compartments have leak, T, SK, and HVA Ca\(^{2+}\) conductances. Synaptic inputs arrive stochastically, leading to non-deterministic dynamics.

(D) Voltage dependence of the activation (dashed red curve) and inactivation (solid blue curve) gating variables for the T-type conductance in DCN neurons. At the resting potential (about -58 mV, dashed vertical line), T-currents are largely inactivated. Hyperpolarization deactivates T-currents, allowing activation during subsequent depolarization.

(E) Voltage dependence of the T-channel activation (dashed red curve) and inactivation (solid blue curve) time constants.

Parameter dependencies in D and E are based on (Gauck et al. 2001; McRory et al. 2001).

Figure 4. DCN cell rebounds require a minimum CS-US interstimulus interval and sufficient expression of cerebellar LTP and LTD.

(A) The time course of CS-driven depolarization in Model 1 (Figure 3A). If prior training involved a sufficiently positive ISI, the CS-driven rebound is of large amplitude and occurs at time \(\sim t_{\text{LTD}}\) before the expected US (red traces). If training involved an insufficient ISI, CS-driven rebounds do not occur (blue traces). For short ISI values, rebounds are diminished in amplitude (orange trace). The color bar indicates the ISI values, which are also marked above the graph with the color corresponding downward triangles for each voltage trace. Rebounds occur prior to the expected US, indicating anticipatory responses.

(B) Rebound amplitude varies with the degree to which the CS drives biphasic Purkinje cell activity. This in turn depends on having sufficient expression of both PF-Purkinje cell LTP and LTD (Figure 2). Driving a large amplitude rebound in the DCN cell requires that during the first phase of biphasic activity the Purkinje cell spiking rate rises well above the spontaneous frequency of 40 Hz. The three
voltage traces (blue, cyan, red traces) in panel B₁ occurred with the color corresponding, Purkinje cell peak spiking rates shown in B₂. Lower peak spiking rates reflect lower expression levels of LTP. The downward triangle indicates the ISI value of 200 ms.

(C) Driving a large amplitude rebound in the DCN cell also requires that during the second phase of biphasic activity the Purkinje cell spiking frequency drops below the 40 Hz spontaneous rate. The three voltage traces in panel C₁ (blue, cyan, red) were created using the color corresponding, Purkinje cell minimum spiking rates shown in C₂. The higher rates reflect lesser degrees of LTD. The downward triangle indicates the ISI value of 200 ms.

Figure 5. Readout of rebounds via Ca²⁺ spikes leads to dependence of response reliability on the CS-US interstimulus interval.

(A) Sample voltage traces during CS presentation in Model 2 (Figure 3B) in the presence of membrane potential fluctuations from noisy synaptic inputs. At an intermediate ISI of 70 ms, a T-current mediated rebound depolarization triggers a Ca²⁺ spike during one trial (dashed red line) but not another (solid blue line).

(B) The reliability of learned responses in Model 2 (closed green triangles) and Model 3 (closed blue circles), defined as the probability of generating a dendritic Ca²⁺ spike in response to a test CS, plotted as a function of the ISI. Classic data on the reliability of conditioned blinks in trained rabbits are re-plotted from Figure 1 (open red symbols) (Salafia et al. 1980; Smith 1968; Smith et al. 1969), showing the similarity to the model data. A t_LTD of 75 ms was used for the model data, which is consistent with empirical data indicating t_LTD is in the ~50-200 ms range (Wang et al. 2000).

(C, D) Example voltage traces from the dendritic and somatic compartments of Model 3 (Figure 3C) during CS presentation. A T-mediated rebound depolarization leads to a high-voltage activated dendritic Ca²⁺ spike (C) that drives a rise in the somatic Na⁺ spike rate (D).

(E) The corresponding time courses of the activation (n, solid red curve) and inactivation (i, dashed blue curve) gating variables during the Ca²⁺ spike.

Figure 6. Phase plane analysis of CS-driven rebounds.

(A) Membrane voltage time course (blue curve) in response to a CS that initiates at time t=0 in Model 1, under the approximation of instantaneous relaxation of the T-channel activation variable to its asymptotic value. The rebound peaks at a time ~40 ms prior to the expected US at 200 ms after CS onset. Dashed vertical lines delineate three stages of the phase plane trajectory in B.

(B) The state trajectory (blue curve) in the 2-D phase plane defined by the voltage (V) and T-type inactivation variable (i), corresponding to the voltage trace in A. The open black circle marks the fixed-
point in the resting state (stage 1). The open green triangle marks the fixed-point from CS onset until $t_{LTD}$ prior to the expected US (stage 2). The open red square marks the fixed-point during the remainder of the CS (stage 3). According to longstanding convention, channels are completely inactivated when $\ell$ equals zero (Hodgkin and Huxley 1952).

(C) A color plot conveying the amplitude of the rebound that occurs during stage 3 for the state trajectory passing through each point in the phase plane of $B$ and converging towards the stage 3 fixed-point (open red square). Warmer hues indicate the larger rebounds (color bar) that initiate if during stage 2 the system has successfully entered the ‘memory reliability zone’ near the stage 2 fixed-point (open green triangle). White curves are example state trajectories.

(D) The addition of high-voltage activated (HVA) $\text{Ca}^{2+}$ channels to the phase plane analysis of C reveals those stage 3 trajectories that lead to a $\text{Ca}^{2+}$ spike (red trajectories) and those that do not (blue trajectories). All of the trajectories closely concur with those in Model 1 (panel C) in the voltage range $V < -35$ mV over which the HVA $\text{Ca}^{2+}$ channels are largely closed. The red trajectories, which initiate within the reliability zone near the stage 2 fixed-point (green triangle), cross the $\text{Ca}^{2+}$ spike threshold and allow successful readout of the rebound (Figures 5A and 5B).

Horizontal dotted lines indicate the resting potential of -58 mV in A-D. Solid and dashed black curves in B, C, and D are nullclines during the resting state for the $\ell$ and $V$ variables, respectively, on which the partial derivatives $\partial \ell / \partial t$ and $\partial V / \partial t$ respectively vanish during stage 1.

**Figure 7. Vestibular nuclei cell rebounds lead to temporally asymmetric VOR adaptation.**

(A) Vestibulo-cerebellar pathways for VOR horizontal gain adaptation involve Purkinje cells (PkJ) that project to target neurons within the medial vestibular nucleus (MVN). Neurons in the MVN project to brainstem motor nuclei (MN) that generate eye movement. Slip of the visual scene on the retina is conveyed to the cerebellum via climbing fibers (CFs). Information about head velocity arrives via mossy fibers (MFs) originating in the vestibular ganglia (VG), is processed within the Golgi (Go) and granule (Gr) cell network, and reaches Purkinje cells by way of parallel fibers (PFs). Conjunctive arrival of CF and PF signals is thought to induce synaptic plasticity at the PF-PkJ synapse that underlies gain adaptation.

(B) A one-compartment model of an MVN Purkinje target neuron that contains $h$- ($g_h$) and leak ($g_l$) conductances and receives glutamatergic mossy fiber and GABAergic Purkinje cell input. Membrane voltage follows a deterministic time course.

(C) Primate behavioral data from well-known studies in which pulses of head rotation (top panel, black bars) were paired during training with moving dot visual stimuli (top panel, grey bars) at three distinct...
interstimulus intervals. During later testing with pulsed head rotations in the dark, the learned component of VOR expression increased markedly with greater ISI values (bottom panel, green, blue, and red curves) (Raymond and Lisberger 1996).

(D) Voltage traces from the model MVN cell in response to a test pulse of head rotation following training with the three different ISI values shown in C. Horizontal dashed lines in D indicate the resting potential of -58 mV.

(E) The three state trajectories in the 2-D phase plane defined by the voltage, \( V \), and the activation level of the h-current relative to that at rest, \( \zeta \), corresponding to the three voltage traces in D. The solid and dashed black curves are the nullclines during the resting state for \( V \) and \( \zeta \), respectively, on which their respective partial time derivatives vanish.

Vertical dashed lines in panels C and D mark the period of head rotation.

**Figure 8. A lock-and-key description of memory recall.**

(A) Schematic of memory formation within the lock-and-key description. Paired PF and CF activity induce synaptic plasticity in the cerebellar cortex. This shapes Purkinje ‘key’ activity in response to subsequent presentations of the learned sensory input.

(B) Schematic of memory recall, in which the lock resides in the DCN. Key activity driven by learned sensory input is sent to the DCN via Purkinje cell axons. Not all keys will be successful at driving DCN cell activity and learned motor responses. The lock prevents inappropriate motor responses by filtering out non-matching key activity. Partially matching key activity leads to unreliable recall or responses of diminished amplitude.

(C) An implementation of a lock-and-key mechanism using a linear-nonlinear (L-N) filter model, similar to those used to describe sensory receptive fields. Key activity, \( K(t) \), that is input to the lock passes through a linear filter, \( F(t) \), and then a nonlinear threshold function, \( G(x) \).

(D) The L-N model enables responses, equal to \( G(K(t) * F(t)) \), to be made selectively to only those keys, \( K(t) \), that are shaped by training with sufficiently positive interstimulus interval (ISI) values.

(E) Response of the L-N model as a function of ISI in eyeblink conditioning. The results mimic those of DCN cell simulations (Figures 4-6) and the classic rabbit behavioral data (Figures 1B and 5B).
Table 1. Parameters for compartmental simulations of DCN neurons. The symbol, description, value, and citations for each parameter in Models 1-3 of DCN cells.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
<th>Model</th>
<th>References</th>
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<tr>
<td>$V_{\text{rest}}$</td>
<td>Resting membrane potential</td>
<td>-58 mV</td>
<td>1,2,3</td>
<td>(Aizenman and Linden 1999; Linas and Muhlethaler 1988)</td>
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<tr>
<td>$V_{\text{Ca}}$</td>
<td>Ca$^{2+}$ reversal potential</td>
<td>140 mV</td>
<td>1,2,3</td>
<td>(Hille 2001)</td>
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<tr>
<td>$V_{\text{syn,Pkj}}$</td>
<td>GABAergic reversal potential, determined by Cl$^{-}$ gradient</td>
<td>-75 mV</td>
<td>1,2,3</td>
<td>(Jahnsen 1986b; Linas and Muhlethaler 1988)</td>
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<tr>
<td>$V_{\text{syn,MF}}$</td>
<td>Glutamatergic reversal potential, determined by Na$^{+}$ and Ca$^{2+}$ gradients</td>
<td>55 mV</td>
<td>1,2,3</td>
<td>(Hille 2001)</td>
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<td>$V_{\text{Na}}$</td>
<td>Na$^{+}$ reversal potential</td>
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<td>(Spain et al. 1987)</td>
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<tr>
<td>$V_{K}$</td>
<td>K$^{+}$ reversal potential</td>
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<td>3</td>
<td>(Jahnsen 1986b; Spain et al. 1987)</td>
</tr>
<tr>
<td>$r_{Pkj,b}$</td>
<td>Background Purkinje cell spike rate</td>
<td>40 Hz</td>
<td>1,2,3</td>
<td>(Berthier and Moore 1986)</td>
</tr>
<tr>
<td>$r_{Pkj,d}$</td>
<td>Reduced Purkinje cell spike rate due to LTD of parallel fiber inputs</td>
<td>20 Hz</td>
<td>1,2,3</td>
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</tr>
<tr>
<td>$r_{Pkj,p}$</td>
<td>Elevated Purkinje cell spike rate due to LTP of parallel fiber inputs</td>
<td>100 Hz</td>
<td>1,2,3</td>
<td>(Berthier and Moore 1986)</td>
</tr>
<tr>
<td>$r_{MF,b}$</td>
<td>Background mossy fiber spike rate</td>
<td>10 Hz</td>
<td>1,2,3</td>
<td>(Freeman and Nicholson 1999; Nicholson and Freeman 2002)</td>
</tr>
<tr>
<td>$r_{MF,CS}$</td>
<td>CS-driven mossy fiber spike rate</td>
<td>50 Hz</td>
<td>1,2,3</td>
<td>(Freeman and Nicholson 1999; Nicholson and Freeman 2002)</td>
</tr>
<tr>
<td>$\bar{g}_{T}$</td>
<td>Maximum T-type Ca$^{2+}$ conductance in Models 1 and 2</td>
<td>0.9 mS/cm$^2$</td>
<td>1,2</td>
<td></td>
</tr>
<tr>
<td>$\bar{g}_{T}$</td>
<td>Maximum T-type Ca$^{2+}$ conductance in Model 3 and phase plane modeling (Fig. 6)</td>
<td>0.6 mS/cm$^2$</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>$W_{\text{syn,Pkj}}$</td>
<td>Maximum total conductance of Purkinje cell synapses</td>
<td>0.1 mS/cm$^2$</td>
<td>1,2,3</td>
<td></td>
</tr>
<tr>
<td>$W_{\text{syn,MF}}$</td>
<td>Maximum total conductance of mossy fiber synapses</td>
<td>1 µS/cm$^2$</td>
<td>1,2,3</td>
<td></td>
</tr>
<tr>
<td>$\bar{g}_{HVA}$</td>
<td>Maximum high-voltage activated Ca$^{2+}$ conductance in one-compartment model 2</td>
<td>0.27 mS/cm$^2$</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>$\bar{g}_{HVA}$</td>
<td>Maximum high-voltage activated Ca$^{2+}$ conductance in two-compartment model 3</td>
<td>0.05 mS/cm$^2$</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>$\bar{g}_{SK}$</td>
<td>Maximum Ca$^{2+}$-dependent K$^{+}$ conductance</td>
<td>0.1 mS/cm$^2$</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>$\bar{g}_{Na}$</td>
<td>Maximum Hodgkin-Huxley type fast Na$^{+}$ conductance</td>
<td>45 mS/cm$^2$</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>$\bar{g}_{Kv}$</td>
<td>Maximum Hodgkin-Huxley type K$^{+}$ conductance</td>
<td>25 mS/cm$^2$</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>$\tau_{m}$</td>
<td>Membrane time constant</td>
<td>12 ms</td>
<td>1,2,3</td>
<td>(Jahnsen 1986a; Linas and Muhlethaler 1988)</td>
</tr>
<tr>
<td>$\tau_{\text{syn,Pkj}}$</td>
<td>GABAergic synaptic time constant</td>
<td>14 ms</td>
<td>1,2,3</td>
<td>(Anchisi et al. 2001)</td>
</tr>
<tr>
<td>$\tau_{\text{syn,MF}}$</td>
<td>Glutamatergic synaptic time constant</td>
<td>31 ms</td>
<td>1,2,3</td>
<td>(Anchisi et al. 2001)</td>
</tr>
<tr>
<td>$N_{\text{syn,Pkj}}$</td>
<td>Number of Purkinje cell inputs</td>
<td>50</td>
<td>2,3</td>
<td></td>
</tr>
<tr>
<td>$N_{\text{syn,MF}}$</td>
<td>Number of mossy fiber inputs</td>
<td>10</td>
<td>2,3</td>
<td></td>
</tr>
<tr>
<td>$R$</td>
<td>Ratio of Purkinje to mossy fiber inputs</td>
<td>5</td>
<td>2,3</td>
<td>(Chan-Palay 1973)</td>
</tr>
<tr>
<td>$g_{c}$</td>
<td>Inter-compartmental coupling</td>
<td>0.05 nS</td>
<td>3</td>
<td>(Mainen and Sejnowski 1996; Pinsky and Rinzel 1994)</td>
</tr>
<tr>
<td>$A$</td>
<td>Total membrane surface area</td>
<td>30,000 µm$^2$</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>$\rho$</td>
<td>Percent of membrane surface area occupied by somatic compartment</td>
<td>5%</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Parameters for compartmental simulations of MVN neurons. The symbol, description, value, and literature citations for each of the parameters used in simulations of MVN cells.

<table>
<thead>
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<th>Parameter</th>
<th>Description</th>
<th>Value</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{rest}}$</td>
<td>Resting membrane potential</td>
<td>-58 mV</td>
<td>(Straka et al. 2005)</td>
</tr>
<tr>
<td>$V_{\text{syn,Pkj}}$</td>
<td>GABAergic reversal potential, determined by Cl$^-$ gradient</td>
<td>-75 mV</td>
<td>(Hille 2001)</td>
</tr>
<tr>
<td>$V_{\text{syn,MF}}$</td>
<td>AMPA reversal potential, determined by Na$^+$ gradient</td>
<td>55 mV</td>
<td>(Chun et al. 2003; Hille 2001)</td>
</tr>
<tr>
<td>$V_h$</td>
<td>$I_h$ mixed-cation reversal potential</td>
<td>-20 mV</td>
<td>(Pape 1996; Santoro et al. 2000)</td>
</tr>
<tr>
<td>$r_{\text{Pkj,b}}$</td>
<td>Background Purkinje cell spike rate</td>
<td>40 Hz</td>
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</tr>
<tr>
<td>$r_{\text{MF,CS}}$</td>
<td>Mossy fiber spike rate during head rotation</td>
<td>50 Hz</td>
<td>(Freeman and Nicholson 1999; Nicholson and Freeman 2002)</td>
</tr>
<tr>
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<td>Maximum h-type cation conductance</td>
<td>0.5 mS/cm$^2$</td>
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</tr>
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<td>$\tau_m$</td>
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<td>12 ms</td>
<td>(du Lac and Lisberger 1995a; b)</td>
</tr>
<tr>
<td>$\tau_{\text{syn,Pkj}}$</td>
<td>GABAergic synaptic time constant</td>
<td>8.9 ms</td>
<td>(Chun et al. 2003)</td>
</tr>
<tr>
<td>$\tau_{\text{syn,MF}}$</td>
<td>Glutamatergic synaptic time constant</td>
<td>5.5 ms</td>
<td>(Chun et al. 2003)</td>
</tr>
</tbody>
</table>
References


Hesslow G. Correspondence between climbing fibre input and motor output in eyeblink-related areas in cat cerebellar cortex. J Physiol 476: 229-244, 1994a.


Porcello DM, Smith SD, and Huguenard JR. Actions of U-92032, a T-type Ca2+ channel antagonist, support a functional linkage between I(T) and slow intrathalamic rhythms. J Neurophysiol 89: 177-185, 2003.


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Figure 1
Figure 2

A

\[ \Delta \text{synaptic strength} \]

\[ \text{CF before PF} \quad 0 \quad \text{PF before CF} \]

\[ \text{CS–US time interval} \]

B

Purkinje cells

Input to DCN

PF activity

Purkinje cell

C

Purkinje cell spiking rate

Time (ms)
Figure 3

Wetmore et al 2007

A

Model 1

B

Model 2

C

Model 3

D

E

Membrane voltage (mV)
Wetmore et al., 2007. "Lock-and-key mechanisms of cerebellar memory recall based on rebound currents" .

Figure 4

A

B1

C1

Membrane Voltage (mV)

Time (ms)

ISI = 0 ms

ISI = 400 ms

Spiking rate (Hz)

Low LTP → High LTP

Low LTD → High LTD

Wetmore et al 2007
Wetmore et al. 2007
Figure 7
Figure 8

A. Storage

- Cerebellar cortex
- Deep nuclei
- Sensory information
- Learning rules, synaptic plasticity

B. Recall

- Cerebellar cortex
- Deep nuclei
- Sensory information
- Keys
- Motor output

C. Lock

- Keys, $K(t)$
- Filter amplitude $F(t)$
- Filter amplitude $G[x]$
- Motor output, $M(t)$

D. Interstimulus interval (ms)

- $K(t)$
- $x(t) = K(t) * F(t)$
- $M(t) = G[x(t)]$

E. Normalized response

- Interstimulus interval (ms)
Legends to Supplemental Movies

Supplemental Movie 1. Voltage trace and phase plane trajectory of a CS-driven rebound in Model 1 of a DCN neuron, following training with a long interstimulus interval (ISI).

(A) The DCN cell membrane voltage (red curve) as a function of time during presentation of a CS lasting 200 ms (black bar), under the mathematical approximation of instantaneous relaxation of the T-channel activation variable to its asymptotic value. The US was presented during prior training (gray bar) with an ISI of 190 ms between the CS and US onset times. The value of $t_{\text{LTD}}$ was set at 75 ms. The dotted horizontal line indicates the resting potential (-58 mV). At the start of the CS, increased spiking by Purkinje cells hyperpolarizes the DCN cell. About 35 ms prior to the time of the expected US, release of the hyperpolarizing drive leads to a rebound depolarization. Dashed vertical lines delineate the three stages of the phase plane trajectory in (B).

(B) The corresponding state trajectory (red curve) in the 2-D phase plane defined by the voltage ($V$) and the T-type inactivation variable ($I$). The black solid and dashed lines are the $I$ and $V$ nullclines, on which the partial time derivatives $\partial I/\partial t$ and $\partial V/\partial t$ respectively vanish. The intersection of the two nullclines is an attractive fixed-point of the dynamical system. The open black circle marks the initial fixed-point in the resting state (stage 1). The open green triangle marks the fixed-point from CS onset until $t_{\text{LTD}}$ before the expected US (stage 2). The open red square marks the fixed-point for the remainder of the CS (stage 3). The $V$ nullcline shifts at the start of the CS due to the increased Purkinje cell input, attracting the state trajectory from its location at the resting fixed-point (stage 1) towards the more hyperpolarized fixed-point (stage 2). We defined the instantaneous position of the $V$ nullcline by solving the equation $\partial V/\partial t = 0$ for $V$, using the steady state values of the synaptic conductances, $g_{\text{syn}}$, that would be attained given constant Purkinje cell and mossy fiber spiking at rates equal to their instantaneous values. Approximately $t_{\text{LTD}}$ before the expected US, the attractive fixed-point shifts again to a location that is depolarized compared to rest (stage 3), triggering a rebound. The color map near the stage 3 fixed-point encodes the amplitude of the rebound depolarization for trajectories passing through each point in phase space. Warmer hues indicate larger rebounds. Thus, with the interpretation of rebound amplitude as setting the reliability of a conditioned response (Figures 5 and 6), the neighborhood around the stage 3 fixed-point can be viewed as a zone of reliable memory recall, with warmer hues indicating greater reliability.

Supplemental Movie 2. Voltage trace and phase plane trajectory of a CS-driven rebound in Model 1 of a DCN neuron, following training with a short interstimulus interval (ISI).

(A,B) This movie presents the same material as Movie 1, except that the ISI value is 85 ms. Since $t_{\text{LTD}}$ is 75 ms, stage 2 lasts only 10 ms. As a result, the rebound amplitude is diminished and the phase
plane trajectory does not move as far into the reliable retrieval zone as with a long ISI value.

**Supplemental Movie 3. Voltage trace and phase plane trajectory of a CS-driven rebound in Model 2 of a DCN neuron, following training with a moderate interstimulus interval (ISI).**

(A) Using Model 2, we simulated the membrane potential of a DCN neuron. The stochastic arrival times of synaptic inputs leads to variations in the voltage dynamics between the 20 traces shown, despite identical initial conditions at $t = -100$ ms (red and blue traces). We chose an intermediate ISI value of 90 ms, for which 6 out of the 20 simulated trials result in a Ca$^{2+}$ spike (red traces). On the 14 other trials no Ca$^{2+}$ spike occurs (blue traces). Thus, this ISI value leads to unreliable retrieval of the stored memory. For comparison, the deterministic trajectory of the model cell given synaptic inputs occurring uniformly at the same average rate as for the noisy traces is also shown (large black dot moving along black trace).

(B) Each of the 20 model trajectories is depicted through the dynamics of a moving dot in the $(V, I)$ phase plane. The resulting cloud of 20 points moves under the influence of synaptic input and active conductances. As in Movies 1 and 2, the black solid and dashed lines are the $V$ and $I$ nullclines. When the $V$ nullcline shifts at the start of the CS due to increased Purkinje cell input, all 20 trajectories begin to move from the resting (stage 1) fixed-point towards the more hyperpolarized (stage 2) fixed-point. However, noisy synaptic inputs lead some trajectories (red dots) closer to the reliable retrieval zone (warm colors in color map) and toward a higher level of deinactivation of T-type Ca$^{2+}$ channels, while other trajectories are more distant from the reliable retrieval zone (blue dots). Approximately $t_{\text{TD}}$ before the expected US, the fixed-point shifts to a more depolarized location (stage 3). Trajectories that have attained a higher level of deinactivation generate larger amplitude rebounds, resulting in a large Ca$^{2+}$ spike (red dots). Trajectories attaining lower levels of deinactivation do not trigger a Ca$^{2+}$ spike (blue dots). The large black dot traces the deterministic phase plane trajectory for a cell receiving synaptic inputs uniformly in time at the same average rate as for the noisy traces.