Regulation of the Rebound Depolarization and Spontaneous Firing Patterns of Deep Nuclear Neurons in Slices of Rat Cerebellum

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Aizenman, Carlos D. and David J. Linden. Regulation of the rebound depolarization and spontaneous firing patterns of deep nuclear neurons in slices of rat cerebellum. *J. Neurophysiol.* 82: 1697–1709, 1999. Current-clamp recordings were made from the deep cerebellar nuclei (DCN) of 12- to 15-day-old rats to understand the factors that mediate intrinsic spontaneous firing patterns. All of the cells recorded were spontaneously active with spiking patterns ranging continuously from regular spiking to spontaneous bursting with the former predominating. A robust rebound depolarization (RD) leading to a Na^+ spike burst was elicited after the offset of hyperpolarizing current injection. The voltage and time dependence of the RD was consistent with mediation by low-threshold voltage-gated Ca^{2+} channels. In addition, induction of a RD also may be affected by activation of a hyperpolarization-activated cation current, I_h. A RD could be evoked efficiently after brief high-frequency bursts of inhibitory postsynaptic potentials (IPSPs) induced by stimulation of Purkinje cell axons. IPSP-driven RDs were typically much larger and longer than those elicited by direct hyperpolarizing pulses of approximately matched amplitude and duration. Intracellular perfusion of the Ca^{2+} buffer bis-(*o*-aminophenoxy)-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) dramatically enhanced the RD and its associated spiking, sometimes leading to a plateau potential that lasted several hundred milliseconds. The effects of BAPTA could be mimicked partly by application of apamin, a blocker of small conductance Ca^{2+}-gated K^+ channels, but not by paxilline, which blocks large conductance Ca^{2+}-gated K^+ channels. Application of both BAPTA and apamin, but not paxilline, caused cells that were regularly spiking to burst spontaneously. Taken together, our data suggest that there is a strong relationship between the ability of DCN cells to elicit a RD and their tendency burst spontaneously. The RD can be triggered by the opening of T-type Ca^{2+} channels with an additional contribution of hyperpolarization-activated current I_h. RD duration is regulated by small-conductance Ca^{2+}-gated K^+ channels. The RD also is modulated tonically by inhibitory inputs. All of these factors are in turn subject to alteration by extrinsic modulatory neurotransmitters and are, at least in part, responsible for determining the firing modes of DCN neurons.

INTRODUCTION

The deep cerebellar nuclei (DCN) comprise the core of the cerebellar circuitry, integrating a variety of converging excitatory and inhibitory inputs representing several streams of sensory-motor information. DCN neurons receive inhibitory projections from Purkinje cells, which are the sole output of the cerebellar cortex. In addition, the DCN also receive excitatory inputs from mossy fibers and climbing fibers. These two afferent systems originate, respectively, in various precerebellar nuclei and in the inferior olive. As a consequence, the output of all the neural computations that are performed in the cerebellum is reflected in the firing patterns of DCN neurons, which then are translated into different motor functions through projections to a variety of premotor centers including the thalamus, red nucleus, and superior colliculus. Moreover, the DCN now are thought to play an increasingly important role in some forms of cerebellar-mediated motor learning (see du Lac et al. 1995; Kim and Thompson 1997 for review). Several converging lines of evidence, based on lesion, inactivation, and extracellular recordings, have pointed to the DCN as a possible locus for information storage during associative eyeblink conditioning, and to their analogous nuclei, the vestibular nuclei, in adaptation of the vestibuloocular reflex (Mauk 1997; Raymond et al. 1996). It is therefore important to bring to light some of the factors that control and modulate the intrinsic spiking behavior of the DCN cells, so that we may begin to understand how its output is regulated, as well as for understanding the cellular substrates that may underlie information storage at this site.

The intrinsic electrophysiological properties of DCN neurons have been studied with intracellular recordings in a variety of different preparations, including isolated brain stem (Llinás and Mühlethaler 1988), cerebellar slices (Aizenman et al. 1998; Gardette et al. 1985b; Jahnsen 1986a,b), and organotypic cultures (Mouginot and Gähwiler 1995; Muri and Knöpfel 1994; see Sastry et al. 1997 for review). A general characteristic of DCN neurons, which has been observed in each preparation, is that they exhibit a pronounced rebound depolarization (RD), often accompanied by a burst of Na^+ spikes, immediately after a hyperpolarizing current pulse. This has been attributed to the opening of low-threshold, voltage-gated Ca^{2+} channels, which become deinactivated during the hyperpolarizing pulse and open on return to more depolarized membrane potentials at the offset of the pulse. The RD and its associated spike burst have been shown to induce large intracellular Ca^{2+} transients in DCN neurons; something that is revealed by the use of Ca^{2+}-sensitive dyes (Aizenman et al. 1998; Muri and Knöpfel 1994). Hyperpolarizing inhibitory postsynaptic potentials (IPSPs), originating from Purkinje cell inputs, can also transiently hyperpolarize the DCN cells and thereby elicit a RD (Aizenman et al. 1998; Gardette et al. 1985a; Llinás and Mühlethaler 1988). Therefore the RD provides an efficient mechanism by which inhibitory Purkinje cell inputs can drive postsynaptic excitation and Ca^{2+} entry. This mechanism has a central role in some cerebellar learning models (Mauk and Donegan 1997) and may be the basis for use-dependent plasticity at the Purkinje cell to DCN synapse (Aizenman et al. 1998).

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Little is known about the exact parameters necessary for induction of a RD or about the interplay between the various currents which underlie it. We performed microelectrode recordings in rat cerebellar slices to understand the factors that underlie the induction and modulation of the RD and the role the RD plays in determining the spontaneous spiking behavior of the DCN cells.

METHODS

Rat pups (12–15 days old) were decapitated, and the brains promptly removed and immersed in ice-cold standard artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgSO_4$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, and 20 D-glucose, continuously bubbled with 95% O2–5% CO_2. Coronal slices (400-μm thick) from the cerebellum were cut using a Vibratome and were incubated in ice-cold ACSF. Although rats of this age still are considered juveniles, the intrinsic properties of these neurons are remarkably similar to that previously reported in mature guinea pigs (Jahnsen 1986a; Llinás and Mühlethaler 1988) and in slice cultures (Muri and Knöpfel 1994), suggesting that at 2 wk the DCN neurons already have a mature electrophysiological phenotype. In addition, there is evidence in the literature that after the first postnatal week, DCN cells already express both intrinsic and synaptic conductances that are present in mature neurons (Gardette et al. 1985a,b). Slices then were incubated for ≥1 h at room temperature in a homemade interface chamber; after this recordings were performed in a Haas-style interface chamber at 33°C that was perfused with ACSF containing 2 mM kynurenate to block ionotropic glutamate receptors. Borosilicate glass microelectrodes (80–150 MΩ) were filled with 3 M KAc. Where indicated, 100 μM bis-(*o*-aminophenoxy)-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) or 100 μM *N*-(2,6-dimethylphenylcarbamoylmethyl) triethylammonium bromide (QX-314) were included in the electrode solution and were allowed to passively diffuse into the cell until a consistent effect was seen (typically for 10 min.). To evoke IPSPs, concentric bipolar stimulating electrodes were placed in the white matter immediately adjacent to the nuclei. Recordings were made from either the medial or lateral group of the DCN, and no obvious differences were observed between the two nuclei. Membrane voltage was measured using an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA) and analyzed with a Macintosh computer using AxoGraph (Axon Instruments) and Igor Pro software (WaveMetrics, Lake Oswego, WI). Bicuculline methiodide (BMI), picrotoxin, and gabazine (SR-95531) were obtained from Research Biochemicals International (Natick, MA). Paxilline and apamin were obtained from Alomone Labs (Jerusalem, Israel). All other chemicals were from Sigma.

RESULTS

Spontaneous firing properties

Sharp electrode, current-clamp recordings from 68 DCN neurons in coronal slices of rat cerebellum were included in the total data pool. DCN cells had resting membrane potentials of −58 ± 1 (SE) mV, and input resistances of 51 ± 3 MΩ. All cells were spontaneously active at rest. All experiments were performed with 2 mM kynurenate in the bath to eliminate excitatory drive mediated by ionotropic glutamate receptors. This afferent input has been shown in different preparations of this tissue (isolated cerebellum/brainstem and cultured slice) to drive spiking in DCN cells (Llinás and Mühlethaler 1988; Mouginot and Gahwiler 1995). Thus most of the spontaneous activity can be attributed to the intrinsic properties of the DCN neurons. An example of a typical cell is shown in Fig. 1*A*. At rest, this neuron fired action potentials regularly at a fairly high

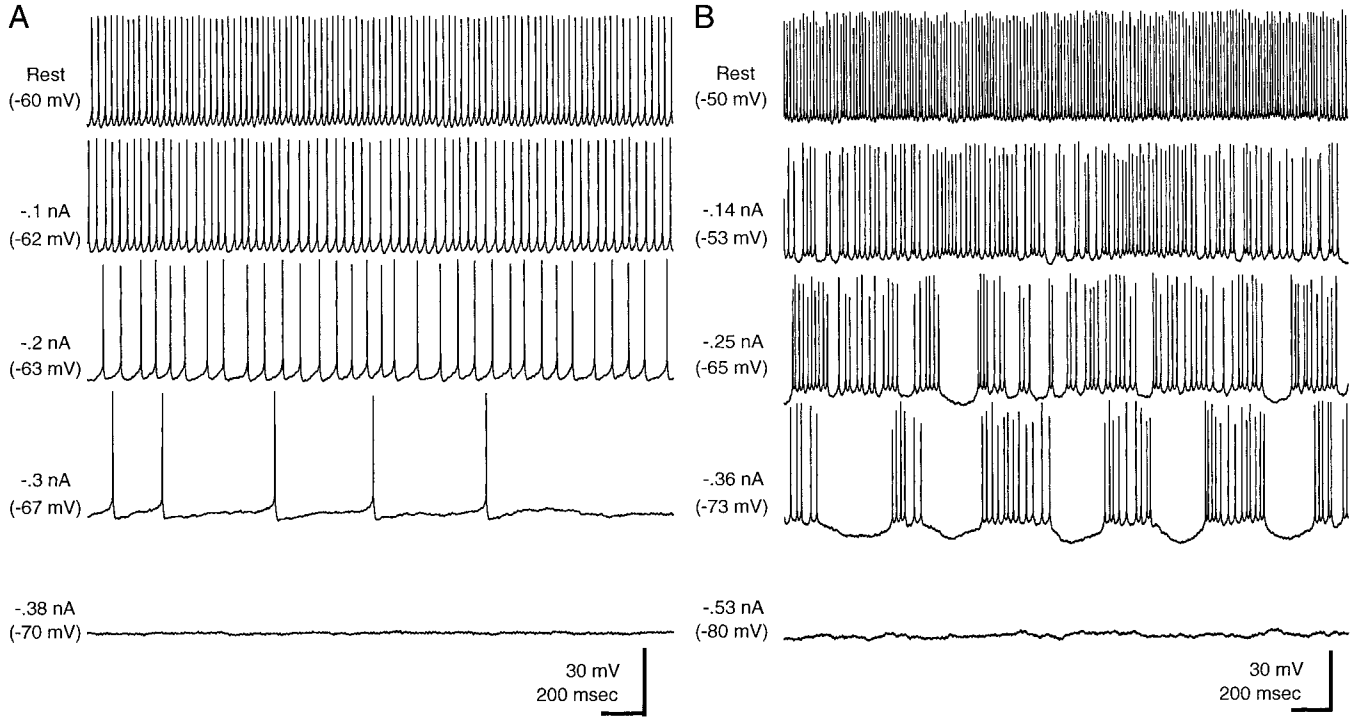

FIG. 1. Spontaneous firing properties of deep cerebellar nuclei (DCN) neurons. *A*: sharp microelectrode recordings of spontaneous activity in a regular-spiking DCN neuron at various membrane potentials. *Top*: spiking at resting membrane potential. *Bottom traces*: spiking recorded with various amounts of hyperpolarizing current tonically injected via the recording electrode. *B*: recordings from a spontaneously bursting cell, at resting membrane potential (*top*), and with varying amounts of tonic hyperpolarization (*bottom traces*).

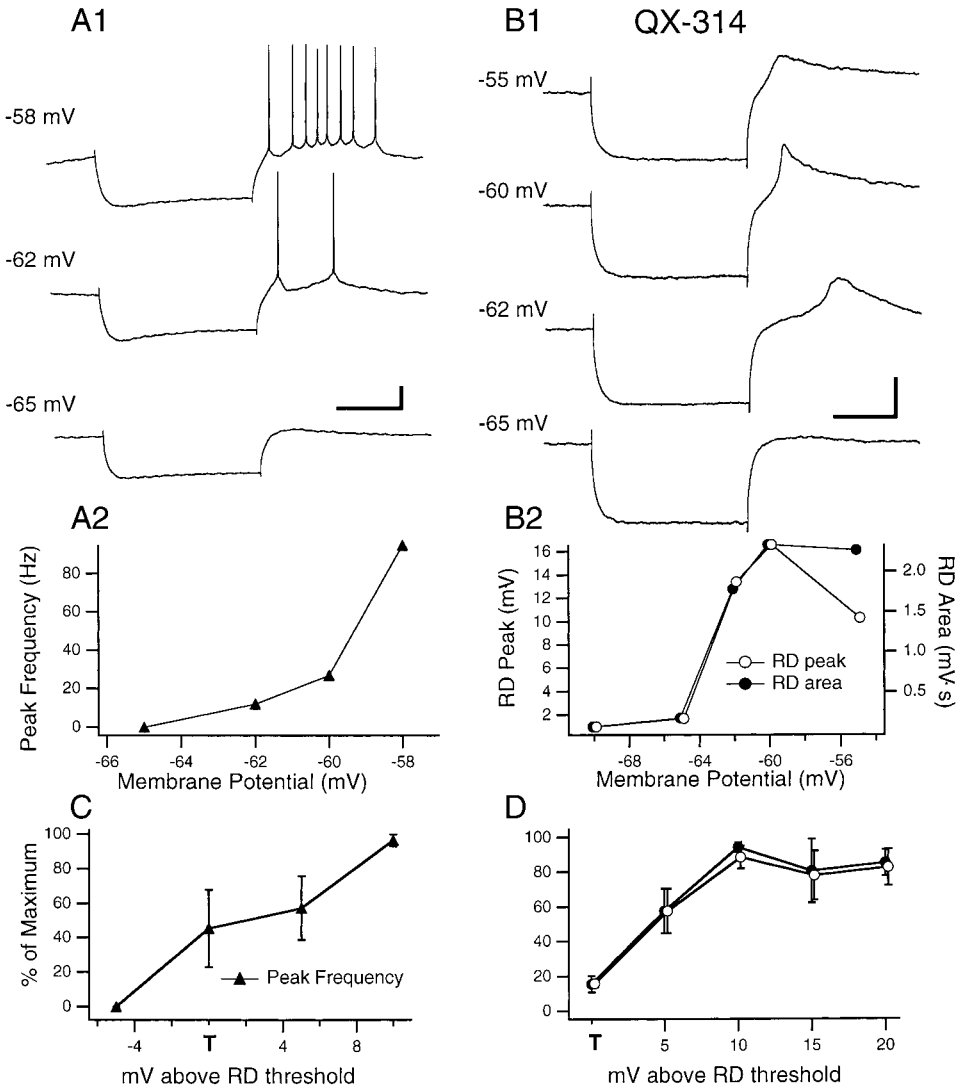

FIG. 2. Rebound depolarization (RD) induction is dependent on the membrane potential. *A1*: sample traces of a RD and associated spike burst evoked by a hyperpolarizing pulse at different membrane potentials. *A2*: plot of RD-evoked peak spiking frequency as a function of membrane holding potential. *B1*: sample traces of a RD in the same cell as in *A1* but after 40 min of intracellular QX-314 perfusion, at different membrane potentials. *B2*: RD area (●) and peak amplitude (○) plotted as a function of membrane holding potential. *C* and *D*: corresponding average data for 4 and 8 DCN cells, respectively. *x* axes were normalized to millivolts above the threshold for RD induction, and the *y* axes were normalized to percent of maximum. T (threshold) is defined as the membrane potential at which a hyperpolarizing pulse 1st evokes a RD. Size of the hyperpolarizing pulse was 250 ms and −0.5 nA. Scale bars are 20 mV and 100 ms.

frequency (~35 spikes/s; *top*). On the application of tonic hyperpolarizing current through the recording electrode, the firing rate slowed in a manner proportional to the amount of current injected (*bottom traces*), until firing finally subsided at approximately −70 mV. This intrinsic, regular spiking behavior was typical of most of the cells recorded. However, in a small subpopulation of neurons [9 of 136 cells, from all DCN cells ever recorded by our group, including those in this study as well as cells from another study (Aizenman et al. 1998)] a different spontaneous firing pattern was observed. An example of this pattern is shown in Fig. 1*B*. This neuron also appears to fire regularly at rest (*top*). Yet when tonic hyperpolarizing current is injected, the firing frequency not only slows but also shifts mode to reveal brief, spontaneously occurring bursts of spikes (*2nd* and *3rd traces*). The bursts become clearly resolved with larger amounts of hyperpolarizing current (*4th trace*). Although these spontaneously bursting cells are a minority, the behavior of many cells falls somewhere in between the two types shown here, forming a continuum between regular spiking cells and spontaneously bursting cells. There were no obvious differences in the resting membrane potential and input resistance between the bursting (−60 ± 3 mV, 61 ± 20 MΩ, $n = 9$) and the regular spiking cells.

Intrinsic responses to current pulses

After application of a hyperpolarizing pulse, a prominent RD was evoked that typically was accompanied by an associated burst of action potentials. (Figs. 2*A1*, 3*A1*, and 4*A1*; see also Aizenman et al. 1998; Jahnsen 1986a,b; Llinás and Mühlethaler 1988; Muri and Knöpfel 1994). In this manuscript, we use "rebound depolarization" as an operative term to describe the overall depolarization that follows a transient period of hyperpolarization and that may consist of several underlying component currents (see following text). The RD and associated spike burst are believed to be triggered by the opening of low-threshold Ca^{2+} channels (T-type) and are accompanied by a large intracellular Ca^{2+} transient (Aizenman et al. 1998; Muri and Knöpfel 1994). To further characterize the stimulation parameters that control the RD, we performed several experimental manipulations shown in Figs. 2–4. It should be noted that there was significant variability in the size, time course, and activation threshold of the RD among different cells. Thus we have attempted to portray this variability in our choice of sample voltage traces for these figures while at the same time extracting common elements of the RD that are present in all of the recorded DCN cells.

The RD is dependent on the membrane potential of the cell, being more strongly activated at more depolarized membrane potentials and usually peaking between −60 and −70 mV, consistent with the activation range of T-type Ca^{2+} channels (Carbone and Lux 1984, 1987). In Fig. 2*A1,* responses to a −0.5-nA hyperpolarizing pulse are shown while the cell is being held at different membrane potentials by tonic hyperpolarization. The peak spiking frequency, as well as the number of RD-evoked spikes are used as an index of RD strength (Fig. 2, *A2* and *C*). Note that the RD is larger at more depolarized membrane potentials. The average membrane potential at which the RD was most strongly activated, as assessed by the number of evoked spikes and peak firing frequency observed across a population of cells, was −61 ± 4 mV and −65 ± 4 mV ($n = 4$), respectively. The average number of evoked spikes for a maximally elicited RD was 28 ± 16 spikes, and the average peak frequency was 77 ± 25 Hz ($n = 4$).

The RD itself is not abolished by blockade of voltage-gated Na^+ channels by intracellular perfusion of QX-314 (Fig. 2*B1*). The RD observed in the presence of QX-314 is nearly indistiguishable from that which is observed in the presence of TTX (see Aizenman et al. 1998). In Fig. 2, *B2* and *D,* the RD area and peak amplitude are used to assess RD strength in the absence of spiking. Again, the RD is more strongly evoked when the cell is held at more positive membrane potentials. The average membrane potential at which the RD was most strongly activated in QX-314-filled cells, as assessed by RD area and peak amplitude, was −58 ± 2 mV and −59 ± 3 mV ($n = 8$), respectively. This value is slightly more positive than that measured in cells without QX-314 and can perhaps be attributed to a mild blockade of T-type channels by QX-314 (Talbot and Sayer 1996). The average maximum RD area was 3 ± 1 mV/s, and the peak amplitude was 15 ± 3 mV ($n = 8$).

A well-known property of T-type Ca^{2+} channels is that they rapidly inactivate in a time- and voltage-dependent manner, and to remove the inactivation, the cell first must be hyperpolarized. One prediction that derives from this is the observation that increasing hyperpolarization to remove more inactivation will elicit a stronger RD. This is illustrated in Fig. 3*A,* where the cell was tonically hyperpolarized below spike threshold (−65 mV) to suppress spontaneous spiking while increasingly larger hyperpolarizing steps are applied. As expected, the RD is also increasingly stronger, as assessed by the number and frequency of the associated spike burst (Fig. 3, *A, 1* and *2,* and *C*). The same is observed for a QX-314-filled cell held at −55 mV (Fig. 3*B, 1* and *2,* and *D*). Another way in which deinactivation of T-type channels can be increased is by increasing the duration of the hyperpolarizing pulse rather than the amplitude. In Fig. 4, we can see that increasingly long hyperpolarizing pulses, applied while the cell was tonically hyperpolarized below spike threshold, elicited a proportionally larger RD in cells recorded both with (Fig. 4, *B* and *D*) and without (Fig. 4, *A* and *C*) QX-314.

In response to a hyperpolarizing current pulse, the membrane voltage deflection had both a transient and a sustained component. The sustained component appears as a "sag" in the voltage deflection (Fig. 5*A*) and increases in amplitude as the cell is increasingly hyperpolarized (Fig. 5, *A* and *B*). This hyperpolarization-activated sag can be suppressed in part by extracellular application of 2 mM CsCl ($n = 4$; Fig. 5*C*). At this concentration, CsCl reduced the absolute amplitude of the sag by 86 ± 3% ($n = 3$) and by 66 ± 6% if expressed as percent of the total hyperpolarization-induced change in V_m. Notice also that QX-314 perfusion seems to almost completely abolish the sag (Figs. 2*B1,* 3*B1,* and 4*B1*), consistent with a previous report where this drug was seen to block hyperpolarization-activated currents (Perkins and Wong 1995). This observation, together with the strong voltage dependence of the sag, suggests that it is at least partly mediated by I_h (McCormick and Pape 1990). The incomplete blockade by CsCl, however, suggests that additional hyperpolarization-activated currents also may be involved (see Williams et al. 1997). When hyperpolarizing pulses were given when the cell was held subthreshold to the activation range of T-type Ca^{2+} channels, there still was a small RD, presumably mediated by Ih (Fig. 5*D, top*), suggesting that it may play a role in boosting the RD as has been observed previously in thalamic neurons (see Pape 1996 for review). Unfortunately, this is difficult to assess directly because application of external CsCl has additional nonspecific effects (presumably mediated via blockade of various K^+ conductances) that potentiate the RD (Fig. 5*D, bottom*).

In summary, these manipulations are consistent with the notion that the RD is mediated, at least in large part, by opening of T-type Ca^{2+} channels. First, the membrane potentials at which the RD is activated maximally are consistent with the activation range of these channels. Second, increasing the size and duration of the hyperpolarizing pulse elicits a stronger RD, consistent with the reported kinetics of T-channel deinactivation (Carbone and Lux 1984, 1987). Third, the RD is still present when voltage-gated Na^+ channels are blocked. And fourth, the RD is a transient event, similar to low-threshold spikes observed in other cell types (Bal and McCormick 1993; Llinás and Yarom 1981). Unfortunately, because of a lack of a specific and potent antagonist of T-type Ca^{2+} channels, it has not been possible to test the role of these channels directly. Application of several putative specific T-channel antagonists (mibefradil, 1 μM; nickel, 100 μM; ethosuximide, 1 mM) failed to alter significantly the RD (data not shown). Activation of I_h also potentially could contribute to the RD induction by boosting the membrane potential when it is subthreshold to T-channel activation. This is supported by the fact there is still a small RD at hyperpolarized membrane potentials as well as by the observation that when the hyperpolarization-induced sag is blocked by QX-314 the induction threshold for the RD is more depolarized. One possible factor that may complicate the interpretation of the RD voltage dependence in these experiments is the observation that QX-314 can have nonspecific effects on several voltage-gated conductances, including T-type Ca^{2+} currents (Talbot and Sayer 1996). Nevertheless, the voltage-dependent properties of the RD in QX-314-filled cells are consistent with those observed in control neurons (Fig. 2, *A* and *B*), in experiments performed with TTX (Aizenman et al. 1998) and with those reported by others (Llinás and Mühlethaler 1988). This suggests that application of QX-314 should not significantly alter the main interpretation of these results.

Synaptically driven RDs

Because all experiments were performed in the presence of kynurenate, stimulating the white matter adjacent to the DCN elicited a pharmacologically isolated IPSP. This IPSP

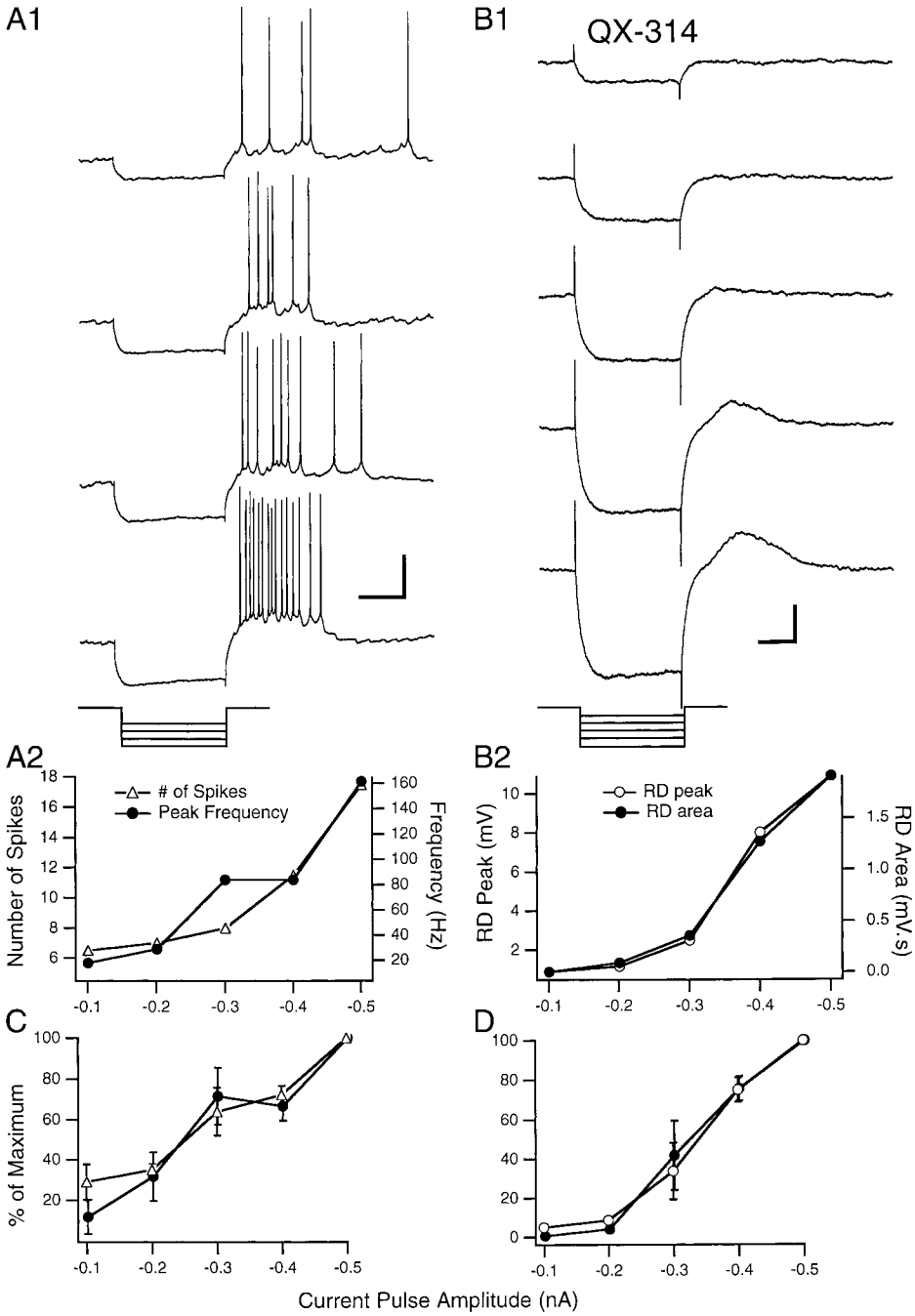

FIG. 3. RD induction is dependent on the amplitude of the hyperpolarizing pulse. *A1*: sample traces of RDs and associated spike bursts evoked by hyperpolarizing pulses with amplitudes ranging from −0.2 to −0.5 nA in a cell held at −65 mV. *A2*: plot of number of RD-evoked spikes (△) and peak spiking frequency (●) as a function of hyperpolarizing pulse amplitude. *B1*: sample traces of RDs in a QX-314-filled cell held at −55 mV, evoked by hyperpolarizing pulses with amplitudes ranging from −0.1 to −0.5 nA. *B2*: RD area (●) and peak amplitude (○) plotted as a function of pulse amplitude. *C* and *D*: corresponding average data for 4 DCN cells. To suppress spontaneous spiking, the cells were tonically hyperpolarized below spike threshold throughout the experiment. Duration of the hyperpolarizing pulse was 250 ms. Scale bars are 20 mV and 100 ms in *A1* and 10 mV and 100 ms in *B1*.

is mediated by $GABA_A$ receptors (Aizenman et al. 1998), has a reversal potential of approximately −75 mV, and is reported by others to be unaffected by $GABA_B$ receptor antagonists (see Sastry et al. 1997 for review). Although a single IPSP was usually not sufficient to evoke a RD, a high-frequency train of IPSPs was quite effective (Fig. 6, *A* and *C*). Like a hyperpolarizing pulse-driven RD, the IPSP-driven RD was larger at depolarized membrane potentials and was attenuated by tonic hyperpolarization (Fig. 6, *B* and *D*). The RD is therefore a mechanism by which hyperpolarizing IPSPs can drive subsequent postsynaptic excitation and their associated intracellular Ca^{2+} transients, processes that have been shown to be important for inducing use-dependent changes of inhibitory synaptic strength in the DCN (Aizenman et al. 1998).

Interestingly, IPSP trains were much more effective at strongly activating a RD than were hyperpolarizing current pulses of similar amplitude and duration. An example is shown in Fig. 7*A*, where a train of 10 IPSPs evokes a robust RD and an associated spike burst, whereas a current pulse of similar amplitude and duration completely fails to elicit a RD in the same neuron. This was a consistent observation and data are summarized for three different cells in Fig. 7*B*. One possible explanation is that because the synaptic input from inhibitory fibers is widely distributed throughout a DCN cell (Chan-Palay 1977), the hyperpolarization pro-

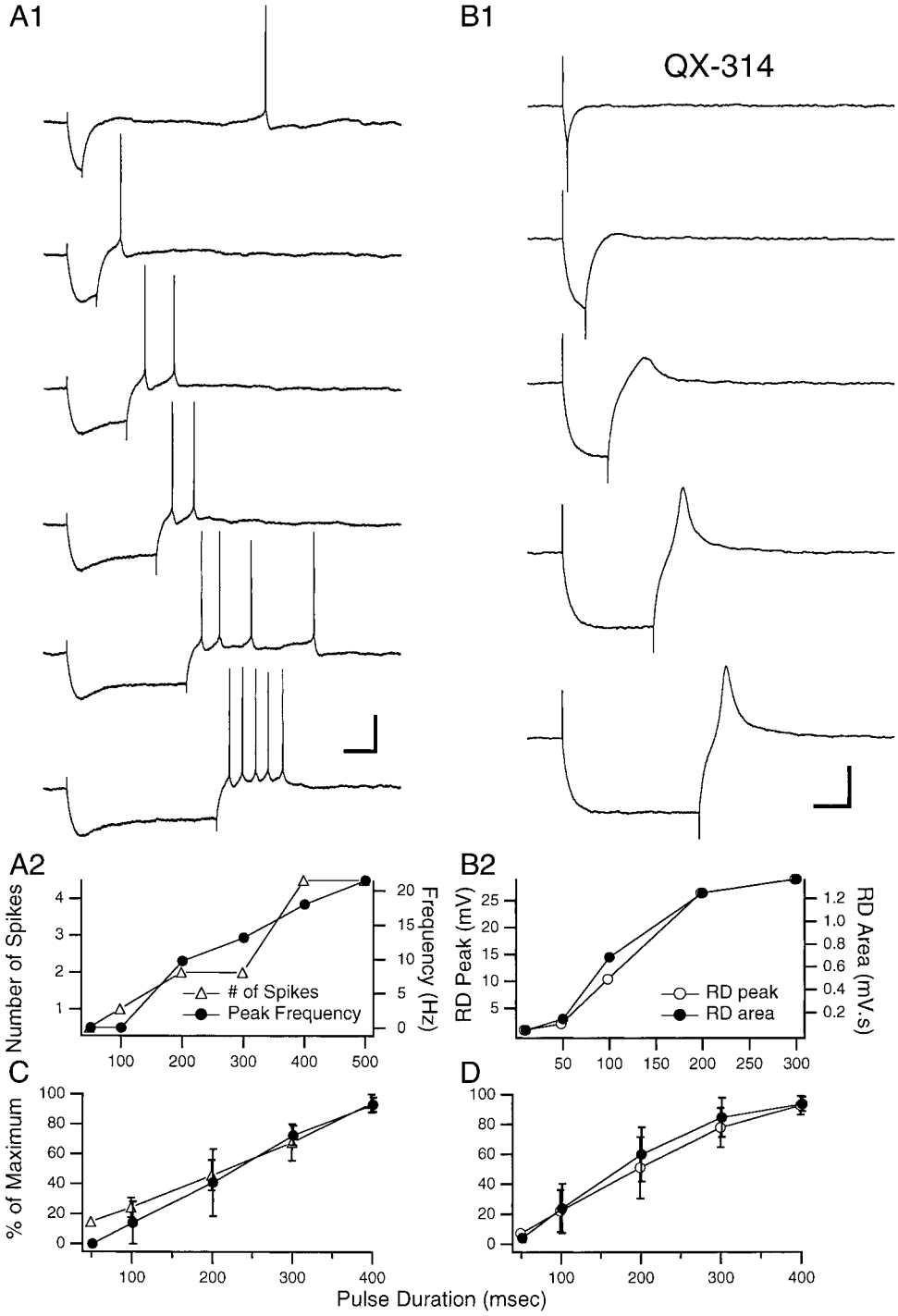

FIG. 4. RD induction is dependent on the duration of the hyperpolarizing pulse. *A1*: sample traces of RDs and associated spike bursts evoked by hyperpolarizing pulses of different durations ranging from 50 to 500 ms in a cell held at −68 mV. *A2*: plot of the number of RD-evoked spikes (△) and peak spiking frequency (●) as a function of hyperpolarizing pulse duration. *B1*: sample traces of RDs in a QX-314-filled cell held at −54 mV, evoked by hyperpolarizing pulses with duration ranging from 10 to 300 ms. *B2*: RD area (●) and peak amplitude (○) plotted as a function of pulse duration. *C* and *D*: average data for 3 DCN cells. To suppress spontaneous spiking, the cells were tonically hyperpolarized below spike threshold. Amplitude of the hyperpolarizing pulse was −0.5 nA. Scale bars are 20 mV and 100 ms in *A1* and 8 mV and 80 ms in *B1*.

duced thereby is more effective in deinactivating T channels as compared with a hyperpolarization delivered from a point source, such as an intracellular electrode. Another possibility is that the synaptically induced RD also may be facilitated by the activation of nonionotropic glutamate receptors or by the tetanically induced release of additional modulatory neurotransmitters. In fact, a slow depolarizing synaptic component is observed even when the IPSP burst is nearly reversed, as in Fig. 6*B*, *bottom*. The identity of this component will prove to be an important element in further studies that consider the nature of excitatory synaptic inputs to the DCN.

Modulation of the RD

Although the induction of the RD is consistent with the activation of T channels, the majority of the intracellular Ca^{2+} transient has been shown, with the aid of Ca^{2+}-sensitive dyes, to be dependent on postsynaptic spiking (Aizenman et al. 1998; Muri and Knöpfel 1994). It is possible then that the time course

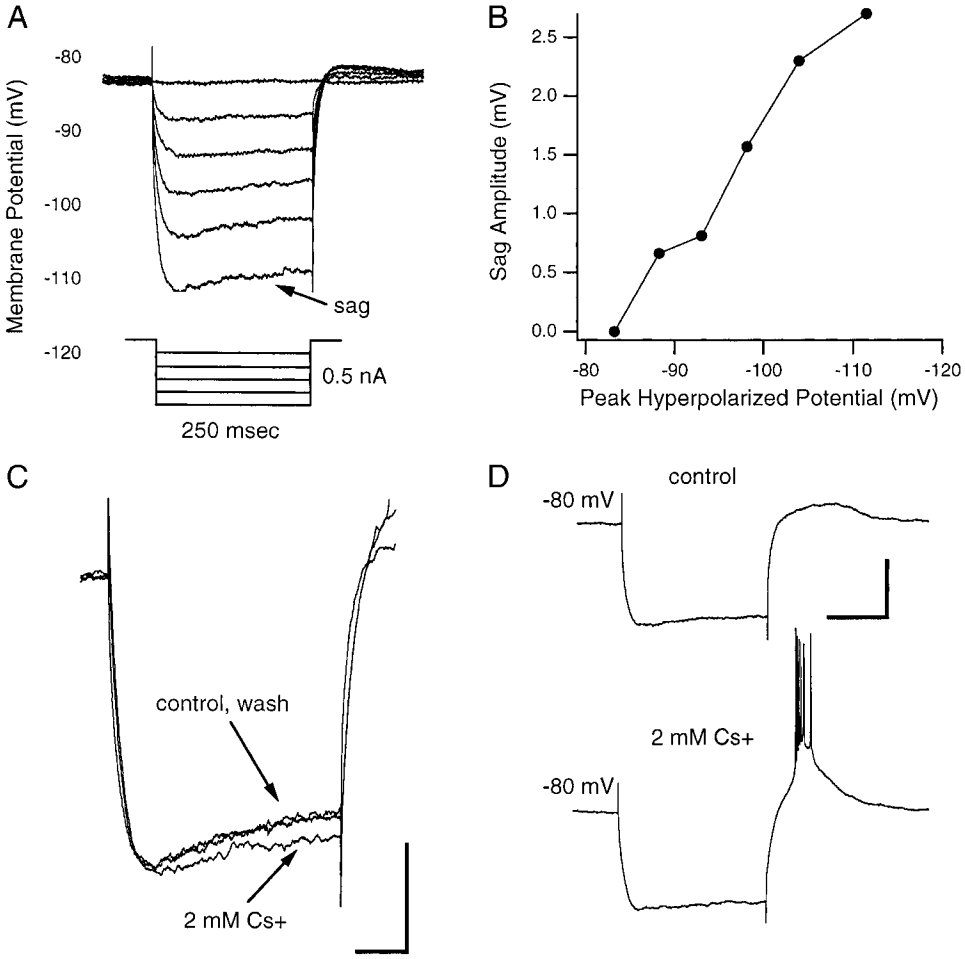

FIG. 5. I_h may contribute to the generation of a RD. *A*: responses of a DCN neuron held at −85 mV (−0.65 nA) to 250-ms steps of hyperpolarizing current, ranging from −0.1 to −0.5 nA. Notice the hyperpolarization-induced sag in the membrane voltage deflection. *B*: absolute amplitude of the sag is plotted against the initial membrane potential in response to the hyperpolarizing step. Notice that as the cell is being increasingly hyperpolarized, the sag increases in amplitude. *C*: amplitude of the hyperpolarization-induced sag can be reduced by the extracellular application of 2 mM CsCl. This suggests that, at least in part, this current is constituted by I_h. *D*: I_h may contribute to the RD as seen by the small depolarization caused by a −0.7-nA hyperpolarizing step in a cell held well below the threshold for T-type Ca^{2+} channels (*top*). It is difficult to accurately assess the contribution of I_h to the RD because bath application of 2 mM CsCl tended to increase the evoked RDs, presumably by a nonspecific blockade of K^+ channels. Scale bars in *A* are 10 mV and 50 ms, in *B* are 20 mV and 100 ms.

of the RD and associated spike burst may be mediated in part by Ca^{2+}-dependent processes. Intracellular perfusion with the Ca^{2+} chelator BAPTA markedly increased the excitability of the cells within 10–20 min of impalement ($n = 9$). An example is shown in Fig. 8*A*. After 25 min of intracellular perfusion of BAPTA, a depolarizing current pulse caused the cell to fire a few spikes at a very high frequency, followed by a plateau response (*bottom left*). A hyperpolarizing pulse induced a RD that also elicited a plateau response lasting several hundred milliseconds (*bottom right*). This suggests that Ca^{2+}-dependent processes, particularly activation of Ca^{2+}-gated K^+ channels, may regulate the duration and amplitude of the RD. To

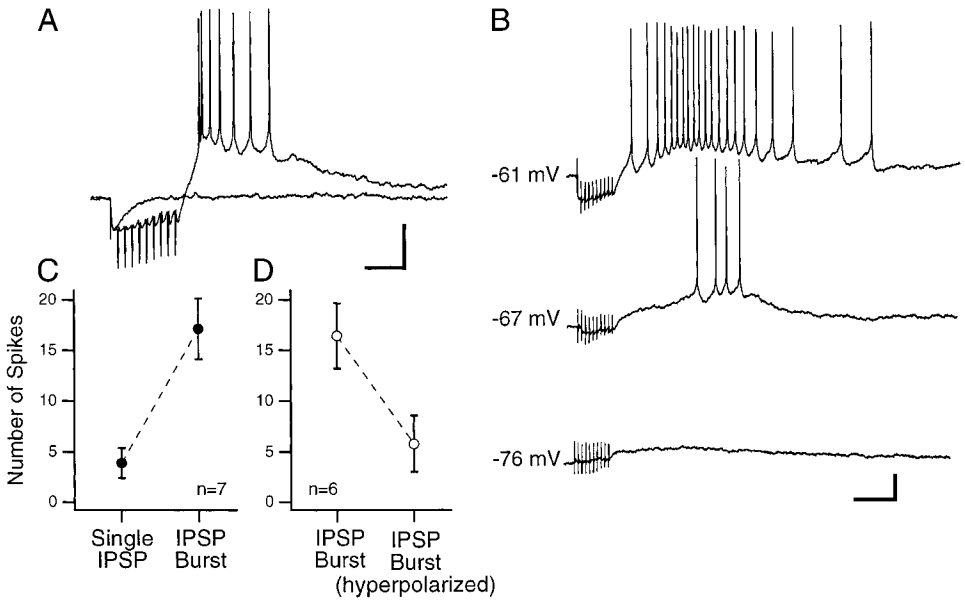

FIG. 6. Synaptically driven RDs. *A*: comparison between a single inhibitory postsynaptic potential (IPSP) evoked by stimulation of Purkinje cell axons and a 10 pulse × 100 Hz IPSP train. Notice that the IPSP train evokes a robust RD, whereas a single IPSP does not. *B*: IPSP train evoked RDs at different membrane holding potentials. *C*: average data from 7 DCN cells, showing the number of RD-driven spikes elicited by a single IPSP as compared with with a 10 pulse × 100 Hz IPSP train. *D*: average data from 6 cells, comparing the number of RD-driven spikes elicited by an IPSP train at different membrane potentials. Like hyperpolarizing pulse-driven RDs, synaptically driven RDs are suppressed with tonic hyperpolarization. Scale bars in *A* are 10 mV and 50 ms, in *B* are 10 mV and 100 ms.

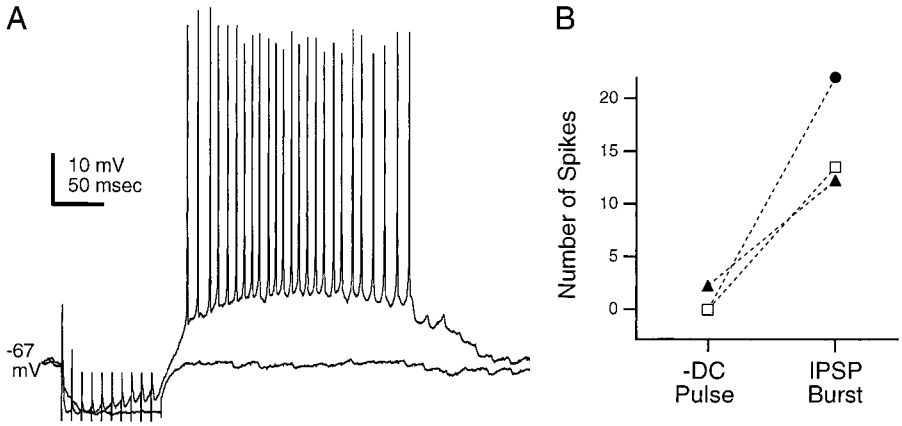

FIG. 7. Comparison of IPSP-train- and hyperpolarizing-pulse-evoked RDs. *A*: sample trace of a RD evoked by a 100-Hz train of 10 IPSPs compared with a hyperpolarizing pulse of similar amplitude and duration applied to the same cell. Note that the IPSP train elicited a large RD, whereas the hyperpolarizing pulse did not. *B*: number of RD-driven spikes elicited by a hyperpolarizing pulse and by an IPSP train in 3 separate cells is shown, each cell is indicated by a different plot symbol. To suppress spontaneous spiking, the cells were tonically hyperpolarized below spike threshold.

test this hypothesis, we bath applied apamin, a specific blocker of small conductance (SK) Ca^{2+}-gated K^+ channels (Blatz and Magleby 1986) ($n = 5$). Figure 8*B* shows the effect of 1 μM apamin on the intrinsic properties of a DCN cell. Apamin caused the cell to fire action potentials at extremely high frequencies ($\leq$300 spikes/s, ~4 times the control rate) during a depolarizing step and during a hyperpolarizing-pulse evoked RD. In four of five cells, apamin also caused the cell to exhibit plateau potentials lasting for hundreds of milliseconds, similar to those seen with BAPTA perfusion (data not shown). Bath application of paxilline (100 nM, $n = 3$), a blocker of the large conductance (BK) Ca^{2+}-gated K^+ channels (Sanchez and McManus 1996) did not cause the cell to fire plateau potentials nor did it substantially increase the firing rate elicited by either a depolarizing step or a RD (Fig. 8*C*).

Another interesting effect of BAPTA and apamin is that they both induce spontaneous, regularly occurring spike bursts while the neuron is at rest. With tonically injected hyperpolarizing current, the interval between the bursts increases, until finally the cell stops firing (Fig. 9, *A* and *B*). This was observed in all the BAPTA-perfused cells ($n = 8$) as well as all of the cells in which apamin was applied ($n = 5$). Paxilline did not cause the cells to burst spontaneously (Fig. 9*C*, $n = 3$). Interestingly, all of the cells in these experiments were regularly spiking before BAPTA or apamin were applied, suggesting that any cell has the capability of bursting if it is subjected to the appropriate type of modulation. Moreover it shows that modulation of the RD affects the spontaneous firing properties of the cell and determines its propensity to fire spike bursts. It is interesting to consider the source of the interburst hyperpolarization when the Ca^{2+}-gated K^+ channels are blocked. One possibility is that both BAPTA and apamin fail to completely block these channels, resulting eventually in sufficient K^+ flux. Alternatively, the cell could be hyperpolarized by slowly activating, voltage-gated K^+ channels.

Role of tonic inhibitory drive

The neurons of the DCN receive large amounts of inhibitory drive, as they are the sole target of Purkinje neurons. In addition, they receive inhibitory drive from local interneurons. It has been shown that Purkinje cell inputs can exert a powerful control over DCN cell activity and affect spontaneous firing properties (Mouginot and Gähwiler 1995). We examined the role of inhibitory drive in regulating the RD and consequently the propensity of the DCN cells to fire spike bursts. Bath application of the $GABA_A$ antagonist bicuculline methiodide (BMI, 20 μM, $n = 7$) caused a substantial increase in the excitability of the cell (Fig. 10*A*). On average, BMI caused an approximately threefold increase in the firing rate produced by a depolarizing pulse, relative to control levels. Peak frequencies of $\leq$370 spikes/s were achieved in one particularly dramatic case. It also caused a substantial enhancement of the RD, increasing the firing rate, on average, by approximately sevenfold, achieving frequencies >200 spikes/s. In addition, BMI caused five of seven cells to fire high-frequency plateaus much like those seen with apamin (compare Figs. 10*A* and 8*B*). Spontaneous bursts were seen in four of seven BMI-treated cells (Fig. 10*B*). One might suspect initially that tonic inhibition is playing a strong modulatory role on the intrinsic firing modes of DCN cells. This spontaneous bursting cannot be attributed to recurrent excitation because all experiments were performed with an ionotropic glutamate receptor antagonist in the bath. However, recent reports have indicated that quaternary salts of bicuculline, such as BMI, in addition to blocking $GABA_A$ receptors, also block SK channels (Debarbieux et al. 1998; Johnson and Seutin 1997; Seutin et al. 1998). In addition, it has been reported that other compounds containing at least one quaternary ammonium group are potent inhibitors of SK channels (Castle et al. 1993; Dunn et al. 1996). This possibly could explain the apamin-like effect caused by BMI application.. To address this potential complication, we tested the effects of two other $GABA_A$ antagonists, picrotoxin (200 μM, $n = 3$) and gabazine (100 μM, $n = 4$). Although both antagonists caused an increase in firing rate in response to a depolarizing pulse (~1.5-fold) and a more substantial enhancement of RD-driven spiking (approximately threefold), neither drug induced plateaus nor spontaneous bursts (Fig. 10, *C* and *D*). This suggests that although there is some degree of tonic synaptic inhibition in the slice, blocking the inhibition does not change the firing mode of DCN cells. Therefore the effect of BMI on DCN cell firing properties can likely be attributed to blockade of SK channels. In addition, this observation strengthens the view that DCN cells can spontaneously burst using purely intrinsic mechanisms, as these cells are isolated both from excitatory (kynurenate) and inhibitory inputs (BMI).

DISCUSSION

In summary, our data show that the spontaneous firing pattern of DCN neurons can range from regular spiking, in

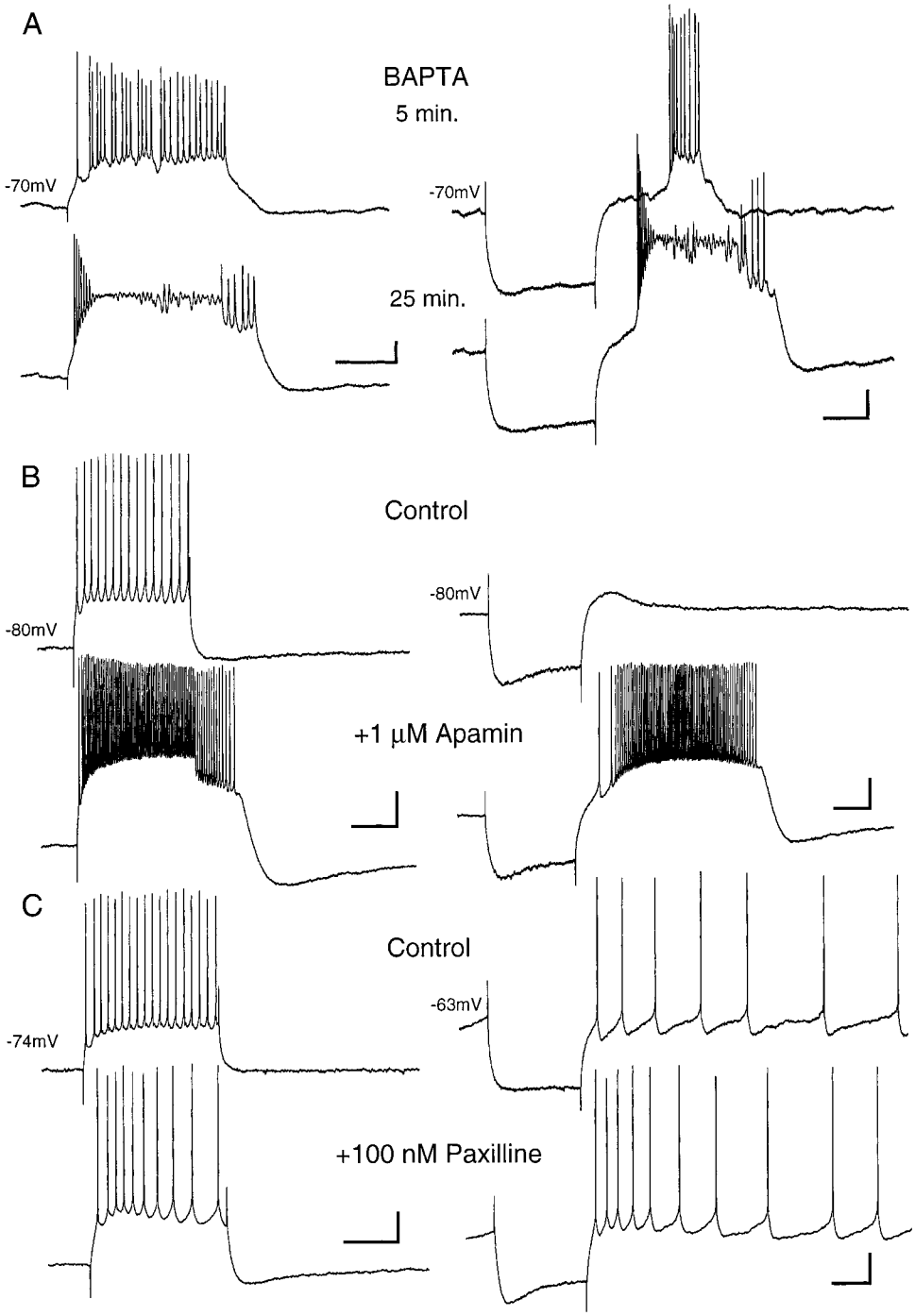

FIG. 8. Modulation of the RD by Ca^{2+}-dependent K^+ channels. *A*: responses to a depolarizing (0.3 nA, *left*) and a hyperpolarizing (−0.5 nA, *right*) pulse measured 5 and 25 min after impalement with a bis-(*o*-aminophenoxy)-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA)-containing microelectrode. Note the enhancement of the RD and the induction of plateau potentials and high-frequency spiking after 25 min. *B*: responses to a depolarizing (0.4 nA, *left traces*) and a hyperpolarizing pulse (−0.5 nA, *top right*, and −0.3 nA, *bottom right*) before and after bath application of 1 μM apamin, an SK channel blocker. Apamin also enhanced the RD, and plateau potentials were present. In contrast to the effect of BAPTA, apamin produced plateau potentials that were not accompanied by significant spike accommodation. *C*: responses to a depolarizing and hyperpolarizing pulse (0.5 and −0.5 nA, respectively) were measured before and after bath application of 100 nM paxilline, a BK channel blocker. No plateau potentials were evoked. To suppress spontaneous spiking, the cells were tonically hyperpolarized below spike threshold. All scale bars are 10 mV and 100 ms. These traces were representative examples from a pool of 8 cells tested in the case of BAPTA, 4 for apamin and 3 for paxilline.

which the rate depends on the membrane potential of the cell, to spontaneously bursting. Most cells that we recorded from had characteristics that more closely resembled regular spiking. DCN cells also exhibited a prominent RD, typically associated with a Na^+ spike burst, which could be elicited either by the offset of a hyperpolarizing pulse or by a train of IPSPs. IPSPs were markedly more effective in eliciting a RD than was a hyperpolarizing pulse of similar magnitude. The properties of RD induction, such as the voltage and time dependence, transient duration, and resistance to Na channel blockade, are consistent with mediation by T-type Ca^{2+} channels. In addition, there is some evidence that hyperpolarization-activated current I_h may play a role in boosting subthreshold RDs. There was a strong modulation of the amplitude and duration of the RD by SK channels. Blockade of these by apamin, or by intracellular Ca^{2+} chelation with BAPTA, resulted in a robust enhancement of the RD and caused regular spiking cells to display spontaneous bursts. Although an enhancement in firing frequency was observed after blockade of $GABA_A$ receptors, this did not cause the cells to burst spontaneously.

Are there multiple cell types in the present DCN recordings?

It is interesting to consider the question of whether the variability we see in the RD and in the propensity to burst

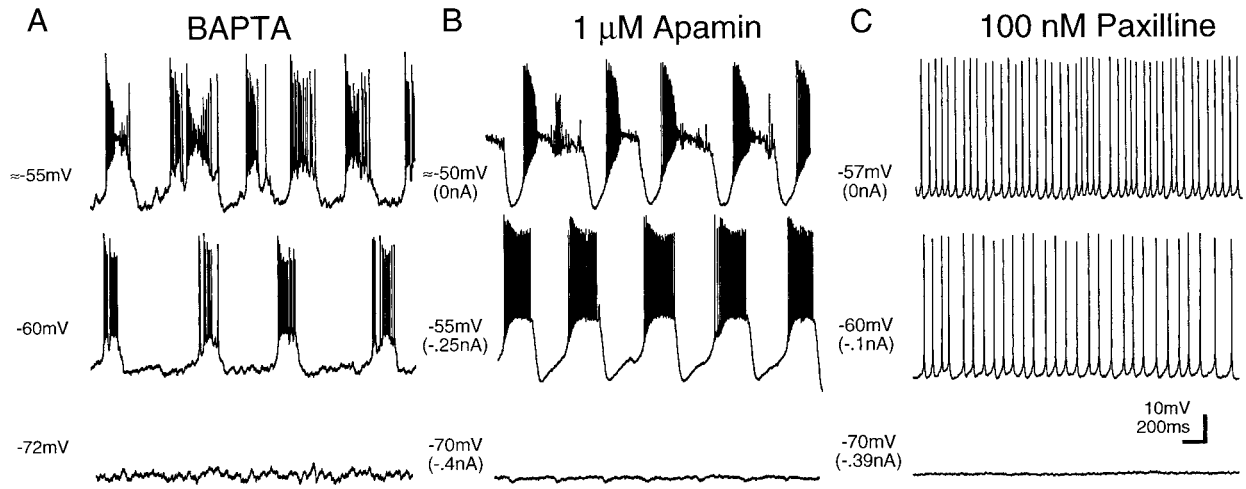

FIG. 9. Modulation of spontaneous spike firing by Ca^{2+}-gated K^+ channels. Effects of intracellular perfusion of BAPTA (*A;* 5 and 25 min after impalement) and bath application of apamin (*B*) and paxilline (*C*) on the spontaneous firing mode of DCN cells. Traces were collected while the cell was held a different membrane potentials with continuous injection of hyperpolarizing current. All cells showed regular spiking at the start of the experiment. Both BAPTA and apamin, but not paxilline, shifted the cells into a spontaneously bursting mode.

reflects a difference in cell type or whether we are recording from a single cell type but under different modulatory influences. Anatomic data suggest that there are three populations of neurons in the DCN, the small neurons that are GABAergic and send projections to the inferior olive, small GABAergic local interneurons as well as somewhat larger glutamatergic neurons, which project to premotor areas (see Chan-Palay 1977; Voogd et al. 1996 for review). Because our recordings were performed "blind," it is possible that we have recorded from multiple cell types. The predominance of regular spiking cells in our sample may reflect a bias toward the large cells, which we are presumably more likely to successfully impale. At present, there is no evidence that the different cell types of the DCN have different electrophysiological characteristics. Another possibility is suggested by the present observation that bath application of apamin or BMI, or intracellular perfusion with BAPTA, could shift the firing mode of a regular spiking cell into a spontaneous burst mode. This suggests that the variability we observed may depend on the different modulatory states of the cells encountered rather than morphologically distinct populations of cells. We currently are filling, staining, and reconstructing microelectrode-recorded DCN cells to address this issue directly.

Modulation of the RD

The present data suggest that there is a relationship between the strength of an evoked RD and the tendency of the cells to burst spontaneously. This suggests that factors that modulate the RD therefore can play an important role in defining the spiking pattern of the cell. We have identified several of these factors. First, we have shown that trains of IPSPs can be highly effective in eliciting a RD. Indeed, the anatomic distribution of Purkinje cell inputs, which have been shown to densely innervate the cell soma and principal dendrites of DCN neurons (Chan-Palay 1977), is highly favorable to causing a cell-wide change in membrane potential in response to IPSPs. Thus inhibitory drive can modulate the induction of a RD in several ways. One way is illustrated by the fact that IPSP trains are significantly more effective in driving a RD as compared with a hyperpolarizing current pulse injected at a single point source through the recording electrode (Fig. 7). Changes in the amplitude and duration of inhibitory inputs will evoke RDs of different sizes by removing different amounts of T-channel inactivation. In addition, tonic inhibitory drive can regulate the membrane potential of the DCN cell, making it more or less likely to exhibit a RD depending on its level of depolarization. This is supported by the observation that pharmacological blockade of $GABA_A$ receptors leads to an increase in cell excitability (Fig. 10) (see also Mouginot and Gähwiler 1995). These observations, taken together with evidence that inhibitory Purkinje cell synapses can undergo activity-dependent long-term depression and long-term potentiation (Aizenman et al. 1998), suggest that plasticity at this synapse may play a homeostatic role in controlling levels of cell excitability, helping to normalize the output levels of the cell. Previous work has shown that large amounts of postsynaptic spiking will cause a sustained potentiation of Purkinje cell-DCN IPSPs (Aizenman et al. 1998), perhaps making the cell more tonically inhibited and less likely to fire action potentials. Conversely, smaller amounts of postsynaptic activity will induce a sustained depression of IPSPs, presumably making the cell less inhibited and more likely to spike. Thus the regulation of synaptic efficacy by postsynaptic spiking may serve, in turn, to regulate the spiking itself.

Another factor that can modulate the RD is activation of Ca^{2+}-gated K^+ channels. Intracellular perfusion of BAPTA caused previously regular-spiking cells to exhibit spontaneous bursts. This effect was mimicked by blockade of SK, apamin-sensitive K^+ channels. These channels underlie I_{ahp}, which follows an action potential and is critical in determining the firing rate of a neuron (Sah 1996). Blockade of the large-conductance (BK) channels does not have the same effect, probably because they do not significantly contribute to the afterhyperpolarizing potential that follows a Na^+ spike (Sah 1996). Thus our data suggest that the DCN cells are under tonic modulation by a Ca^{2+}-gated K^+ current, which is being intermittently but frequently activated by an influx of Ca^{2+} ions,

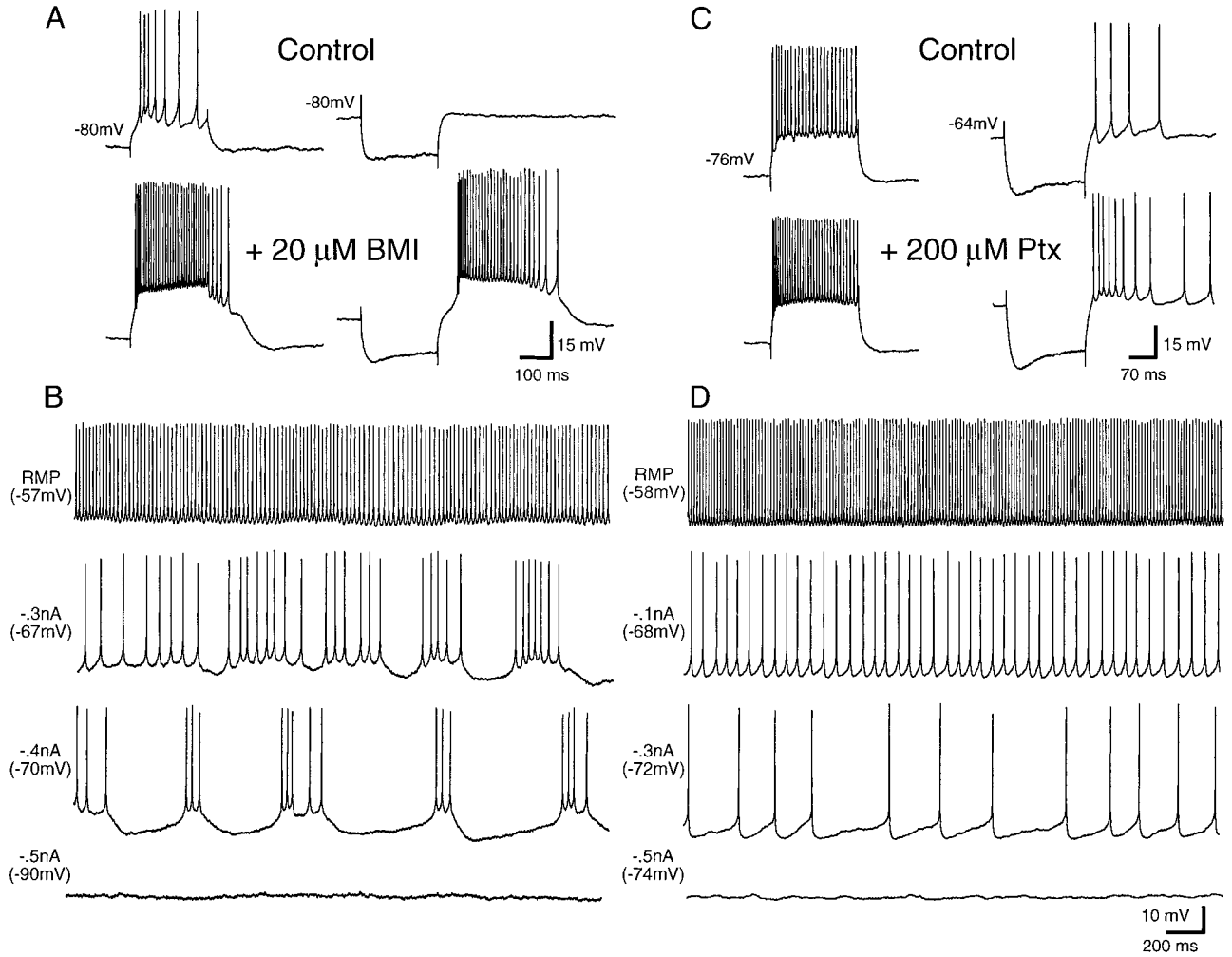

FIG. 10. Role of tonic inhibitory drive in modulating the RD and spontaneous spike firing. *A*: response to a depolarizing (*left*) and a hyperpolarizing (*right*) pulse before (*top*) and after (*bottom*) bath application of bicuculline methiodide (BMI; 20 μM), a $GABA_A$ receptor antagonist. Note the robust enhancement of the RD and of spiking frequency, as well as the induction of plateau potentials. *B*: effect of BMI on spontaneous spike firing at different membrane potentials. Like apamin and BAPTA, BMI converted a cell from regular spiking to bursting mode. *C*: effect of picrotoxin (200 μM), a different $GABA_A$ receptor antagonist, on the responses to current pulses. Layout is the same as in *A*. Picrotoxin causes an enhancement in spiking frequency, but no plateau potentials. *D*: effects of picrotoxin on the spontaneous spiking of a DCN cell. Note that picrotoxin causes no bursting or oscillations, suggesting that the effect of BMI may be due to its nonspecific blockade of Ca^{2+}- gated K^+ channels (see text). These traces were representative examples from a pool of 7 cells tested in the case of BMI and 3 for picrotoxin. Cells shown in *A* and *C* were tonically hyperpolarized to supress spontaneous spiking.

either via RD-driven T-type Ca^{2+} channels or by high-threshold voltage-gated Ca^{2+} channels activated by spontaneous or RD-evoked action potentials. Ca^{2+}-gated K^+ channels have been shown to be modulated by several different neurotransmitter types via various second-messenger cascades (Müller et al. 1992; Nicoll 1988; Pedarzani and Storm 1993). Thus they may be possible targets for modulation by serotonergic, noradrenergic, dopaminergic, and peptidergic fibers innervating the DCN (Voogd 1996). In addition, calmodulin, which is required for the Ca^{2+} gating of SK channels (Xia et al. 1992), may itself be subject to modulation by calmodulin-binding proteins such as MARCKS, GAP-43, and Ca^{2+}/calmodulin-dependent protein kinases. The observation that Ca^{2+}-gated K^+ currents modulate spontaneous spiking also has been observed in other cell types (Llinás and Suguimori 1980a,b; Llinás and Yarom 1981). It is important to note that analysis of the contribution of specific Ca^{2+} channel subtypes to the RD, using the present recording techniques, is complicated by the dual contribution of Ca^{2+} to both the initiation and in the termination of the RD. Pharmacological agents that partially reduce Ca^{2+} flux into the cell will result on the one hand in a reduced depolarizing current but also in an enhanced RD due to reduced activation of Ca^{2+}-gated K^+ channels.

A third factor that may affect the RD is modulation of the T-type Ca^{2+} channels and the channels mediating I_h that underlie its induction. T channels have been shown to be modulated by acetylcholine, serotonin, dopamine, norepinephrine, and some peptides, as well as by protein kinase C (see Huguenard 1996; Linden and Routtenberg 1989). I_h has been shown to be strongly regulated by $[Ca^{2+}]_i$ (Lüthi and McCormick 1998) as well as by various neurotransmitters (Pape 1996). Some evidence supporting the presence of I_h in our recordings is the fact that a sustained sag in the membrane voltage deflection was observed during the administration of hyperpo-

larizing current pulses. This sag was partially suppressed by Cs^+ and by QX-314, both of which are known to block I_h. Interestingly, in the cells filled with QX-314, the membrane voltage at which the RD was maximally evoked was slightly shifted (from -65 ± 4 mV to -59 ± 4 mV), suggesting that I_h may be boosting the induction of the RD. Finally, the BAPTA- and apamin-induced plateaus may be carried in part by noninactivating Na^+ currents previously described in DCN cells (Gardette et al. 1985b; Jahnsen 1986b; Llinás and Mühlethaler 1988). This conductance also is subject to modulation by various second messengers and G proteins (Crill 1996).

Our results support the recent observations that BMI causes a significant blockade of apamin-sensitive, Ca^{2+}-gated K^+ channels (Debarbieux et al. 1998; Johnson and Seutin 1997; Seutin et al. 1998). Because this highly water soluble form of bicuculline often is used to block $GABA_A$ receptors, it is very easy to confuse the effects of reduced inhibition with the effects of blocking apamin-sensitive K+ channels, making any interpretation ambiguous because both will result in increased neuronal excitability. Thus we suggest that BMI is not a useful drug for studying $GABA_A$ receptor function and that other more specific compounds should be considered (e.g., picrotoxin, gabazine, bicuculline free-base).

Function of the RD

The RD endows the DCN neurons with several interesting computational properties. For example, the RD might allow the DCN cells to distinguish between different types of inputs received by the cerebellum. Although the continuous barrage of parallel fiber inputs to Purkinje cells, which is translated as simple spikes (see Ito 1984), may present itself to the DCN as a tonic inhibitory drive; a climbing fiber input arriving at the Purkinje cell will cause it to fire a complex spike and be presented to the DCN as a brief, high-frequency burst of IPSPs followed by a pause (see Ito 1984), which then will elicit a RD. Thus because of its intrinsic membrane properties, a DCN cell may be able to distinguish between climbing fiber and mossy fiber input to the Purkinje cells and modify its output accordingly.

RDs are by no means unique to DCN neurons. In fact they are known to be critical in controlling the spiking patterns of several types of neurons exhibiting rhythmic behavior. Thalamic relay neurons, for example, fire a RD in response to IPSPs originating in the perigeniculate nucleus, which then causes them to feed back inputs to this nucleus and thus generate a pacemaker type circuit (Bal et al. 1995). Indeed, inferior olivary neurons as well as Purkinje cells, both of which are interconnected with the DCN, have been shown to exhibit RDs, and fire rhythmic bursts of action potentials (Llinás and Sugimori 1980a,b; Llinás and Yarom 1981). This circuit has been proposed to underlie different types of coordinated motor behavior and tremor (Llinás 1985a,b). In addition, there is a striking similarity between the bursting capabilities of DCN cells and B-type cells of the medial vestibular nuclei (MVN), which are the analogous cell type in the vestibulo-cerebellum. Both express low-threshold spikes (or RDs), in both cases a small population was seen to burst spontaneously, and both have a high propensity to burst in the presence of apamin (de Waele et al. 1993; Serafin et al. 1991a,b). This rhythmic behavior in MVN cells has been proposed to be important in controlling certain types of eye movements. It remains to be determined whether intrinsic bursting plays an analogous role in a DCN-dependent motor behavior.

A third role for the RD in the DCN may be important for activity-dependent synaptic plasticity at this site. A computational model for eye-blink conditioning has been proposed where the RD acts as a type of Hebbian-style coincidence detector, which can compare the timing between excitatory and inhibitory inputs and induce changes in the excitatory drive accordingly (Mauk and Donegan 1997). Thus some of the learned behavior can be stored in the inputs to the DCN instead of exclusively occurring in the cerebellar cortex. In addition, there is experimental evidence that shows that the degree of RD-driven spiking determines the polarity of synaptic plasticity at the Purkinje cell to DCN inhibitory synapse (Aizenman et al. 1998). Thus it is possible that the spiking patterns of the DCN may act as a framework within which excitatory and inhibitory inputs arrive and are modified accordingly.

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