Ionic Factors Governing Rebound Burst Phenotype in Rat Deep Cerebellar Neurons

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Molineux ML, Mehaffey WH, Tadayonnejad R, Anderson D, Tennent AF, Turner RW. Ionic factors governing rebound burst phenotype in rat deep cerebellar neurons. J Neurophysiol 100: 2684–2701, 2008. First published September 3, 2008; doi:10.1152/jn.90427.2008. Large diameter cells in rat deep cerebellar nuclei (DCN) can be distinguished according to the generation of a transient or weak rebound burst and the expression of T-type Ca2+ channel isoforms. We studied the ionic basis for the distinction in burst phenotypes in rat DCN cells in vitro. Following a hyperpolarization, transient burst cells generated a high-frequency spike burst of ≤450 Hz, whereas weak burst cells generated a lower-frequency increase (<140 Hz). Both cell types expressed a low voltage-activated (LVA) Ca2+ current near threshold for rebound burst discharge (~50 mV) that was consistent with T-type Ca2+ current, but on average 7 times more current was recorded in transient burst cells. The number and frequency of spikes in rebound bursts was tightly correlated with the peak Ca2+ current at ~50 mV, showing a direct relationship between the availability of LVA Ca2+ current and spike output. Transient burst cells exhibited a larger spike depolarizing afterpotential that was insensitive to blockers of voltage-gated Na+ or Ca2+ channels. In comparison, weak burst cells exhibited larger afterhyperpolarizations (AHPs) that reduced cell excitability and rebound spike output. The sensitivity of AHPs to Ca2+ channel blockers suggests that both LVA and high voltage-activated (HVA) Ca2+ channels trigger AHPs in weak burst compared with only HVA Ca2+ channels in transient burst cells. The two burst phenotypes in rat DCN cells thus derive in part from a difference in the availability of LVA Ca2+ current and spike output. Transient burst cells expressed Cav3.1 channel isoform and were putative excitatory neurons, whereas weak burst cells expressed the Cav3.3 channel isoform and are putative excitatory (non-GABAergic) cells (Molineux et al. 2006).

INTRODUCTION

The cerebellum is a highly organized brain structure responsible for the coordination, learning, and timing of motor movements. The neurons of the deep cerebellar nuclei (DCN) provide the last stage of cerebellar information processing by integrating an array of excitatory and inhibitory sensory motor input. The dominant projection to the DCN is an inhibitory input from axons of Purkinje cells in the cerebellar cortex (Chan-Palay 1977; Ito et al. 1970; Teune et al. 1998). The DCN also receives lateral inhibitory inputs from the two major excitatory inputs to the cerebellum: the mossy fibers and climbing fibers (Eller and Chan-Palay 1976; Kitai et al. 1977; Shinoda et al. 2000). As the sole output of the cerebellum, all neural processing accomplished by the cerebellum is filtered through the firing dynamics of DCN neurons before their projection onto numerous centers throughout the brain stem and thalamus (Teune et al. 2000).

Morphologically, the neurons of the rat DCN have been distinguished according to a large or small soma diameter, presumably representing projection neurons or local GABAergic interneurons, respectively (Czubayko et al. 2001; Sultan et al. 2003). A spontaneous, tonic discharge is present in DCN neurons at rest that is independent of synaptic input (Raman et al. 2000). Alternatively, DCN cells can fire bursts of action potentials that can be correlated with specific movements (Chen and Evinger 2006; Ohtsuka and Noda 1991, 1992; Raman et al. 2000). The ability to generate a rebound depolarization in vitro can show considerable variability but has been reported for large diameter cells (Aizenman and Linden 1999; Czubayko et al. 2001; Jahnsen 1986). The rebound depolarization involves low voltage-activated (LVA) T-type (Cav3.x) Ca2+ channels, allowing a sufficient hyperpolarization to deinactivate Cav3 channels and on release generate a rebound depolarization and burst of action potentials (Aizenman and Linden 1999; Czubayko et al. 2001; Llinas and Muhlethaler 1988). We recently distinguished two distinct phenotypes of large diameter DCN cells based on the properties of rebound bursts (transient burst or weak burst) and their expression of specific Cav3 Ca2+ channel isoforms (Molineux et al. 2006). The rebound depolarization of transient burst neurons is characterized by a brief high-frequency burst of action potentials followed by a return to tonic firing over ~1 s, whereas weak burst cells produce a lower-frequency burst of spikes that can persist for several seconds. Transient burst neurons express Cav3.1 Ca2+ channels and can correspond to either GABAergic or non-GABAergic cells, whereas weak burst cells express the Cav3.3 channel isoform and are putative excitatory (non-GABAergic) cells (Molineux et al. 2006).

The role(s) for rebound burst discharge in DCN neurons has not been fully elucidated. In vivo recordings have begun to identify DCN cells with different firing patterns that discharge with respect to specific aspects of the eyelid blink response or saccadic eye movements (Chen and Evinger 2006; Ohtsuka and Noda 1991, 1992). Some theories of cerebellar function also depend on DCN rebound spiking to drive reverberating loops that have implications to the acquisition and timing of motor movement (Kistler and De Zeeuw 2003; Kistler et al. 2000). Studies in vitro have further shown that plasticity of Purkinje cell inhibitory postsynaptic potentials (IPSPs) and of mossy fiber input depends on rebound depolarizations in DCN neurons (Aizenman et al. 1998; Pugh and Raman 2006). The distinct burst phenotypes apparent in DCN cells will therefore

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govern many important responses to synaptic input and determine their role in cerebellar output.

Our previous tests uncovered differences between transient and weak burst cells in terms of the contribution of Ca\(^{2+}\) spike responses to the rebound depolarization (Molineux et al. 2006). Specifically, when recorded in the presence of blockers of Na\(^{+}\) and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, only the transient burst cell type routinely exhibits an active Ca\(^{2+}\) spike response as part of the rebound from a hyperpolarization. Nonetheless, both cell types proved capable of generating Ca\(^{2+}\) spikes during the rebound in the presence of K\(^{+}\) channel blockers. The dramatic differences in rebound spiking characteristics between cells may reflect differences in the underlying Cav3 current or their interplay with voltage- and/or Ca\(^{2+}\)-activated K\(^{+}\) (K\(_{Ca}\)) channels. This study used patch-clamp recordings in rat cerebellar slices to identify the ionic basis of rebound burst firing behavior between cells exhibiting either a transient or weak burst phenotype. We found that the two burst phenotypes derive primarily from differences in the availability of LVA Ca\(^{2+}\) current near threshold for the rebound response and the influence of two families of K\(_{Ca}\) channels underlying spike afterhyperpolarizations (AHPs).

**METHODS**

**Animal care**

Sprague-Dawley rats over the range of P14–P20 were obtained from Charles River, and procedures were conducted according to guidelines approved by the local Animal Care Committee and the Canadian Council for Animal Care.

**Electrophysiology**

All chemicals were obtained from Sigma (St. Louis, MO) unless otherwise noted. Whole cell current-clamp recordings were obtained using the Axoclamp 2A, 700A, or 700B amplifiers (Axon Instruments, Sunnyvale, CA) and whole cell voltage-clamp recordings with the Axoclamp 700B. Data were collected and digitized at 20 kHz and filtered at 10 kHz using a Digidata 1322A and pClamp 9 or 8 software (Axon Instruments). Tissue slices of cerebellum were prepared as detailed in Molineux et al. (2006). Briefly, artificial cerebrospinal fluid (ACSF) was composed of (in mM) 125 NaCl, 3.25 KCl, 1.5 CaCl\(_2\), 1.5 MgCl\(_2\), 25 NaHCO\(_3\), and 25 d-glucose preoxygenated with carbogen (95% O\(_2\)-5% CO\(_2\)) gas. Rats were anesthetized with pentobarbital sodium (MTC Pharmaceuticals, Cambridge, Ontario, Canada), and procedures were conducted according to guidelines approved by the local Animal Care Committee and the Canadian Council for Animal Care.

**Data analysis**

Analysis of electrophysiological data were accomplished using custom software written in MatLab R2006a (MathWorks, Natick, MA). Spike threshold was determined through analysis of the voltage derivative and all other spike parameters measured relative to spike threshold. Voltage-clamp analysis was restricted to determining the amplitude and peak latency of inward current evoked by a single step to −50 mV from a holding potential of −90 mV. Any residual capacitance artifact was removed digitally by subtracting inverted and scaled transients. Spike parameters, such as frequency, spike width, fast AHP (IAHP), depth, etc., were measured using 5 s of uninterrupted spiking at −14 Hz for tonic data or the average of the first five spikes for rebound burst data. A calculated junction potential of 11 mV was subtracted from all current-clamp recordings. In voltage-clamp recordings, the junction potential was not subtracted because the value was small (−2 mV) for the internal KCl solution. Significant differences were assessed using one- or two-way ANOVA using Tukey’s honestly significant difference criterion. Observed versus expected frequencies were assessed using a χ\(^2\) test. Averaged data are presented as means ± SE, and statistical significance was assessed at P < 0.05.
**Immunocytochemistry**

Rats were deeply anesthetized with an overdose of pentobarbital sodium and perfused intracardially with 250 ml of 0.1 M phosphate-buffer (PB, pH 7.4) followed by 100 ml of 4% paraformaldehyde (PARA, pH 7.4) at room temperature. Brains were postfixed in 4% PARA at room temperature for 1 h and overnight at 4°C. Free-floating 30 to 40 µm sections were cut by vibratome in ice-cold PB and transferred to PB at room temperature. To identify cells filled with neurobiotin during recordings, slices were immediately transferred to a 4% PARA solution for ≥1 h at room temperature. All sections were transferred to a working solution consisting of 3% normal donkey or horse serum (Jackson Immuno-Research, West Grove, PA), 0.1% TWEEN, and 1% DMSO in PB, with gentle agitation throughout all reactions. Primary antibodies were added to the working solution [Kv3.1 (1:330), Kv3.3 (1:500), K_v,2.1 (1:300), K_v,2.2 (1:300), and K_v,1.1 (1:1,000); Alomone Labs, Jerusalem, Israel] and reacted for 48 h at 4°C and washed in working solution three times for 15 min. Monoclonal antibodies to microtubule-associated protein (MAP-2; 1: 500) were further used as a counterlabel to identify cell structure. Secondary antibodies consisted of Alexa Fluor 488–conjugated donkey anti-mouse IgG (1:1,000) or Cy3-conjugated donkey anti-rabbit IgG (1:1,000) (Molecular Probes, Eugene, OR) incubated for 4 h at room temperature. After washing in PB, sections were mounted on gel-coated slides, coverslipped with anti-fade medium, and stored at −20°C. Controls consisted of omitting the primary antibodies. Immunoreactivity was assessed using an Olympus BH-2 research microscope or an Olympus FV300 BX50 confocal microscope. Images were first processed using Fluoview software (Olympus America, Melville, NY) and transferred to Adobe Photoshop and Illustrator for figure preparation. All image adjustments were confined to brightness/contrast and intensity levels.

**RESULTS**

**Electrophysiological characteristics define two burst phenotypes in large diameter DCN neurons**

Previous physiological studies distinguished two types of DCN neurons according to a large or small somatic diameter and firing patterns (Czubayko et al. 2001). The large diameter cell type was assumed to correspond predominantly to glutamatergic projection neurons (type I) and the smaller type to local GABAergic interneurons (type II) (Sultan et al. 2003). It is recognized that both large and small diameter cell populations include non-GABAergic and GABAergic cells (McMahon and Joho 2002; Molineux et al. 2006; Uusisaari et al. 2007). It was later shown that the large diameter cells in rat cerebellar nuclei could be distinguished on the basis of two distinct rebound burst phenotypes (Molineux et al. 2006). A transient burst phenotype corresponds to either GABAergic or non-GABAergic large diameter cells, whereas a weak burst phenotype was only identified in non-GABAergic (putative excitatory) cells. A recent study in a green fluorescent protein (GFP) transgenic mouse line carried out an extensive analysis of DCN cell firing properties with respect to the expression pattern of GAD-GFP (Uusisaari et al. 2007). This study attributed specific spike shapes and firing patterns to GAD⁺ and GAD⁻ cells. In particular, it was reported that spike shape and a fAHP or slow AHP (sAHP) could be used to distinguish between GAD⁺ and GAD⁻, with all GAD⁺ cells exhibiting both a fAHP and sAHP. The presence of a fAHP and sAHP in all of our recorded cells is consistent with the classification of Uusisaari et al. (2007) in representing a population of large diameter GAD⁻ cells. An attempt to identify other distinguish-

![Figure 1](http://jn.physiology.org/)

**FIG. 1.** A comparison of spike properties and key morphological characteristics between large diameter cells exhibiting either a transient or weak burst phenotype in rat deep cerebellar nuclei (DCN). A and B: histogram plots (A) and mean values of spike amplitude and halfwidths (B) of spikes recorded during tonic discharge at a resting state of 14 Hz. A high degree of overlap in these spike properties prevent them from being used to distinguish between cells exhibiting either burst phenotype or between non-GABAergic and GABAergic cell types within the transient burst cell population. C: mean values of the number of dendritic branches leaving the soma and somatic area measured in cells recorded in vitro and filled with neurobiotin for subsequent histological analysis. Control spike parameters were calculated from populations of transient (n = 47) and weak burst cells (n = 62) in this and all subsequent figures. Values for dendritic branching and somatic area are drawn from a population of neurobiotin-filled and labeled transient (n = 34) and weak burst cells (n = 45).
Therefore we recorded from 284 large diameter cells from the three DCN nuclei that we expect to be comprised primarily of non-GABAAergic and presumed projection neurons. An extensive comparison of cell properties across nuclei has found that for the initial rebound response the difference in rebound burst frequency is always maintained at a level 8–20 times greater for transient than weak burst cells. No changes are found for a host of membrane properties or spike responses over the developmental time frame examined here (P14–P20). We therefore pooled all recordings according to burst phenotype to focus on the underlying ionic basis of rebound discharge. As described below, we find that several differences in rebound discharge and spike repolarization reflect essential differences in the ionic basis for transient or weak burst firing patterns.

Rebound discharge differs between transient and weak burst cells

DCN cells are known to fire action potentials spontaneously both in vitro and in vivo (Llinas and Muhlethaler 1988; Thach 1970). Recordings taken in on cell voltage-clamp mode and whole cell current-clamp mode with no applied bias current showed that, under our recording conditions, the tonic spike frequency for DCN neurons was ~14 Hz, consistent with other observations in on cell mode in mouse and rat DCN in vitro (Alvina and Khodakhah 2008; Uusisaari et al. 2007). For consistency, we adjusted each cell to this frequency using small amounts (<60 pA) of amplifier bias current (see METHODS). To distinguish between cells exhibiting either burst phenotype, we used a hyperpolarizing current pulse to evoke a membrane potential shift to approximately ~90 mV for 1 s to ensure availability of any inactivating channels. On release from the hyperpolarizing influence, a rebound depolarization was generated that allowed us to distinguish the burst phenotype.

Transient burst cells responded immediately after a membrane hyperpolarization with a brief high-frequency burst of two to six action potentials at frequencies that ranged from 169 to 448 Hz (average of 234 ± 14 Hz; n = 47). Spike frequency during the rebound burst in transient burst cells was thus significantly higher than either the tonic firing or rebound burst frequency of any other cell type (Fig. 2, A–C). The rebound burst in these cells was usually followed by a brief pause before gradually returning to the tonic level of spiking over the course of ~1.5 s. In contrast, cells exhibiting a weak burst never displayed a discrete high-frequency or transient rebound burst phase. Rather, these cells responded with a modest frequency increase of 37 ± 3 Hz (n = 62) that was significantly different from the original tonic firing frequency (14.3 ± 1 Hz, P < 0.05; Fig. 2, B and C), and significantly slower than rebound bursts of transient burst cells (Fig. 2D). Weak burst cells showed some variability in peak spike frequency and burst duration during a rebound, with a frequency increase of ≤137 Hz (n = 62) above the tonic firing rate (Fig. 2, A–C). A comparison between a transient burst cell and three representative weak burst cells is shown in Fig. 2, A and B. The differences in initial spike frequency are seen more clearly by plotting the instantaneous frequency before and after a hyperpolarizing step sufficient to induce an intense burst in a representative transient burst cell (Fig. 2C). We could find no justification for separating the weak burst cells further at this time, because there were no statistically significant differences between any other spike parameters (i.e., spike height, half-width or threshold, fAHP depth, or sAHP depth; data not shown). We therefore included all weak burst cells in a single group for analysis, yet recognize that differences may be identified in future studies to distinguish between cells exhibiting this burst phenotype. It is important to note that the elevated rebound spike frequency in transient burst cells also slowly decayed to baseline levels in a manner not unlike that of weak burst cells, yet typically within only ~1.5 s. This spike accommodation process could well arise through similar mechanisms between transient and weak burst cells but was not examined here.

LVA $\text{Ca}^{2+}$ current is of greater amplitude in transient than weak burst cells

Previous work established that large diameter DCN neurons express at least one of the $\text{Ca}_{3.3}$ T-type channel isoforms (McKay et al. 2006; Molineux et al. 2006). A combined approach of electrophysiology, cell fills, and immunocytochemistry established that transient burst neurons are specifically associated with the expression of $\text{Ca}_{3.1}$ and weak burst neurons with $\text{Ca}_{3.3}$. Furthermore, a low threshold $\text{Ca}_{2+}$-dependent rebound depolarization could be readily evoked in the presence of TTX in transient burst but not weak burst cells. However, when $\text{K}^+$ channels were globally blocked, both transient and weak burst cells were capable of exhibiting a low threshold $\text{Ca}_{2+}$-dependent depolarization during the rebound that was completely blocked with 1 mM Ni$^{2+}$ (Molineux et al. 2006). These results suggest that the difference in burst phenotypes is the result of either a difference in the density or voltage-dependent properties of $\text{Ca}_{3.3}$ channel isoforms or a difference in voltage- and/or $\text{Ca}_{2+}$-activated $\text{K}^+$ channels that are active during a rebound depolarization.

The procedure for examining LVA rebound current in most cases was to first assess the properties of the rebound spike burst in current-clamp mode and then bath perfuse TTX (200 nM) before switching to voltage clamp. In this way, we could first assess the properties of spike output and then test for the availability of LVA $\text{Ca}^{2+}$ current using a single step from ~90 to ~50 mV, a voltage equivalent to the threshold for rebound bursts in these cells. Voltage-clamp recordings were carried out at 33–35°C to allow direct comparisons of inward currents evoked at physiological temperatures. We note that this voltage protocol had the potential to recruit the hyperpolarization-activated current $I_h$ that is also expressed in large DCN cells. However, $I_h$ has a relatively high threshold for activation in DCN neurons (more than ~100 mV) and would be expected to be <10 pA at ~90 mV (Raman et al. 2000). In agreement with this, we found that the inward current evoked at ~50 mV was fast inactivating, suggesting that there is no contamination from $I_h$ in our protocol (Fig. 3A). Steps more depolarized than ~40 mV began to activate outward $\text{K}^+$ currents and eventually high-voltage-activated (HVA) $\text{Ca}^{2+}$ currents with a variability in activation that suggested deterioration of our voltage clamp at more depolarized potentials. Restricting our analysis to a single ~50 mV step potential thus provides a realistic estimate of the total LVA $\text{Ca}^{2+}$ current available at the threshold for burst discharge to examine possible differences in rebound...
Ca$^{2+}$ current available to drive a transient versus weak burst discharge.

These studies showed an LVA, fast activating and inactivating inward current in both transient and weak burst cells, but with differences in properties (Fig. 3A). The Ca$^{2+}$ current in transient burst cell types was faster activating in reaching a peak in $17 \pm 0.9$ ms ($n = 10$) compared with $23 \pm 2.8$ ms ($n = 11$, $P < 0.05$) in weak burst cells (Fig. 3, A and B). There was a prominent difference in the amplitude of the transient LVA current between cells of either phenotype. The Ca$^{2+}$ current expressed in transient burst cells at $-50$ mV was on average 7.4 times greater in peak amplitude than in weak burst cells, with an average of $806 \pm 63$ pA in transient burst cells ($n = 10$) compared with $108 \pm 17$ pA in weak burst cells ($n = 11$, $P < 0.05$; Fig. 3, A and B).

A separate set of experiments examined the sensitivity of LVA currents to Ca$^{2+}$ channel blockers using CsCl as the internal electrolyte in the presence of external TTX (200 nM) and CsCl (1 mM). Although rebound spike discharge could not be reasonably assessed under current clamp in these conditions, LVA currents again fell into two groups of less than $\sim 200$ pA ($n = 5$) and more than $\sim 400$ pA ($n = 3$); a distribution consistent with identified transient and weak burst phenotypes. Lee et al. (1999) have shown that Cav3 Ca$^{2+}$ channel isoforms exhibit a differential sensitivity to external Ni$^{2+}$, with Cav3.2 sensitive to concentrations as low as 100 $\mu$M, whereas the IC$_{50}$ for Cav3.3 is $\sim 300$ $\mu$M. Because our previous immunolabeling studies failed to identify an association of either of the two burst phenotypes with Cav3.2 expression, we chose to use 300 $\mu$M Ni$^{2+}$ to test for putative T-type channel contribution to the LVA current. Initial perfusion of 50 $\mu$M Cd$^{2+}$ to block HVA Ca$^{2+}$ channels had no significant effect on LVA current in either group (Fig. 3A, inset; $n = 8$), whereas subsequent perfusion of 300 $\mu$M Ni$^{2+}$ nearly abolished the transient current in all cases (Fig. 3A, inset; $n = 7$). We can also rule out the action of LVA Cav1.3 channels given previous work showing no effect of 5 $\mu$M nifedipine on the rebound Ca$^{2+}$ spike in the presence of TTX or rebound spike bursts in DCN cells (Molineux et al. 2006). The combination of low threshold for activation, fast activation

**FIG. 2.** Differences in rebound discharge properties can be distinguished in transient and weak burst DCN neurons. A and B: DCN neurons were grouped into 1 of 2 categories according to the properties of rebound discharge following a membrane hyperpolarization: (A) transient burst and (B, i–iii) weak burst cells. Each case shows a representative example of tonic spiking at rest (left column) and the rebound burst following a hyperpolarizing step to approximately $-90$ mV (right column). Three representative examples of weak burst cells are shown in B (i–iii) to show the variability in rebound frequencies that can be found among weak burst cells. C: plots of instantaneous spike frequency before and after the hyperpolarization for the cells shown in A and B. Spike numbers $-1$ to $-3$ refer to spikes immediately preceding the hyperpolarization and numbers 1–15 to spikes immediately after. Note the large difference in rebound spike frequency for transient burst compared with weak burst neurons. D: comparison of the average spike frequencies during tonic and rebound discharge in transient and weak burst cells.
The peak Ca²⁺ current of transient and weak burst cells and the frequency of the first five spikes in the immediate rebound phase was also highly correlated ($R = 0.95$; Fig. 3D).

It should be noted that these experiments cannot distinguish differences in the voltage dependence or expression density of LVA Ca²⁺ channels between transient and weak burst cells. Nor can they clarify the role of K⁺ currents or Ca²⁺ currents that will be active during the larger voltage excursion of the rebound spike burst. However, they do emphasize that the magnitude of LVA Ca²⁺ current available at burst threshold translates directly to a change in the pattern of rebound burst firing. The ability to generate strong rebound bursts in transient burst cells can thus be attributed in part to a faster activating and larger net LVA (T-type) Ca²⁺ current at burst threshold.

Afterpotential characteristics distinguish transient and weak burst cell types

We have previously determined that the lower frequency of rebound bursts in weak burst cells was caused at least in part by the activation of K⁺ channels, because blocking K⁺ channels consistently uncovered a Ca²⁺-sensitive rebound depolarization (Molineux et al. 2006). The difference in rebound burst capabilities between transient and weak burst cells should also reflect a difference in the expression of K⁺ channel subtypes. We thus compared the characteristics of spike repolarization and afterpotentials in transient and weak bursts neurons during tonic firing and after a 1 s membrane hyperpolarization to approximately −90 mV (Fig. 4). All spike parameter measurements were taken in reference to spike threshold, which was used to define the fAHP, DAP, and sAHP (Fig. 3A; see METHODS). We found no significant difference in the absolute spike threshold between transient and weak burst cell types (transient burst, −46 ± 0.7 mV; n = 47; weak burst, −45 ± 0.7 mV, n = 62, $P > 0.05$). Similarly, no significant differences were found between transient and weak burst cells for several other parameters, including input resistance, spike rate of rise, spike repolarization rate, or peak DAP latency (data not shown; see also Fig. 1).

A comparison of spike afterpotentials showed that repolarizing currents were stronger in weak burst cells in terms of a larger fAHP (−14.1 ± 0.6 mV; n = 62) than in transient burst cells (−6.7 ± 0.4 mV; $n = 47$; Fig. 4, A–C). This difference was maintained during the rebound burst phase, with the fAHP depth being more negative with respect to spike threshold in weak burst cells (−13.7 ± 0.6 mV; n = 62) than transient burst cells (−6.2 ± 0.6 mV; $n = 47$; Table 1). Therefore one distinguishing characteristic between the two burst phenotypes is the fAHP during either tonic or burst firing.

DAPs can be an important factor in promoting burst discharge during high-frequency firing in a variety of neurons (Azouz et al. 1996; Bourque et al. 1986; Fernandez et al. 2005; Stuart et al. 1997). Both transient and weak burst cell types had readily identifiable DAPs (Fig. 4, A and B). A comparison of the absolute amplitude of the DAP during tonic spiking (determined from the trough of the fAHP to the peak of the DAP) showed no significant difference between transient and weak burst cell types (Fig. 4D). However, the DAP depolarized the membrane much closer to spike threshold in transient compared to weak burst cells. Thus the peak of the DAP during tonic firing in transient burst cells was only −4.5 ± 0.4 mV...
The frequency of tonic spike firing in a neuron is often determined by the magnitude of the sAHP. We again found that the sAHP attained a larger amplitude and thus more hyperpolarized peak value during tonic firing in weak burst than in transient burst cells (weak burst sAHP amplitude, 20.5 ± 0.6 mV, n = 62; transient burst, 16.5 ± 0.4 mV, n = 47; Fig. 4E). The sAHP in transient burst cells could not be measured during rebound spiking (Fig. 4B). However, during the rebound spiking phase, the sAHP was reduced in weak burst cells to 15.7 ± 2.1 mV (n = 47) compared with tonic spiking (Fig. 4E). It cannot be resolved with the current information as to whether the decrease in sAHP during the rebound reflects a reduction of Ca\(^{2+}\)-mediated K\(^+\) current after membrane hyperpolarization or simply competition with the depolarization of the underlying rebound currents. In any case, these shifts likely determine, at least in part, the frequency of rebound discharge.

**DAP does not require voltage-gated Na\(^+\) or Ca\(^{2+}\) currents**

A DAP can arise from any of several active current sources that vary according to cell type, including Ca\(^{2+}\) (Jung et al. 2001; Metz et al. 2005; Nelson et al. 2005b; Yuste et al. 1994; Zhang et al. 1993), Na\(^+\) (Afshari et al. 2004; Azouz et al. 1996; Yue et al. 2005), Ca\(^{2+}\)-activated Cl\(^-\) (Martinez-Pinna et al. 2000), or Ca\(^{2+}\)-activated nonspecific cation current (Ghamari-Langroudi and Bourque 2002). Bursting neurons in the DCN have been shown to express sufficient LVA Ca\(^{2+}\) channels in dendritic regions to exhibit an increase in internal dendritic Ca\(^{2+}\) concentration during high-frequency Na\(^+\) spike bursts (Gauck et al. 2001). These findings would be consistent with an active backpropagation of Na\(^+\) spikes into dendrites and the subsequent activation of voltage-gated Ca\(^{2+}\) channels, as observed in other cells (Golding et al. 1999; Yuste et al. 1994). To test the Ca\(^{2+}\) dependence of the DAP, we bath-applied the broad-spectrum Ca\(^{2+}\) channel blockers Cd\(^{2+}\) (50 \(\mu\)M) and Ni\(^{2+}\) (1 mM) but found no effect on the DAP in either transient or weak burst neurons (Fig. 5, A and B; n = 12 and 16, respectively). These tests show that the DAP cannot be attributed to a depolarization arising through either LVA or HVA Ca\(^{2+}\) channels.

We next attempted to eliminate the DAP by selectively blocking dendritic Na\(^+\) channels. Given the multipolar orientation of DCN cell dendrites and axon, we could not adopt the approach of pressure ejecting a drug in the dendritic versus somatic region (Turner et al. 2002). Therefore we perfused all processes of a recorded cell by bath applying TTX (200 nM) while protecting the soma with a constant stream of control ACSF from a pressure electrode placed immediately adjacent to the soma. We established that this procedure could be effective for ~2 min in that the somatic Na\(^+\) spike amplitude and rate of rise remained stable, while stopping the pressure ejection allowed TTX in the bath medium to quickly block the spike. By bath applying TTX while protecting the soma, we could find no evidence for a block of the DAP while the somatic spike remained intact, suggesting that TTX-sensitive ion channels in dendritic or distal axonal membrane are not involved in generating the DAP. Eventually the TTX in the bath ACSF penetrated the protective stream of control ACSF perfusing the soma and slowly reduced the somatic Na\(^+\) spike amplitude (Fig. 5C). Under these condi-
tions, the amplitude of both the Na\(^+\) spike and DAP decreased in tandem, with evidence for a vestigial DAP even when spike amplitude had been reduced to a small prepotential. These effects were consistent for both transient and weak burst neurons (n = 6 and 5).

One explanation for the close association between the Na\(^+\) spike and DAP could be the activation of a slow Na\(^+\) current or the resurgent Na\(^+\) current that is blocked by flufenamic acid (Ghamari-Langroudi et al. 2002). We repeated this test in DCN cells and found no effect on the DAP under either control conditions or when using the spike-like transient waveform in 200 nM TTX (n = 3; data not shown). Therefore it seems that the DAP does not require voltage-gated Ca\(^{2+}\) or Na\(^+\) currents or the Ca\(^{2+}\)-activated currents we tested here. The primary remaining alternative is that the DAP is generated through a passive discharge of membrane capacitance following charging by the somatic Na\(^+\) spike (see DISCUSSION).

Expression of K\(^+\) channels for spike repolarization and AHPs

Maintaining a high frequency of firing or a rapid shift to a rebound burst of spikes depends on the expression of ion channels that regulate spike repolarization and AHPs (fAHP and sAHP). Spike repolarization and the fAHP are often mediated by the high-threshold class of Kv3 K\(^+\) channels or Ca\(^{2+}\)-dependent big conductance (BK) channels (Lu et al. 2006; McKay and Turner 2004; Rudy and McBain 2001). The sAHP is most often an apamin-sensitive Ca\(^{2+}\)-dependent small conductance (SK) channel or fAHP (Sah 1996). The identity of voltage-dependent Ca\(^{2+}\) channels that can functionally couple the entry of Ca\(^{2+}\) to BK or SK channel activation can vary substantially between cells (Berkefeld et al. 2006; Smith et al. 2002).

Previous studies have indicated the expression of Ca\(^{2+}\)-activated K\(^+\) channel family subunits BK, SK1, and SK2 in the DCN (Chang et al. 1997; Knaus et al. 1996; Sailer et al. 2004; Stocker and Pedarzani 2000). The cerebellum has also been reported to express members of the high-threshold K\(_{\text{Ca}}\) 3.1 K\(^+\) channel family (Chang et al. 2007; McMahon and Joho 2002). To determine the distribution of these ion channel types in large diameter DCN cells, we used antibodies directed against the delayed rectifier K\(^+\) channels K\(_{\text{Ca}}\) 3.1 and K\(_{\text{Ca}}\) 3.2, the inactivating K\(_{\text{Ca}}\) 3.3 and K\(_{\text{Ca}}\) 3.4, and the Ca\(^{2+}\)-activated K\(_{\text{Ca}}\) channels BK, SK1, and SK2 (Fig. 6). In general, immunolabel was evident in all three DCN nuclei. In all cases, omission of the primary antibody eliminated the immunolabel. The expression of two subtypes of the Kv3 K\(^+\) channel family are shown in Fig. 6, A and B, in relation to MAP-2 immunolabel that was used as a general cytoskeletal marker to visualize channel distribution over the soma and dendritic axis. These images show Kv3.1 and Kv3.3 immunolabel primarily over somatic membranes and the proximal 50 \(\mu\)m of dendrites of large diameter cells. Labeling was detected as a diffuse signal in the cytoplasmic region but also as a membrane-associated label that delineated the membranes of the soma and proximal dendrites. The dendritic label was distinct in the proximal region and appeared to dissipate rapidly beyond this point despite the ability to resolve extended dendritic segments in the MAP-2 immunolabel. However, some individual dendritic

### Table 1. Ionic basis of AHPs and rebound burst frequency in large DCN neurons

<table>
<thead>
<tr>
<th></th>
<th>Transient Burst</th>
<th>Weak Burst</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>fAHP Tonic Spiking, mV</td>
<td>fAHP Rebound Spiking, mV</td>
</tr>
<tr>
<td>Control</td>
<td>6.7 ± 0.4</td>
<td>6.2 ± 0.6</td>
</tr>
<tr>
<td>Ni(^{2+}) (1 mM)</td>
<td>4.3 ± 0.4</td>
<td>7.1 ± 1.6</td>
</tr>
<tr>
<td>Cd(^{2+}) (50 (\mu)M)</td>
<td>1.7 ± 0.7*</td>
<td>1.9 ± 1.0*</td>
</tr>
<tr>
<td>Ni(^{2+}) and Cd(^{2+})</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CBTX (100 mM)</td>
<td>1.5 ± 0.4*</td>
<td>1.4 ± 0.9*</td>
</tr>
<tr>
<td>MIB (1 (\mu)M)</td>
<td>7.7 ± 0.9</td>
<td>7.3 ± 0.9</td>
</tr>
<tr>
<td>TEA (500 (\mu)M)</td>
<td>−18 ± 4.7*</td>
<td>−5.2 ± 1.8*</td>
</tr>
<tr>
<td>APA (100 (\mu)M)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Values are mean ± SE. AHP, afterhyperpolarizations; DCN, deep cerebellar nuclei; fAHP, fast AHP; sAHP, slow AHP, CBTX, charybdotoxin; MIB, Mibefradil; TEA, tetraethylammonium; APA, apamin. *Significance from control at P < 0.05.

J Neurophysiol • VOL 100 • NOVEMBER 2008 • www.jn.org
branches positive for Kv3.1 immunolabel could be detected over \( \geq 60 \, \mu m \) from the soma. We also attempted to immuno-stain for Kv3.2 and Kv3.4 channel distribution, but the results were ambiguous in providing no clearly defined cellular label (data not shown). By comparison, a punctate and putative membrane-associated label was detected for the KCa1.1 (BK) class of KCa channel around large diameter cells (Fig. 6C).

Finally, antibodies directed against members of the SK channel family (\( K_{\text{C}a,2.1} \) and \( K_{\text{C}a,2.2} \)) showed immunolabel in large diameter DCN cells as at least a diffuse cytoplasmic pattern (Fig. 6, D and E). Thus the large diameter DCN cells seem to express members of the SK, Kv3, and BK families of channels.

**Ionic basis of tonic and rebound spike firing in transient and weak burst neurons**

**Transient burst cell fAHP.** To identify currents generating the fAHP in transient and weak burst cells, we applied blockers of the Kv3 and KCa families of K\(^+\) channels. We first examined the contribution of BK channels by applying Ni\(^{2+}\) (1 mM) to abolish LVA currents recorded at burst threshold. For transient burst cells, bath application of Ni\(^{2+}\) had no effect on fAHP depth during tonic firing or during the rebound phase of spike discharge (Fig. 7, A and F). However, Ni\(^{2+}\) application was effective at blocking the underlying rebound depolarization, because the rebound burst frequency was reduced by 60\% (Fig. 7, A and F). The more selective T-type Ca\(^{2+}\) channel blocker Mibefradil (1 \( \mu M \)) again had no effect on the fAHP during tonic or rebound phases of spike firing but reduced the frequency of the rebound burst (Fig. 7, D, F, and G; Table 1). This suggests that T-type Ca\(^{2+}\) channels in transient burst cells serve to initiate rebound depolarizations but do not act as a significant Ca\(^{2+}\) source for BK channels involved in spike repolarization. The fact that some rebound depolarization persists in the presence of 1 mM Ni\(^{2+}\) further suggests the sequential activation of more than just LVA Ca\(^{2+}\) current during the rebound phase.

We next applied 50 \( \mu M \) Cd\(^{2+}\) because this concentration blocks HVA Ca\(^{2+}\) channels without affecting T-type Ca\(^{2+}\) channels (Lacinova et al. 2000). We found that Cd\(^{2+}\) significantly reduced the fAHP in transient burst cells during the tonic and rebound phase of spiking, leading to Na\(^+\) spike failure during the rebound from membrane hyperpolarization (Fig. 7, B and F; Table 1). To examine the role of KCa\(^{-}\) currents we applied charybotoxin (CBTX; 100 nM) to block BK channels (Coetzee et al. 1999). The effects of CBTX were nearly identical to those of Cd\(^{2+}\) in significantly reducing the fAHP during the tonic and rebound phase but had no significant effect on rebound burst frequency (Fig. 7, C, F, and G). The similar results of Cd\(^{2+}\) and CBTX application led us to believe that they were acting predominantly on BK channels (Fig. 7F; Table 1) (Coetzee et al. 1999; Gao and Garcia 2003).

Finally, we applied TEA at a concentration of 500 \( \mu M \), a level that will act on BK channels, Kv3 channels, and some members of the Kv1 K\(^+\) channel family (Coetzee et al. 1999).

**FIG. 5.** The DAP in transient burst and weak burst neurons occurs independently of voltage-gated Ca\(^{2+}\) or Na\(^+\) channels. A and B: in both transient burst (A) and weak burst (B) neurons, the DAP was insensitive to the Ca\(^{2+}\) channel blockers Cd\(^{2+}\) (50 \( \mu M \)) and Ni\(^{2+}\) (1 mM). C: DAP amplitude is linked to spike height. The soma was initially protected by pressure ejection of artificial cerebrospinal fluid (ACSF) and slowly perfused by bath applied TTX (200 nM) to reduce and finally eliminate the Na\(^+\) spike. Arrows indicate DAP. D–G: the ability to evoke a DAP was tested while bath applying 200 nM TTX (D, E, and G) or 1 \( \mu M \) TTX (F). A transient current waveform evoked a spike-like response and a DAP in the presence of 200 nM TTX in both weak burst (D) and transient burst cells (E) but not small diameter cells (G) but not small diameter cells (E) but not small diameter cells (G) but not small diameter cells (E). Subsequent perfusion of 1 \( \mu M \) TTX onto a representative transient burst cell (F) to block resurgent Na\(^+\) current did not block the DAP.
TEA significantly reduced spike repolarization in transient burst cells to the point of even preventing full repolarization to a subthreshold voltage during the tonic or rebound phase of spike firing (Fig. 7, E and F; Table 1). As a result, rebound burst frequency was substantially reduced by TEA (Fig. 7G).

The above results are all consistent with a significant contribution by Kv3 and BK K⁺ channels to the fAHP in transient burst cells. The BK-mediated currents involved in spike repolarization further seem to be functionally associated with Ca²⁺ influx through Cd²⁺-sensitive Ca²⁺ channels that are distinct from the LVA (Mibebradil-sensitive) Ca²⁺ channels involved in generating the rebound depolarization.

**WEAK BURST CELL fAHP.** The fAHP of weak burst cells reaches a significantly more hyperpolarized level than in transient burst cells (Fig. 4, A and B). T-type Ca³⁺,3,3 Ca²⁺ channels are known to be expressed in weak burst cells, but their contribution to the rebound depolarization is dampened by K⁺ channel activation (Molineux et al. 2006). We therefore studied the possibility that T-type Ca³⁺,3,3 channels might instead act as a source of Ca²⁺ for BK channel activation contributing to the strongly hyperpolarizing fAHP. We found that application of Ni²⁺, Mibebradil, or CBTX significantly reduced the fAHP of weak burst cells during tonic spiking (Fig. 8, A, D, E, and G; Table 1). This suggests that T-type channels can act as a source of Ca²⁺ for a KCa channel involved in spike repolarization in weak burst cells. Application of the HVA Ca²⁺ channel blocker Cd²⁺ (50 µM) also significantly reduced the fAHP during tonic firing (Fig. 8, B and G). Furthermore, co-applying Ni²⁺ and Cd²⁺ had an additive effect, producing an even greater reduction of the fAHP during tonic spiking (Fig. 8, C and G). Finally, TEA substantially reduced the fAHP of weak burst neurons equally in both the tonic and rebound phase of spike discharge (Fig. 8, F and G; Table 1). Therefore the fAHP in weak burst cells is mediated by the potential combination of BK channels and TEA-sensitive Kv3 K⁺ channels. Furthermore, a Mibebradil-sensitive (putative T-type current) and Cd²⁺-sensitive (spike-activated HVA) Ca²⁺ channel subtype serves as the source of Ca²⁺ to activate KCa channels in weak burst cells during tonic firing.

By comparison, the effects of Ca²⁺ channel blockers on fAHP amplitude during rebound spiking were diminished even though CBTX reduced the fAHP during both tonic and rebound spiking (Fig. 8; Table 1). There are several potential explanations for this result. One possibility is that the lack of spiking during the hyperpolarized phase may reduce the intracellular concentration of Ca²⁺ and thus BK channel function during rebound, as seen in medial vestibular nucleus (MVN) neurons under similar experimental conditions (Nelson et al. 2003). In fact, Ca²⁺ imaging has shown that the frequency of tonic spike discharge in DCN cells regulates the baseline levels of intracellular Ca²⁺ (Muri and Knopfel 1994), such that membrane hyperpolarization and loss of spike discharge will lead to a decrease in Ca²⁺ availability. Alternatively, voltage-gated K⁺ channels may be partially inactivated during tonic spiking and subsequently recover during hyperpolarization, resulting in a larger available K⁺ current and hence an increased fAHP.

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**Fig. 6.** Immunolocalization of voltage- and Ca²⁺-activated K⁺ channels involved in spike repolarization and generation of AHPs. In each case, ion channels (left column, Cy3) are co-localized with MAP-2 (right column, Alexa-488) as a general structural counterlabel. A and B: immunolabel for the Kv3.1 and Kv3.3 subtypes of high-threshold voltage-activated K⁺ channels are located on the soma and proximal dendrites of large diameter DCN neurons. C: immunolabel for KCa1.1 (BK) K⁺ channels can be visualized as a punctate label on the membranes of MAP-2-labeled DCN neurons. D and E: DCN cells label for 2 of the SK KCa channel isoforms KCa2.1 (SK1) and KCa2.2 (SK2). Scale bars = 20 µm.
Given the effects of CBTX during both tonic and rebound discharge, one potential contributor would be the highly CBTX-sensitive Kv1.3 \(K^+\) channel that has been localized to DCN cells (Coetzee et al. 1999; Veh et al. 1995). These mechanisms are not necessarily mutually exclusive and may operate concurrently even though our results cannot distinguish between them.

The currents mediating the fAHP also regulated burst frequency in weak burst cells (Fig. 8H). We found that spike frequency during the rebound phase was significantly increased by \(\text{Cd}^{2+}\), co-applied \(\text{Ni}^{2+}/\text{Cd}^{2+}\), or CBTX. Because \(\text{Cd}^{2+}\) and/or \(\text{Ni}^{2+}\) did not significantly reduce the rebound fAHP but did increase rebound spiking, we believe this effect was caused largely by a reduction in the sAHP that often regulates firing rate (Fig. 9) (Smith et al. 2002). Another possibility may be that the slight reduction of the fAHP is able to augment rebound spiking through an increase in gain (Table 1) (Me-

**Fig. 7.** Ionic conductances underlying the fAHP in transient burst neurons. A–E: representative traces showing the effects of ion channel blockers on a single spike during tonic firing (left column; superimposed control and test responses) and on rebound burst spiking (right column). Shown are the effects of perfusing (A and B) the general Ca\(^{2+}\) channel blockers Ni\(^{2+}\) (1 mM) and Cd\(^{2+}\) (50 \(\mu\)M), (C) the voltage- and Ca\(^{2+}\)-activated K\(^+\) channel blocker CBTX (100 nM), (D) putative T-type channel blocker Mibefradil (1 \(\mu\)M), and (E) the voltage- and Ca\(^{2+}\)-activated K\(^+\) channel blocker TEA (500 \(\mu\)M). F: plots of the effects of ion channel blockers on fAHP depth (measured from spike threshold) during tonic and rebound spike firing. G: plots of the average change in rebound spike frequency in the presence of the indicated ion channel blockers.
These data affirm that a KCa current (responsible for the fAHP and/or sAHP) restrains rebound discharge in weak burst cells.

**Weak Burst cell class**

Rebound Spiking

![Rebound Spiking Diagram]

A–F: representative traces showing the effects of ion channel blockers on a single tonic spike (left column; superimposed control and test responses) and on rebound burst spiking (right column). Shown are the effects of perfusing (A–C) the general Ca\(^{2+}\) channel blockers Ni\(^{2+}\) (1 mM) and/or Cd\(^{2+}\) (50 μM), (D) the voltage- and Ca\(^{2+}\)-activated K\(^+\) channel blocker CBTX (100 nM), (E) putative T-type channel blocker Mibefradil (1 μM), and (F) the voltage- and Ca\(^{2+}\)-activated K\(^+\) channel blocker TEA (500 μM). G: plots of the effects of ion channel blockers on fAHP depth (measured from spike threshold) during tonic and rebound spike firing. H: plots of the average change in rebound spike frequency in the presence of the indicated ion channel blockers.

**FIG. 8.** Ionic conductances underlying the fAHP in weak burst neurons. A–F: representative traces showing the effects of ion channel blockers on a single tonic spike (left column; superimposed control and test responses) and on rebound burst spiking (right column). Shown are the effects of perfusing (A–C) the general Ca\(^{2+}\) channel blockers Ni\(^{2+}\) (1 mM) and/or Cd\(^{2+}\) (50 μM), (D) the voltage- and Ca\(^{2+}\)-activated K\(^+\) channel blocker CBTX (100 nM), (E) putative T-type channel blocker Mibefradil (1 μM), and (F) the voltage- and Ca\(^{2+}\)-activated K\(^+\) channel blocker TEA (500 μM). G: plots of the effects of ion channel blockers on fAHP depth (measured from spike threshold) during tonic and rebound spike firing. H: plots of the average change in rebound spike frequency in the presence of the indicated ion channel blockers.
to regulate the interspike interval and promote stable low-frequency firing (Aizenman and Linden 1999; Shakkottai et al. 2004). A strong reduction of the sAHP in either transient or weak burst cells thus destabilized tonic firing and promoted slow oscillations that alternated between very high-frequency firing (≈200 Hz) for seconds at a time, followed by long periods of quiescence (Fig. 9, C and D, insets) (Alvina and Khodakhah 2008). Indeed, a single experiment perfusing apamin could induce this effect in subsequent experiments for days unless perfusion lines were cleaned or replaced, suggesting an exquisite sensitivity to this SK channel blocker. We also found that any disruption of resting internal Ca²⁺ levels (i.e., using an EGTA concentration >0.1 mM in the whole cell pipette solution) or

**FIG. 9.** Ionic conductances underlying the sAHP in transient burst and weak burst neurons. A–F: representative control and test traces superimposed to show the effects of ion channel blockers on the sAHP during tonic spiking in transient burst (left column) and weak burst (right column) neurons. Shown are the effects of perfusing (A–C) the general Ca²⁺ channel blockers Ni²⁺ (1 mM) and/or Cd²⁺ (50 μM), (D) the SK1 and SK2 channel blocker apamin (100 nM), (E) the voltage- and Ca²⁺-activated K⁺ channel blocker CBTX (100 nM), and (F) T-type channel blocker Mibefradil (1 μM). G: plots of the average effects of ion channel blockers on sAHP depth (measured from spike threshold) during tonic firing.
recording at room temperature promoted these slow oscillations.

We examined the effects of Ca\(^{2+}\) and K\(^+\) channel blockers to identify the currents underlying the sAHP. We found that Ni\(^{2+}\) significantly reduced the sAHP only in weak burst neurons (Fig. 9, A and G; Table 1). By comparison, Cd\(^{2+}\) significantly decreased the sAHP in all cases (Fig. 9, B and G). When Ni\(^{2+}\) and Cd\(^{2+}\) were combined, the reduction of the sAHP was significantly greater than either drug alone (Fig. 9, C and G; Table 1), suggesting at least two sources of Ca\(^{2+}\) for the sAHP with different Ni\(^{2+}\) and Cd\(^{2+}\) sensitivities. The elimination of the sAHP by Ni\(^{2+}/Cd^{2+}\) was replicated by the selective SK channel blocker apamin (Fig. 9, D and G), confirming that Ni\(^{2+}/Cd^{2+}\) acts by blocking Ca\(^{2+}\) activation of SK K\(^+\) channels. Mibebradil had no effect on the sAHP of either transient or weak burst neurons (Fig. 9, F and G; Table 1). The Ca\(^{2+}\) influx that supports the sAHP in weak burst neurons thus does not likely correspond to T-type Ca\(^{2+}\) conductance (Fig. 9, A and G), suggesting that the effects of 1 mM Ni\(^{2+}\) on the weak burst sAHP (Fig. 9A) could reflect a secondary block of N-type Ca\(^{2+}\) channels (Alvina and Khodakhah 2008). Another drug that had a differential effect between transient and weak burst cells was CBTX, which significantly reduced the sAHP in weak burst but not transient burst cells (Fig. 9, E and G; Table 1). This indicates that CBTX-sensitive (BK) K\(^+\) channels also contribute to at least the early phase of the sAHP in weak burst neurons.

**DISCUSSION**

Neurons in the DCN occupy a critical position for motor control in representing the final output of all regions of cerebellum outside of vestibular function. Physiological studies have begun to identify different subtypes of DCN cells based on spike output patterns in vitro and in the context of behavioral stimuli in vivo (Aizenman and Linden 1999; Chen and Evinger 2006; Czubayko et al. 2001; Molineux et al. 2006; Ohtsuka and Noda 1991, 1992; Uusisaari et al. 2007). This study focused on the large diameter cells that exhibit two phenotypes of rebound burst output in response to current-evoked hyperpolarizations in vitro (Molineux et al. 2006). Although it is uncertain under what conditions DCN neurons exhibit rebound bursts in vivo, it is clear that they respond in a different manner to specific aspects of sensory input and motor output. DCN neurons are recognized to respond selectively to different aspects of muscular control of saccadic eye movements (Ohtsuka and Noda 1991, 1992), as well as opposing muscle actions on a given joint (Soteropoulos and Baker 2008). Studies in the interpositus nucleus of the cat showed two populations of neurons that respond to eyelid conditioning: one that increase firing frequency and another that slow or pause spike firing (Delgado-García and Gruart 2005; Gruart et al. 1997; Jimenez-Díaz et al. 2004). A recent study confirmed these differences in the rat interpositus nucleus and noted that the pause cell lacked high-frequency burst activity following Purkinje cell–induced hyperpolarizations (Chen and Evinger 2006). It is interesting to note that the ratio of pause to burst neurons found in the rat interpositus nucleus in vivo was reported as 69:31 (n = 131). This proves to be essentially identical to a ratio of 68:32 (n = 84) for weak burst compared with transient burst neurons we found in the interpositus nucleus in vitro, providing a close parallel between at least these results. The different responses observed in vivo may reflect at least in part differences in the intrinsic cellular properties of DCN neurons.

**Ionic basis for rebound burst discharge**

This study identified several key intrinsic properties that underlie the two burst phenotypes of large diameter DCN neurons. Because both phenotypes can be recorded from any slice and age level examined here (P14–P20), we have no data at this time to suggest that these patterns reflect a developmental transition from one phenotype to the other, as several measured parameters of membrane excitability, spike, or burst discharge do not change over this time frame (data not shown). We also lack evidence to suggest that modulation of an ion channel (i.e., K\(^+\)) could dynamically switch a given cell between rebound burst phenotypes, although this remains a possibility. We have now identified several essential differences between transient and weak burst cells in the availability of LVA Ca\(^{2+}\) current at burst threshold and in the magnitude and contribution of afterpotentials that offset membrane excitability and rebound burst capability. In terms of rebound discharge, transient burst cells proved to have ≈7 times more rebound LVA Ca\(^{2+}\) current available at burst threshold (approximately −50 mV) than weak burst cells. Given that this inactivating rebound current was completely blocked by a low dose of Ni\(^{2+}\), the results are consistent with a Cav3.3-mediated T-type Ca\(^{2+}\) current. We note that another recent study reported that DCN cells can exhibit either of two forms of rebound inward currents (Pugh and Raman 2006). Our data now indicate that cells with differing LVA currents can correspond at least to different phenotypes of rebound discharge in large diameter cells. The inward Ca\(^{2+}\) current in transient and weak burst cells consistently differed in terms of amplitude, peak latency, and rates of inactivation. In fact, these differences are consistent with Ca\(_{3.1}\) and Ca\(_{3.3}\) currents recorded in heterologous expression systems, in which Cav3.1 current peaks at a shorter latency than Cav3.3 currents (Itinica et al. 2006).

Further analyses will be required to distinguish any differences in voltage-dependent properties or membrane channel densities between cells exhibiting either of these forms of rebound discharge. It is clear, however, that at −50 mV, there is significantly less LVA Ca\(^{2+}\) current available to drive bursting in weak burst DCN cells. The amount of LVA current recorded in any given cell showed a remarkably strong correlation to the intensity of rebound bursts, such that an increase in Ni\(^{2+}\)-sensitive (T-type) current translates directly to an increase in the number and frequency of rebound spikes (Fig. 3). These results also serve to validate the accuracy of our measurements of LVA current available at −50 mV and emphasize that even small variations in T-type current can exert an effect on cell output. A strong relationship between T-type current density and cell output was also recently reported in inferior olivary cells (Chorev et al. 2006).

**Spike afterpotentials**

Previous comparisons of spike output in DCN cells identified the presence of a fAHP, DAP, and sAHP (Aizenman and
Linden 1999; Czubayko et al. 2001), although these studies did not differentiate between large cell bursting phenotypes. Two recent studies indicate that large diameter cells in the mouse or rat DCN characteristically exhibit all three of these afterpotentials (Molineux et al. 2006; Uusisaari et al. 2007). Our work now shows key differences in spike afterpotentials between excitant and weak burst cells that set relative membrane excitability and the propensity for rebound burst discharge.

DAP generation

Many bursting cells generate a DAP that can contribute to high-frequency firing and thus drive a burst of action potentials. The DAP recorded in transient or weak burst cells proved to be quite small and of similar absolute amplitude (~1.5 mV). However, its interaction with an fAHP that is generated over a similar time frame allowed the DAP in transient burst cells to approach much more closely to spike threshold than in weak burst cells. Although assigning a specific role for the DAP in burst discharge was complicated by the underlying rebound depolarization, the DAP may allow faster firing during rebound spike bursts in transient burst cells. By comparison, the DAP in weak burst cells was less able to drive a burst under physiological conditions given that the hyperpolarizing effect of the fAHP outweighed the depolarizing influence of the DAP.

This work established that the DAP in both transient and weak burst cells is not actively mediated through voltage-gated Na\(^+\) or Ca\(^{2+}\) channels. This was surprising given the prevalence of Ca\(^{2+}\)- or Na\(^+\)-dependent DAPs in other cells (Azouz et al. 1996; Ghamar-Langrudi and Bourque 2002; Jung et al. 2001; Nelson et al. 2005b) and the established expression of resurgent Na\(^+\) current in DCN cells (Afshari et al. 2004). We also found that it is not caused by a Ca\(^{2+}\)-activated Cl\(^-\) conductance or nonspecific cation channel. However, the amplitude of the DAP was tightly linked to that of the recorded somatic Na\(^+\) spike (Fig. 5C). In this regard, it has been established that a DAP can arise through current flow from distant regions of a cell because of differences in voltage that arise during spike conduction (Barrett and Barrett 1982; Fernandez et al. 2005, 2007; Turnier et al. 2002; Yuste et al. 1994; Zhang et al. 1993). Often the propagating spike is aided by active Na\(^+\) or Ca\(^{2+}\) conductances, although this does not seem to be necessary in DCN cells. The data at this time are consistent with the DAP in large diameter DCN neurons arising through a passive discharge of distal regions of cell structure (dendritic or axonal) after charging by a somatic Na\(^+\) spike. In support of this, a compartmental modeling study of vestibular neurons showed that DAPs could be generated by the addition of passive dendritic compartments (Quadroni and Knopfel 1994). Although alternative explanations do not present themselves at this time, we recognize that this interpretation may be modified with future experimentation.

Afterhyperpolarizing potentials

The ability for the DAP or rebound depolarization to bring a cell to spike threshold and trigger a burst of spikes proved to be differentially controlled by AHPs in transient versus weak burst cells. Pharmacological tests traced the differences in AHPs to the relative contribution of different K\(^+\) channel subtypes and the potential Ca\(^{2+}\) channels that provide the necessary Ca\(^{2+}\) influx.

fAHP. The fAHP was much greater in amplitude in weak burst compared with transient burst cells, thus maintaining the DAP at a substantially lower level with respect to spike threshold. To summarize our pharmacological results, the fAHP in both transient and weak burst cells was similar in incorporating at least Kv3 and BK K\(^+\) channel activation. The fAHPs differed in that BK channels were activated only via HVA (Ca\(^{2+}\)-sensitive) Ca\(^{2+}\) channels in transient burst cells but involved both T-type Ca\(^{2+}\) channels (Ni\(^{2+}\) and Mibefradil-sensitive) as well as Cd\(^{2+}\)-sensitive HVA Ca\(^{2+}\) channels in weak burst cells. The multiple potential sources of Ca\(^{2+}\) to activate BK channels in weak burst cells may help account for the larger fAHP.

sAHP. The pharmacology of the sAHP showed further contrasts in hyperpolarizing responses. The sAHP in both cell types was mediated primarily by SK K\(^+\) channels. However, an additional contribution by BK channels to the sAHP exclusively in weak burst cells further increased the membrane hyperpolarization. The differential sensitivity of the weak burst cell sAHP to Ni\(^{2+}\) and Cd\(^{2+}\) implies that more than one source of Ca\(^{2+}\) channel influx serves to activate SK channels (Fig. 9, A–C). Similar conclusions were drawn in the recent study of Alvina and Khodakhhah (2008). These authors reported a functional coupling between N-type Ca\(^{2+}\) channels and SK activation in DCN cells. Moreover, a Cd\(^{2+}\)-sensitive component of the sAHP reflected Ca\(^{2+}\) influx from a source not blocked by classic HVA Ca\(^{2+}\) channel toxins and blockers. The source of this additional Cd\(^{2+}\)-sensitive Ca\(^{2+}\) increase is not currently known and not further examined in this study.

Together these results define several key differences in spike afterpotentials that regulate membrane excitability and rebound responses. The expression of specific T-type Ca\(^{2+}\) channel isoforms, the net LVA Ca\(^{2+}\) current available at burst threshold, and K\(^+\) currents have a significant impact on the ability to evoke rebound discharge. Thus in transient burst cells, K\(^+\) channels repolarize spikes effectively to ensure the ability for Cav3.1 Ca\(^{2+}\) current (and the DAP) to generate fast bursts of spikes. In weak burst cells, K\(^+\) currents play a significant role in generating AHPs that downregulate the ability for a lower functional expression of Cav3.3 channels to generate a rebound depolarization and spike burst.

Burst phenotypes and cell identity

The DCN contains a group of excitatory projection cells as well as inhibitory cells that participate in local network function or as projection neurons to the inferior olive (Chan-Palay 1977; Teune et al. 2000). Identifying which group the transient and weak burst cells correspond to would assist in understanding the functional roles of these bursting neurons. Combined cell fills and immunocytochemistry performed to date show that the large diameter transient burst cells are comprised of non-GABAergic cells and a smaller population of GABAergic cells, whereas weak burst cells were uniformly non-GABAergic (Molineux et al. 2006). Comparisons of membrane and spike properties between transient burst cells failed to identify an electrophysiological signature that could be easily used to distinguish the non-GABAergic and GABAergic members of this population (Fig. 1). We note that these differences may well be present but masked in the larger...
population of non-GABAergic cells. However, a recent study using a transgenic GAD67 mouse line to identify GAD-expressing cells indicated that an important criterion for distinguishing GAD- cells was the presence of a fAHP, DAP, and sAHP. Because all of the cells in our recordings had these responses, the majority of recordings should represent GAD- cells.

The further classification of mouse DCN cells into tonic versus phasic firing patterns by Uusisaari et al. (2007) is similar to that used for cells in the medial vestibular nucleus that also receive direct Purkinje cell inhibitory input. In fact, a great deal of work on the electrophysiological properties of MVN neurons allow for comparisons between cells in these structures (for review, see Straka et al. 2005). Interestingly, work in the MVN defines two cell classes according to spike output: a type A neuron that fires regularly and a type B neuron that is more phasic, with a small percentage of the type B population exhibiting a relatively weak form of rebound discharge (<100 Hz). Others have proposed that type A and B subtypes instead represent the extremes of a continuum in spike output properties (du Lac and Lisberger 1995). We note that rat DCN transient and weak burst cells have electrophysiological properties closer to type B MVNs but more pronounced capability for exhibiting rebound bursts. One difference is that MVN type B rebounding cells only make up ~10% of the population, whereas in the rat interpositus nucleus, transient burst cells make up ~38% (32/84) of recordings. The transient and weak burst phenotypes in rat DCN are unlikely to represent a continuum of cell output, because we have shown an association between burst phenotype and the specific expression of either Cav3.1 or Cav3.3 T-type Ca2+ channels (Molineux et al. 2006), suggesting two distinct populations. However, the ability for K+ channel blockade to increase burst output of weak burst cells (Molineux et al. 2006) leaves open the possibility that modulation of BK or SK channels could transform firing patterns (Nelson et al. 2005a). In addition, a range of burst output in weak burst neurons suggests that there may be multiple cell types with different patterns of ion channel expression that lead to more subtle differences in spike output.

Recent molecular studies on MVN neurons raise another interesting challenge to associating spike output properties with transmitter phenotype. A detailed whole cell recording and single-cell RT-PCR study showed that type A MVN cells typically express GABA (Takazawa et al. 2004). These neurons also share many electrophysiological properties with the small diameter GAD+ cells in mouse DCN (Uusisaari et al. 2007). Type B MVN neurons that express a fAHP, DAP, and sAHP were typically glutamatergic but again included a smaller fraction of GABAergic cells (Takazawa et al. 2004). Moreover, 51% of MVN cells analyzed expressed mRNA for more than two different transmitters, including co-expression of GABA and glutamate or GABA and acetylcholine. Similarly, immunocytochemistry has identified DCN cells that colabel for GABA and glycine (Chen and Hillman 1993), as found for other cerebellar neurons (Dugue et al. 2005; Simat et al. 2007). Understanding the full relationship between burst phenotype and transmitter content in relation to circuit function will thus require further work.

This study has served to identify the ionic basis for several aspects of spike repolarization, afterpotentials, and rebound depolarizations and how these factors interact to generate distinct phenotypes of burst output in large diameter rat DCN cells. Although further work is needed to determine whether these different forms of neuronal output perform distinct computations on Purkinje cell inhibitory input, a characterization of the ionic basis for spike firing in DCN cells is a crucial step toward understanding their possible functional roles.

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