Ion Channels Generating Complex Spikes in Cartwheel Cells of the Dorsal Cochlear Nucleus

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Kim Y. Trussell LO. Ion channels generating complex spikes in cartwheel cells of the dorsal cochlear nucleus. J Neurophysiol 97: 1705–1725, 2007; doi:10.1152/jn.00536.2006. Cartwheel cells are glycinergic interneurons that modify somatosensory input to the dorsal cochlear nucleus. They are characterized by firing of mixtures of both simple and complex action potentials. To understand what ion channels determine the generation of these two types of spike waveforms, we recorded from cartwheel cells using the gramicidin perforated-patch technique in brain slices of mouse dorsal cochlear nucleus and applied channel-selective blockers. Complex spikes were distinguished by whether they arose directly from a negative membrane potential or later during a long depolarization. Ca2+ channels and Ca2+-dependent K+ channels were major determinants of complex spikes. Onset complex spikes required T-type and possibly R-type Ca2+ channels and were shaped by BK and SK K+ channels. Complex spikes arising later in a depolarization were dependent on P/Q- and L-type Ca2+ channels as well as BK and SK channels. BK channels also contributed to fast repolarization of simple spikes. Simple spikes featured an afterdepolarization that is probably the trigger for complex spiking and is shaped by TR-type Ca2+ and SK channels. Fast spikes were dependent on Na+ channels; a large persistent Na+ current may provide a depolarizing drive for spontaneous activity in cartwheel cells. Thus the diverse electrical behavior of cartwheel cells is determined by the interaction of a wide variety of ion channels with a prominent role played by Ca2+.

INTRODUCTION

The dorsal cochlear nucleus (DCN) is a cerebellum-like component of the mammalian auditory system that may play a role in sound localization (Oertel and Young 2004; Young and Davis 2002). Auditory nerve fibers terminate on dendrites of giant cells and fusiform cells in the DCN deep layer, and their input is modified by various interneurons within the cochlear nucleus (Nelken and Young 1994; Zhang and Oertel 1993b,c, 1994). The fusiform cells of the DCN also receive excitation through a system of granule cells and their associated parallel fibers in the DCN molecular layer. Some of the fibers that drive the granule cells originate in medullary somatosensory nuclei and may convey ear and head position. Indeed, one proposed function of the DCN has been that it contributes to orienting toward specific sounds by integrating auditory, somatosensory, and vestibular input (Knol and Young 2001; May 2000; Oertel and Young 2004; Young and Davis 2002). Cartwheel cells (CWCs) are inhibitory interneurons that receive parallel fiber input and form synapses on fusiform cells and among themselves in the molecular and fusiform cell layer of the DCN (Berrebi and Mugnaini 1991; Mugnaini et al. 1987). The output of DCN principal neurons in vivo or in vitro has been characterized by inhibition of background spontaneous activity, and it is believed that CWCs play an important role in this process, being spontaneously active and capable of providing powerful feed-forward inhibition of parallel fiber input (Young and Davis 2002; Zhang and Oertel 1994). However, in vivo recordings have been made in immobilized animals where many of the somatosensory and vestibular inputs to granule cells are not activated, making the physiological activity of the molecular layer poorly understood.

CWCs are unique in the cochlear nucleus for their ability to generate complex spikes (Manis et al. 1994; Zhang and Oertel 1993a). Complex spikes, also called “bursts”, consist of brief (<100 ms), clusters of high-frequency (>100 Hz) action potentials superimposed on an underlying slow depolarization and are seen in several neuronal cell types (Athanasiadis et al. 2005; Brumbach et al. 2000; Chagnac-Amittai et al. 1990; Deschenes et al. 1982; Jung et al. 2001; Kandel and Spencer 1961; Niespodziany and Poulin 1995; Schmoleksy et al. 2002). The slow underlying depolarization of CWC complex spikes is believed to be Ca2+-dependent, whereas the fast action potentials riding on the slow wave are Na+ dependent as are single action potentials (the “simple spike”) in these same cells (Golding and Oertel 1997). The terms “complex spike” and “simple spike” imply electrophysiological similarity of the CWCs to the cerebellar Purkinje cells and are in keeping with other studies highlighting morphological, genetic, and molecular parallels between these two cell types (Berrebi and Mugnaini 1991; Berrebi et al. 1990; Mugnaini and Morgan 1987). However, several features that differentiate these cell types suggest that a more careful investigation of CWC firing properties is needed. For example, Purkinje cells fire complex spikes only in response to climbing fiber activity; this activity is believed to play a key role in induction of synaptic plasticity at climbing fiber and parallel fiber synapses (Hansel and Linden 2000; Ito 2001; Konnerth et al. 1992). By contrast, CWCs may fire complex spikes spontaneously or in response to glutamatergic parallel fibers or glycnergic inputs from other CWCs (Golding and Oertel 1996; Tzounopoulos et al. 2004; Zhang and Oertel 1993a). Thus the computational meaning of complex spikes may be different in the two cell types. We therefore have investigated the channel types underlying firing of complex and simple spikes and what conditions promote each mode of firing, in slices of mouse DCN. Our results identify the role of multiple Ca2+ channels and Ca2+-depen-
dent K⁺ channels as well as Na⁺ currents in promoting and shaping the complex spike.

METH ODS

Slice preparation, recording, and analysis of data

Brain stem slices containing the DCN were prepared from ICR mice aged 16–23 days (Harlan, Indianapolis, IN). Mice were anesthetized with isoflurane and then decapitated in accord with the regulations of the Institutional Animal Care and Use Committee of Oregon Health and Science University. Subsequently, a block of brain stem was isolated and horizontal slices of 210-μm thickness were cut with a vibrating slicer (VT1000S, Leica, Deerfield, IL). Dissection and slicing were done in a warm (~30°C) solution, which was composed of (in mM) 130 NaCl, 3 KCl, 1.2 KH2PO4, 2.4 CaCl2, 1.3 MgSO4, 20 NaHCO3, 3 HEPES, and 10 glucose and was saturated with 95% O2-5% CO2. The chamber containing DCN slices were incubated at 34.5°C for the first hour and left at room temperature thereafter. For recording, a slice was transferred to the recording chamber on the stage of Olympus BX51WI microscope, and DCN cells were visualized with infrared differential interference contrast videomicroscopy. The bathing solution for recording was the same as that used for dissection and was perfused at 2–3 ml/min to the recording chamber by a peristaltic pump (Minipulse 3, Gilson, Middleton, WI). The temperature of the solution at the recording chamber was maintained at 33°C by a heating water jacket around perfusion tubing or by an in-line heating device.

Medium-sized cells in the molecular and fusiform cell layers of DCN were identified as CWCs if they showed complex spikes spontaneously or on injection of depolarizing current. The majority of cells presented in this study were obtained with gramicidin perforated-patch recording (Kyrozis and Reichling 1995; Rhee et al. 1994) unless otherwise specified. The pipette solution for perforated patch recording consisted of (in mM) 140 KCl, 10 NaCl, and 10 HEPES (pH adjusted to 7.25 with KOH), and gramicidin was added just before use at a final concentration of 10–40 μg/ml from a stock solution of 10–30 mg/ml DMSO. The tip of the recording pipette was filled with the same solution but without gramicidin. For examination of the effect of increased intracellular Ca²⁺ buffering, conventional whole cell recording was employed: a standard internal solution containing (in mM) 115 K-glucanate, 4.5 MgCl₂, 14 trisphosphocreatine, 9 HEPES, 0.1 EGTA, 4 Na-ATP, and 0.3 Tris-GTP (pH adjusted to 7.3 with KOH), was modified to have higher EGTA at 5 mM or to include 20 mM BAPTA (tetrapotassium salt) in place of equimolar K-glucanate. Patch pipettes for recording electrodes of 2–6 MΩ resistances were prepared by pulling thick-walled filamented borosilicate glass capillaries (1B120F-4, World Precision Instruments, Sarasota, FL) and wrapped with Parafilm along one-third of length from the tip to reduce capacitance. The liquid junction potentials measured (according to Neher 1992) and then corrected (for the reference junction, with JPCalc) (Barry 1994) were 2.8, 16, and 13.7 mV for the 140 mM KCl-based, 5 mM EGTA-containing, and 20 mM BAPTA-containing pipette solutions, respectively; the values for later two solutions were subtracted from the voltage data obtained with each solution off-line.

Recordings were made with a BVC-700A (Dagan, Minneapolis, MN) or MultiClamp 700B amplifier (Molecular Devices, Sunnyvale, CA) in conjunction with pClamp 9.2 software (Molecular Devices). Data were digitized with Digidata 1322A (Molecular Devices) at 20 kHz (current-clamp) or 10 kHz (voltage-clamp) and low-pass filtered at 10 kHz (current-clamp) or 3 kHz (voltage-clamp). Pipette capacitance was compensated in all current-clamp recordings. For perforated-patch recording, after the electrode had formed a seal (>1 GΩ) on the cell membrane in voltage clamp, the progression of perforation (reduction in series resistance, Rₚ) was monitored in current clamp by periodic bridge balancing and by observing the growth in amplitudes of spontaneous fast spikes (see Terminology). Within 40 min of forming a seal, the Rₚ dropped to 20–40 MΩ; cells in which the Rₚ did not go <40 MΩ were excluded from analysis. Occasionally, the perforated patch spontaneously ruptured, thus establishing whole cell configuration. There were several signs of patch rupture when the recording pipette contained the 140 mM KCl-based solution in the preceding text, listed in the order of occurrence: an abrupt positive shift in voltage trace by ~7–10 mV accompanied with an increase in amplitude of fast spikes (due to Rₚ reduction; supplemental Fig. 1Bii), very large depolarizing glycineric/GABAergic postsynaptic potentials when the corresponding synaptic blockers were not present, and changes in pattern and waveforms of spikes characteristic of whole cell recording with a KCl-based internal solution (supplemental Fig. 1Bi). In some cases, the Vₚ occurred slowly, obscuring detection of patch rupture, but the striking effects of dialysis with KCl eventually confirmed the rupture. Extracellular recordings were done in voltage-clamp mode (Vₑ = 0 mV) with a bath solution-filled recording pipette loosely attached to a cell.

Data were analyzed with Clampfit (pClamp 9.2) and Microsoft Excel. The membrane potential (Vₑ) between spikes of spontaneously active CWCs often fluctuated over a broad (~20 mV) range (see RESULTS). When there were not such fluctuations in basal Vₑ, an approximate level of interspike trough potentials, Vₑtrough (Fig. 1Ei), was used as a representative value of Vₑ. The thresholds of fast spikes were defined by the potential at the inversion point of the rising phase of the spike waveform (Fig. 1Ab, →). For the repolarizing phase of a fast spike, the most negative Vₑ before the afterdepolarization, which may be a consequence of the fast afterhyperpolarization (fAHP), was measured and termed pFR (potential of fast spike repolarization; Fig. 1Ab, ↓). The half-width of a fast spike was measured at the mid-point between the threshold and the peak of the spike. For comparison of spike waveform parameters between different conditions in one cell, the initial spikes in response to step current injections of a given amount were selected to obtain measurements unless otherwise specified. The threshold and pFR of spontaneous simple spikes were measured for some cells for inter-cellular comparison. For cells firing simple spikes with stable Vₑtrough, the threshold and pFR were measured from randomly selected spikes. However, for some cells in which the basal Vₑ during trains of simple spikes fluctuated (e.g., control trace in Fig. 9Aii), spikes sitting on the most negative deflections of basal Vₑ were chosen for measurement of the two parameters.

For analyzing some drugs’ effect on the frequency and duration of spontaneous complex spikes, data obtained by extracellular recording were included. The frequency of spontaneous complex spikes was measured by counting complex spikes in a 25-s period. The duration of complex spikes was quantified by summing interspikelet intervals. The “interspikelet” intervals of an extracellular complex spike were measured between negative peaks as extracellular spikes/spikelets are biphasic. The extracellular spike seen in voltage-clamp mode is considered the sum of the capacitative and tonic current caused by the intracellular action potential (Fenwick et al. 1982). The waveform of our extracellular spikes generally resembled that of the first derivative of the intracellular fast spike; the extracellular interspikelet interval is similar to the interval between the points of maximum rise slope of two adjacent intracellular spikelets. If all the spikelets are uniform in waveform, the extracellular interspikelet interval should be the same as the intracellular ones. For a typical complex spike in which the successive spikelets are smaller in amplitude and slower rising, the extracellular interspikelet interval is likely to be shorter than the intracellularly recorded interspikelet interval at least for later appearing, small spikelets. Thus the sum of interspikelet intervals of an extracellular complex spike may be a slight underestimate of that of the underlying intracellular complex spike. To document changes in the shape of Ca²⁺ spikes, rough indices of amplitude and decay time, the (maximum, if multi-peaked) peak-to-trough amplitude and the (last, if multi-peaked) peak-to-trough duration, respectively, were

The online version of this article contains supplemental data.
were given as means ± SD, and the difference between two groups of data were tested using two-tailed t-test (paired or unpaired) at the 0.05 level of significance.

Drug application

All the pharmacological agents were applied by bath perfusion. Blockers of fast glutamatergic, GABAergic and glycineric transmission, 200 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX), 100 μM 2-amino-5-phosphonovaleric acid (APV), 10 μM SR95531, and 0.5 μM strychnine] were added to the bath solution after an initial examination of spiking properties unless otherwise specified. Drugs were obtained from Sigma-Aldrich (St Louis, MO) with the exception of SR95531 (Tocris Cookson, Ellisville, MO), tetrodotoxin (TTX), ω-conotoxin-GVIA, iberiotoxin, apamin (Alomone Labs, Jerusalem, Israel), ω-agatoxin-TK, ω-agatoxin-IVA and ω-conotoxin-MVIIC (Peptide International, Louisville, KY). When applying peptide toxins, 0.5 mg/ml cytochrome C (for agatoxins, conotoxins), or 0.1 mg/ml bovine serum albumin (BSA, for iberiotoxin) was included in the drug perfusate to reduce nonspecific binding; and control traces were obtained in the presence of cytochrome C or BSA alone before drug application began. Agatoxins, conotoxins, and iberiotoxin were perfused for ≈15 min using recirculation. When CdCl₂ or NiCl₂ was used, KH₂PO₄ in the bathing solution was replaced with KCl to prevent precipitation.

Terminology

Fast action potentials riding on the slow depolarization of a complex-spike waveform became slower and smaller as they arose from more depolarized membrane potentials (Manis et al. 1994). However, the initial fast spikes could have similar thresholds and amplitude to simple spikes. We used the term “spikelets” to refer specifically to the spikes comprising a complex spike and “fast spikes” to indicate both simple spikes and initial spikelets of complex spikes. Figure 12 diagrams several types of spike discussed here.

Requirement for perforated-patch recording and consideration of voltage offsets

Whole cell recording with the standard K-glucuronate solution was generally avoided because of the following changes, which developed within 15 min from the moment of patch break-through in CWCs: $V_m$ became hyperpolarized resulting in loss of spontaneous activity, later, complex spikes would occur more frequently and at a lower stimulus level in response to step depolarizations, and fast spikes depolarized less, the pFR becoming depolarized by 3.2 ± 3.4 mV (n = 24). These changes progressed more slowly when using recording pipettes of smaller bore and therefore must be related to the dialysis of cytoplasmic constituents. Changing the major anion of the internal solution to methysulfate or methanephosphate did not prevent time-dependent changes. On the other hand, with gramicidin perforated-patch recording, spontaneous spike activity was maintained, although slight depolarization of $V_m$, as apparent from increase in spiking frequency, was often seen during early periods of recording. Most importantly, the increase with time in complex spiking on routine step current protocols did not occur, as it did with whole cell recording. Supplemental Fig. 1A illustrates changes in spike properties of a cell that had been recorded initially in perforated-patch mode and then in whole cell mode with the K-glucuronate based solution after rupture of patch. A sudden –8-mV jump in $V_m$ and corresponding shift in spike parameters (threshold, pFR) were observed when the perforated-patch ruptured during recordings with the 140 mM KCl-based internal solution. This shift in potential might suggest that a junction potential existed across the gramicidin perforated patch.
between the pipette solution and the cytoplasm, such that the potential at the recording electrode had been more negative than the true \( V_m \); alternatively, it may be that a Donnan potential developed on patch rupture (Horn and Marty 1988). Similar positive \( V_m \) shifts at patch rupture during gramicidin recording have been reported and considerations for correction of the recorded \( V_m \) by adding the magnitude of shift have been addressed (Atherton and Bevan 2005; Brockhaus and Ballanyi 1998; Hallworth et al. 2003). In our recordings, however, the initial 8-mV upward shift in \( V_m \) later appeared to sag back toward the original potential (supplemental Fig. 1Bii); during the 20-s period after rupture, peaks of fast spikes gradually declined in spiking cells, and in silent cells the \( V_m \) settled to a final level 3–5 mV more depolarized than the potential before the sudden jump. This led us to wonder what correction should be applied to the recorded \( V_m \), if any. We have found that when gramicidin perforated-patch recordings were done with a pipette solution composed of (in mM) 140 K-gluconate, 1 MgCl\(_2\), and 10 HEPES, no shift of \( V_m \) was noticeable at patch rupture; this indicates that there may be no junction potential between this solution and the cytoplasm. To estimate the potential difference between the 140 KCl solution and the cytoplasm, we employed dual perforated-patch recording on single cells with the 140 K-gluconate solution in one recording pipette and the 140 KCl solution in the other. The difference in \( V_m \) read-out between the two recording electrodes was 14.7 ± 1.3 mV (\( n = 10 \)), and when the patch under the K-gluconate pipette was ruptured to form a whole cell configuration, the potential difference did not change. The mean value, 14.7 mV, was corrected for liquid junction potentials of each solution (13.3 and 2.8 mV, respectively) to become 4.2 mV, which is the estimated potential difference across the perforated-patch between the 140 KCl solution and the cytoplasm. Because the offsets to be considered for the \( V_m \) recorded with 140 KCl solution were similar in magnitude and of opposite direction (−2.8 for liquid junction potential, +4.2 mV for the patch potential) leaving 1.4 mV, we opted not to apply any correction.

RESULTS

Spontaneous spiking activity in CWC

CWCs were identified as medium-sized neurons located in the molecular and fusiform cell layers of the DCN that fired complex spikes in response to depolarizing current injection. Of the 335 CWCs recorded with gramicidin perforated-patch method and not previously exposed to pharmacological agents, 33.7% (\( n = 113 \)) were silent and the rest, 66.3% (\( n = 222 \)), were spontaneously spiking. Silent cells had a mean resting potential of −82.3 ± 2.7 mV (range −76 to −88 mV; \( n = 113 \)). Among spontaneously active cells, a range of spiking patterns was observed with respect to the tendency to fire complex spikes: 72.1% (\( n = 160 \)) showed only simple spikes (“all-simple-spiking”), whereas the others (27.9%, \( n = 62 \)) showed different frequencies of complex spikes along with simple spikes (“complex spiking”). Another characteristic feature of CWCs was a slow fluctuation or oscillation in the basal profile of \( V_m \). The \( V_m \) excluding spikes (base \( V_m \)) coursed between levels close to and far from the spike thresholds, generating periods of spiking interposed with deep hyperpolarized periods of silence (Fig. 1A). Such fluctuation in base \( V_m \) was seen in most complex-spiking cells and in 24.5% of all-simple-spiking cells (together, 44.8% of spontaneously spiking cells). The remainder of all-simple-spiking cells maintained relatively stable base \( V_m \) while firing quasi-regularly at 10–30 Hz (Fig. 1Ei) or irregularly at a lower frequency. Silent CWCs were heterogeneous with respect to their tendency to fire complex spikes or exhibit fluctuating base \( V_m \) behavior when made to spike with small depolarizing holding currents. Elimination of spontaneous synaptic potentials by applying a cocktail of blockers of ionotropic glutamate, GABA\(_A\), and glycine receptors did not eliminate spontaneous spike activity of CWCs, suggesting that CWCs’ spiking characteristics—complex spikes and slow \( V_m \) fluctuation—are intrinsically determined.

Variety of complex spikes

In this section, we will describe at some length the appearance and variety of complex spikes as these detailed characteristics helped make clear what factors promote complex versus simple spiking. We noticed that spontaneous complex spikes of individual CWCs were often not uniform: some were distinct, whereas others appeared to blend with neighboring simple spikes, especially on the rising phase of their underlying depolarization, which made the beginning of such complex spikes unclear. However, these are all recognized as complex spikes based on a core motif in their waveform, including the terminal part in the rising phase where the slope was steepest and the presence of two to three spikelets having intervals of <4 ms. We used the term “prompt” spike to refer to a distinct form of the complex spike, which is separated from a previous spike by ≥30 ms and has an initially fast rise consisting of three to four spikelets at intervals <6 ms (Figs. 1Aa, and 12). The less distinct complex spikes, which appeared to follow directly from one or more simple spikes having 7- to 25-ms interspike intervals, were termed “delayed” spikes (Figs. 1A, b–d, and 12). Spontaneous complex spikes were often preceded by a hyperpolarization. Typically, in a given cell, the prompt complex spike occurred after a long (>100 ms) hyperpolarized phase of slow \( V_m \) oscillation, and the delayed spike after a short hyperpolarization (Fig. 1Ai). However, some cells showed only delayed complex spikes even after prolonged hyperpolarizations (Fig. 1Aii). Spontaneous complex spikes occurring in the middle or end of a prolonged spiking phase without a clearly preceding hyperpolarization were also observed in some cells. It was also possible to recognize prompt and delayed complex spikes in extracellular recordings (Fig. 1B; \( n = 65 \) cells) (Tzounopoulos et al. 2004). To summarize, we distinguished two main classes of spontaneous complex spike: prompt spikes arising quickly from a preceding hyperpolarization and delayed spikes arising soon after simple spike activity.

The repolarizing phase of a complex spike was steeper when there were more spikelets, as shown from comparison of prompt complex spikes with random variation in spikelet number observed in same cells (Fig. 1C). Sometimes the extra spikelet(s) was much slower than the preceding ones (“slow spikelet”) and appeared to occur from the repolarizing phase of the underlying slow depolarization (Fig. 1C, 3rd pair). These data suggest that current activated during spikelets drive repolarization of the complex spike; in a later section, we show that part of this current is due to SK \( K^+ \) channels.

The ability to fire complex spikes on depolarizing current injection distinguished all-single-spiking or silent CWCs from other neurons in the DCN (Fig. 1E). Most CWCs, when given incremental depolarizing current steps (Δ20–25 pA, 200–300 ms) from a quiescent state near −80 mV (with...
spikes throughout (the “onset” spike). Then with larger depolarizations, additional complex spikes arose after the onset spike, interspersed with simple spikes, and are termed “late” spikes. Late complex spikes became more frequent as depolarization increased (Fig. 2Aii). Occasional deviations from this pattern included, for example, cases in which the cells first gave rise to late complex spikes (as in Fig. 6Ai, top) and then with more current generated the onset spike. In some cells, the initial spike response to depolarizing steps was one or more complex spikes with or without following simple spikes (Fig. 2Aii). Stronger depolarizations made the next responses follow the more typical pattern. The onset spike for these cells occurred immediately on the stimulus regardless of the magnitude of stimuli, but for other cells, the onset spike appeared delayed when first seen at the threshold stimulus, and the delay decreased on increasing stimuli (Fig. 2Ai). To examine the dependence of complex spikes on the preceding potential, onset spikes were triggered with a two-step current protocol (Fig. 2Bi). As the conditioning $V_m$ was gradually made more positive than $-80 \text{ mV}$, the onset spike became delayed and eventually could not be triggered. The level of $V_m$ above which an onset spike (limited to those arising within 40 ms) could not be evoked with this protocol varied between $-70$ and $-65 \text{ mV}$ across different cells (Fig. 2Bii). A transient depolarization at the onset of a current step was often observed at stimulus levels subthreshold to the appearance of a delayed onset complex spike (Fig. 2Ai, 80 pA trace) or at failure of an onset spike (Fig. 2Bi, right bottom).

**Afterdepolarization as a trigger for complex spikes**

The waveform of CWC simple spikes had a characteristic bump-like afterdepolarization (ADP) following the end of repolarization (Fig. 1Ab) (Manis et al. 1994; Zhang and Oertel 1993a). When the first spikelet of a prompt complex spike and an isolated simple spike having the same threshold belonging to the same cell were superimposed, the waveforms appeared indistinguishable, except that the pFR of the former tended to be slightly (~1.5 mV) less negative than that of the latter. Considering this similarity and that the simple spike’s ADP peaks within 3–6 ms of the peak of the spike, it seemed possible that the prompt complex spike starts from a simple spike having a larger ADP such that the ADP triggers the second spikelet and the rest of a complex-spike waveform, the core motif. In the same manner, the delayed complex spike might arise where a simple spike’s ADP is not depolarized enough to give rise to a fast spike at its peak but enough that another simple spike arises on its decay phase, and so on, until the threshold for the core burst is reached on a spike’s ADP (Fig. 1A, b–d). Examples in which successive ADPs accumulate but decay without reaching such threshold are seen in the beginning of the fourth spike cluster in Fig. 1Aii and the 80-pA response in Fig. 2Ai. Is there a systematic relationship between the threshold for a fast spike, the peak of the ADP, and the probability of firing complex spikes? Spontaneous simple spikes’ thresholds and pFRs were measured for subsets of complex-spiking and all-simple-spiking CWCs (Table 1). The pFR was measured as an indirect index of the basal ADP level of simple spikes because the ADP itself appeared to vary in amplitude in some cells (supplemental Fig. 2). The mean pFR and the mean difference between pFR and threshold of spon-
taneous simple spikes were found to be significantly different between all-simple-spiking cells and complex-spiking cells ($P < 0.001$ for both), such that spontaneous complex spikes were not seen in cells in which the fAHP brought the membrane potential farther from threshold.

To explore this relationship further, we evoked single spikes with a short (<1.5 ms) current pulse while a bias current was maintained to keep the cell silent (at $V_m$ approximately −80 mV) before and after the spike. Although the pFR and the amplitude of ADP (the difference between the base $V_m$ and the peak of ADP) of spikes evoked by short pulses were variable among CWCs (Fig. 1D), the pFR was more negative (by 2.5 ± 1.2 mV, $n = 28$, paired $t$-test, $P < 0.001$), and the bump of the ADP was less apparent for spikes evoked by short pulses than for spontaneous spikes. In cells with more negative pFRs, a slow afterhyperpolarization reaching negative to −80 mV could be observed (12 of 115 cells examined with short pulses; Fig. 1Di). Short pulses elicited only simple spikes in most CWCs, including 2/3 (20 of 29) of the spontaneously complex-spiking cells tested, but they could also elicit complex spikes (Figs. 1Di and 11E, left) from some prompt complex-spiking cells and silent cells. These features will be used in the following sections to explore the effect of selective channel blockers.

**Effect of Na$^+$ channel block**

Application of the voltage-dependent Na$^+$ channel blocker TTX (0.5 μM) eliminated fast action potentials. TTX did not affect the resting potential of silent cells, but in spiking cells the $V_m$ in TTX ranged between −75 and −50 mV. For all-simple-spiking cells that fired quasi-regularly without base $V_m$ oscillation, the $V_m$ in TTX was less negative than the interspike trough potential ($V_{trough}$) before TTX by 10 ± 5.6 mV ($n = 20$). Depolarizing current evoked slow spikes in a pattern reminiscent of complex spikes (109 of 109 cells given TTX; Fig. 3A). Typically, a lone slow spike could be observed at the onset of a step depolarization, and then, with larger depolarizing steps, more slow spikes appeared (Fig. 4Aii). The slow spike at onset of depolarization appeared graded in amplitude with the amount of current injected, whereas the later-occurring ones were all-or-none. The threshold current for the late

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**TABLE 1. Simple spike threshold, pFR, and the difference between them**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Threshold, mV</th>
<th>pFR, mV</th>
<th>Threshold – pFR, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex-spiking cells</td>
<td>52</td>
<td>−60.0 ± 3.8</td>
<td>−65.9 ± 3.3*</td>
<td>5.9 ± 2.8*</td>
</tr>
<tr>
<td>All-simple-spiking cells</td>
<td>135</td>
<td>−60.1 ± 3.2</td>
<td>−68.9 ± 3.7*</td>
<td>8.8 ± 2.8*</td>
</tr>
<tr>
<td>Total</td>
<td>187</td>
<td>−60.1 ± 3.4</td>
<td>−68.0 ± 3.8</td>
<td>8.0 ± 3.1</td>
</tr>
</tbody>
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Values are means ± SD. *$P < 0.001$.
slow spikes was $293 \pm 83 \text{ pA (n = 21, } V_m \text{ held at } -80 \text{ mV})$, and this was $112 \pm 91 \text{ pA more than that required for the late complex spikes before TTX application in the same cell. A more detailed study of slow spikes is presented in later sections of RESULTS.}

CWCs have a persistent $\text{Na}^+$ current. When stepping to potentials less negative to $-70 \text{ mV}$ from a holding potential of $-80 \text{ mV}$, a persistent inward current was observed that was blocked by TTX (Fig. 3Bi). Accordingly, a negative slope conductance region existed in the steady-state current-voltage ($I-V$) plot of CWCs, which started between $-70$ and $-75 \text{ mV}$ extending to about $-55 \text{ mV}$ where the plot terminated due to limitation in voltage control (Fig. 3Bii). The $I-V$ plots varied in their vertical position with respect to the $0$-current axis. For many silent cells, the plot crossed $0$-current axis once at their resting potential with the negative slope region located above the $0$-current axis. On the other hand, the entire plot lay below the $0$-current axis for many spontaneously spiking cells. The current at the crest of $I-V$ plot was $-14 \pm 21 \text{ pA (n = 52)}$ for spiking cells and $78 \pm 51 \text{ pA (n = 32)}$ for silent cells ($t$-test, $P < 0.001$). TTX eliminated, or in some silent cells reduced the negative slope region (Fig. 3B). The difference currents obtained by subtracting the currents in the presence of TTX from those in its absence show that the persistent $\text{Na}^+$ current activated near $-75 \text{ mV}$ and reached $-139 \pm 31 \text{ pA (n = 15)}$ at $-55 \text{ mV}$. In current clamp, the $V_m$ was unstable above $-75 \text{ mV}$ and drifted in the depolarizing direction until cycles of fast spikes were generated. It is likely that this drift arose from activation of persistent $\text{Na}^+$ current. Similar observations were made with sharp electrode recordings (Hirsch and Oertel 1988).

**Effect of general $\text{Ca}^{2+}$ channel block and properties of the slow spikes**

$\text{Ca}^{2+}$ currents are believed to contribute to the underlying depolarization of complex spikes (Golding and Oertel 1997). We thus examined how the removal of regenerative $\text{Ca}^{2+}$ currents with $\text{Cd}^{2+}$ or by replacing $\text{Ca}^{2+}$ with $\text{Mg}^{2+}$ affected firing. As $\text{Cd}^{2+}$ ($200 \mu\text{M}, n = 3$; $100 \mu\text{M}, n = 2$) entered the recording chamber, there was a transient period of enhanced complex spiking in all CWCs (Fig. 5A, middle). After a longer exposure to $\text{Cd}^{2+}$, cells fired broadened simple spikes at high rates and in long clusters. Eventually only a two-spikelet pair remained at the onset of a step depolarization (Fig. 5A, bottom) or at the beginning of spontaneous, simple spike clusters. The simple spikes in $\text{Cd}^{2+}$ appeared broadened more toward their base, and comparison of the spike width at 40% of amplitude
yielded a more significant result than that of the half-width (40%-width, 0.45 ± 0.07 vs. 0.64 ± 0.17 ms, n = 5, paired t-test, P = 0.021; half-width, P = 0.047). Cd2+ also caused a hyperpolarization of \( V_m \) by <10 mV, but this was not studied further. Removal of external Ca2+ (replaced with Mg2+, \( n = 3 \)) also abolished complex spikes and broadened the base of simple spikes (40%-width, 0.52 ± 0.06 vs. 0.63 ± 0.08 ms, paired t-test, \( P = 0.01 \)). However, in the absence of Ca2+, CWCs eventually became depolarized and were unable to fire more than a few spikes even with a hyperpolarizing holding current.

In the presence of TTX, the slow spike triggered at the onset of a depolarizing step only occurred when the preceding potential was more negative than approximately –65 mV (Fig. 4B), suggesting that the onset spike had a lower threshold than later slow spikes (see also Fig. 4Aii). The slow spike at the onset will be referred to as the low-threshold spike (LTS), and the later ones, the high-threshold spike (HTS). Expecting that these TTX-insensitive slow spikes were mediated by Ca2+, Cd2+ was added (50 \( \mu M \), \( n = 2 \); 200 \( \mu M \), \( n = 4 \); 500 \( \mu M \), \( n = 3 \)). HTTs, but not LTS, were abolished by Cd2+ at all the concentrations tried (\( n = 9 \); Fig. 5B). The low-threshold nature and the relative resistance to Cd2+ suggest that T-type Ca2+ channels mediate LTS (Ertel 2004). Ni2+, a more efficient blocker of T-type Ca2+ channels, was therefore applied at 500 \( \mu M \) plus 100 \( \mu M \) Cd2+ (\( n = 1 \)), or alone at 500 \( \mu M \) (\( n = 1 \); Fig. 5Bi), or at 1 mM (\( n = 1 \)). Under these conditions, HTTs were eliminated and the maximum peak-to-trough amplitude of the LTS evoked with a series of hyperpolarizing presteps (as in Fig. 4B) was reduced by 73, 63, and 87%, respectively. Removal of external Ca2+ also eliminated all slow spikes in TTX (\( n = 3 \)). The differences in threshold and in sensitivity to Cd2+ and Ni2+ indicate that the LTS and HTs were Ca2+ spikes that were mediated by different subtypes of Ca2+ channels. The forms of Ca2+ spikes were not as stereotypical as those of Na+ spikes; the amplitude and width of HTs or LTS varied within a CWC depending on the \( V_m \) from which spikes were evoked and on the amount of current injected. HTTs were more frequent and larger when evoked from a more depolarized \( V_m \), and multi-peaked broader forms could be seen in some CWCs with threshold current injection (Fig. 4A). The magnitude of depolarizing current steps just sufficient to elicit HTs or LTS and the maximum amplitude of these spikes differed among CWCs, as did the onset and late complex spikes in control solutions. Moreover, the amplitude of the LTS varied between cells, and could be quite small (Fig. 10Bi).

To determine whether Ca2+ channels shape the ADP and fAHP, spikes evoked with short pulses of current were re-
corded in the presence of 100 μM Cd²⁺ plus 500 μM Ni²⁺ (Fig. 5C; n = 8). The short pulse-evoked fast spikes broadened toward the base of the spike (width at –40 mV, roughly just below half-amplitude level, 0.52 ± 0.06 ms vs. 1.16 ± 0.38 ms, n = 8, paired t-test, P = 0.001) and repolarized to less negative potentials. The changes in repolarization suggested involvement of Ca²⁺-mediated outward currents. To identify the subtype channels underlying these effects, further experiments were conducted with specific blockers of Ca²⁺ channel and Ca²⁺-activated K⁺ channel subtypes.

**Effect of intracellular Ca²⁺ buffering**

To test the possibility that Ca²⁺-activated K⁺ conductances served as repolarizing currents, intracellular Ca²⁺ was buffered using whole cell recording with an EGTA- or BAPTA-containing pipette solutions. As mentioned in METHODS, whole cell recording, with 0.1 mM EGTA, led to hyperpolarization, increased complex spiking, and a small depolarizing shift in pFR. Raising the EGTA to 5 mM or including 20 mM BAPTA caused progressive changes in spike waveforms far larger than those seen with 0.1 mM EGTA.

In recordings using 5 mM EGTA in the patch pipette, the pFR became less negative by 8.1 ± 1.4 mV after 15 min of dialysis (n = 6). Moreover, complex spikes occurred more readily with depolarizing current injection and had a broader appearance and several slow spikelets (Fig. 6Ai). The half-width of fast spikes after 15 min was 0.45 ± 0.03 ms (n = 6), which was not significantly longer than that measured from 6 whole cell recordings with an internal solution containing 0.1 mM EGTA (0.42 ± 0.04 ms, t-test, P = 0.16). In the presence of TTX, 5 mM EGTA, caused the HTSs to become prolonged and to acquire multiple peaks (n = 3; Fig. 6Aii). The LTS did not noticeably change except that an HTS often appeared to occur on top of the LTS (Fig. 6Aii). The effect of a faster Ca²⁺ buffer, BAPTA, at 20 mM, was also examined. Complex spikes broadened in 1 min after patch break-through (Fig. 6Bi, top), and then a marked loss of fAHP ensued. Simple spikes disappeared leaving only complex spikes consisting of a half-repolarizing fast spike and many slow spikelets (Fig. 6Bi, middle; n = 7). Eventually the pFR became very depolarized (by 41.3 ± 4.3 mV after >15 min; n = 7), and the complex spike waveform became narrower, appearing more like a broadened simple spike with inflections in its repolarizing...
phase (Fig. 6Bi, bottom, initial 2 spikes; half-width 1.22 ± 0.53 ms, n = 7). In the presence of TTX, a similar broadening-narrowing sequence in waveforms of HTSs was observed as BAPTA diffused intracellularly (Fig. 6Bii; n = 3).

**Effect of BK and SK channel block**

Because blocking Ca\(^{2+}\) channels or buffering intracellular Ca\(^{2+}\) slowed spike repolarization, we turned to selective blockers of Ca\(^{2+}\)-activated K\(^+\) channels, using iberiotoxin for the large-conductance Ca\(^{2+}\)-activated K\(^+\) (BK) channel and apamin for the small-conductance Ca\(^{2+}\)-activated K\(^+\) (SK) channel. Incubation in iberiotoxin (100 nM) led to spontaneous firing of complex spikes in all-simple-spiking cells (n = 6). In two cells that were silent and gave only simple spikes at rheobase (i.e., the smallest suprathreshold depolarizations), iberiotoxin also led to firing of complex spikes. Iberiotoxin generally increased firing of complex spikes (Fig. 7A), but the degree of increase was quite variable; among five spontaneously complex-spiking cells monitored with intra- or extracellular recording, the increase in spiking was 0, 116, 154, 390, and 536% (paired t-test, n = 5, P = 0.10). Along with this enhancement of complex spiking was a reduction in the fAHP (pFR depolarized by 13.9 ± 4.8 mV, n = 9; Figs. 7Ai, inset, and 7B) and increase in the half-width of fast spikes (0.42 ± 0.08 vs. 0.49 ± 0.11 ms, n = 9, paired t-test, P = 0.007). The less negative pFR in iberiotoxin was accompanied by a shortening of the first interspikelet interval for the one cell that had spontaneous, prompt, complex spikes (Fig. 7Ai, inset) and conversion of delayed onset complex spikes to prompt ones for other cells (Fig. 7Ai iii); prompt complex spikes appeared in spontaneous or evoked activity of cells that did not display such complex spikes in control conditions. The decrease in the first interspikelet interval for spontaneous prompt complex spikes, when jointly assessed with measurements from four extracellularly recorded complex-spiking cells, all of which displayed prompt complex spikes in control conditions (e.g., Fig. 7Aii), was significant (4.0 ± 0.5 vs. 1.8 ± 0.2 ms, paired t-test, n = 5, P < 0.001). The reduced fAHP and boosted complex spiking in iberiotoxin was also observed for spikes evoked by short pulses (n = 3). The three tested cells generated only simple spikes in control conditions; two of these became able to fire complex spikes robustly (Fig. 7B). In the presence of TTX, iberiotoxin increased the amplitudes of Ca\(^{2+}\) spikes (Fig. 7C, n = 7). The peak-to-trough amplitude of HTSs increased by 51 ± 17% (from 17.8 ± 4.4 mV, paired t-test, n = 7, P < 0.001), whereas the peak-to-trough time did not change (9.9 ± 2.0 vs. 10.0 ± 1.7 ms, paired t-test, n = 7, P = 0.8). For the LTS, four of the seven cells showed an increase in the peak-to-trough amplitude (by 70 ± 6%, paired t-test, P = 0.014). Among the other three cells that had no or barely noticeable LTSs, two gained a clear LTS in iberiotoxin. Thus BK channels played a key role in determining the shape of fast spikes and the likelihood of generating complex spikes.

Blockade of SK channels using apamin (100 nM, n = 7, or 50 nM, n = 9), also increased the tendency to fire complex spikes. Four of the five all-simple-spiking cells as well as complex-spiking cells (n = 5) started to fire broad, prompt or short-delay (preceded by just one simple spike with the interval <10 ms) complex spikes, isolated or clustered with more complex spikes and few interposing simple spikes (Fig. 8A). For the five complex-spiking cells and one extracellularly recorded complex-spiking cell, the frequency of spontaneous complex spikes increased in apamin (0.9 ± 0.8 vs. 2.7 ± 1.4 Hz, 15 - 1440% increase, n = 6, paired t-test, P = 0.015). Silent cells’ firing at rheobase also became predominantly complex spiking in apamin (n = 7 of 8 cells). The usual initial response to step current injections, either all simple spiking or an onset complex spike followed by simple spikes, became replaced in apamin with all complex spiking, whereas responses to larger current injections were also dominated by complex spikes (Fig. 8B). For the cell shown in Fig. 8B, a transitional effect of apamin as the drug washed in was also recorded (supplemental Fig. 3A): the train of simple spikes following the onset spike depolarized more steeply, giving rise to late complex spikes at a higher frequency than in control. Thus apamin appeared to impair CWCs’ ability to fire simple spikes with a stable V\(_{\text{th}}\).

We measured interspikelet intervals of spontaneous complex spikes and plotted these for successive spikes in the waveform. To monitor spikes of uniform shape, we only included those spikes preceded by >150 ms of silence; this was done for six cells including one extracellular recording. Figure 8C shows two sets of such plots for two cells, the second one (ii) extracellularly recorded. Both cells in control conditions had delayed complex spikes, starting with an interspikelet/spike interval >5 ms, and the first interval varied rather widely [7.6 ± 4.2 (n = 18) and 15.0 ± 3.6 ms (n = 73), for Fig. 8C, i and ii]. In apamin, both cells showed marked decrease in the duration and variability of the first interspikelet interval [3.8 ± 0.2 (n = 25) and 5.2 ± 0.9 (n = 92) ms, for i and ii], so that the complex spikes in apamin were prompt or of short delay. These changes in the first interspikelet interval in apamin occurred in all of the five complex-spiking cells and one extracellularly recorded cell (n = 6; Δduration, – 4.5 ± 3.5 ms, paired t-test; P = 0.025; F-test, P < 0.001 for every cell). When short pulse-evoked complex spikes were examined (n = 4, 2 of complex-spiking cells and 2 silent cells), the first interspikelet interval was also found significantly decreased by apamin (Fig. 8Dii; Δ – 0.9 ± 0.5 ms, paired t-test, P = 0.041). Compared with the effects of BK channel block, which also include the shortening of first interspikelet interval, the pFR and the half-width of fast spikes were not affected by apamin (Fig. 8D, i–iv; ΔpFR of short pulse evoked spikes, –0.1 ± 0.5 mV, n = 16, paired t-test, P = 0.23). However, the slope of the first interspikelet interval appeared steeper in apamin, and this may have led to a shortening of the interval (Fig. 8D, i and ii). The complex spikes in apamin appeared prolonged with more spikelets but repolarized to a lesser extent (Fig. 8D, i and ii).

To measure complex spikes’ duration, we summed the interspikelet intervals of each complex spike preceded by >150 ms of silent period, beginning from the first interval ≤5 ms to exclude the long intervals before the abrupt rise of underlying slow depolarization in delayed complex spikes. The sum of interspikelet intervals thus obtained from spontaneous complex spikes was compared between control and apamin-treated conditions. For the five complex-spiking cells and one extracellularly recorded cell, the complex spike duration increased from 6.6 ± 1.1 ms in control to 17.6 ± 8.8 ms in apamin (n = 6, 50 – 470% increase, paired t-test, P = 0.034), along with the change in the number of spikelets (number of included inter-
FIG. 7. Effect of BK channel block with iberiotoxin (ibtx), 100 nM. Ai, increased frequency of complex spikes during spontaneous activity in iberiotoxin compared with the control condition. Inset, depolarized pFR (↑) and shorter interspikelet intervals of complex spikes in ibtx (red) were shown by superimposing indicated spikes on the left. Ai: extracellular recording of a complex-spiking cell. Two prompt complex spikes along with simple spikes are shown in the control trace, whereas in ibtx all spikes are complex spikes. Inset: indicated complex spikes shown in expanded scales to reveal a decrease in the first interspikelet interval by ibtx. Aii: less negative pFR and facilitation of complex spiking by ibtx shown in current step responses. Note change from a delayed complex spike to 2 prompt spikes at onset. B: using short stimulus pulses, complex spikes were triggered in ibtx in a cell for which only simple spikes were seen in control solution. Three traces are superimposed for each condition; 50-pA bias current in both conditions. C: ibtx enlarged the amplitudes of both the LTS and HTSs in TTX, 0.5 μM. Traces in A–C are from 5 different cells.
vals +1) from 3.3 ± 0.5 in control to 6.7 ± 2.8 in apamin (n = 6, 40 – 360% increase, paired t-test, P = 0.045).

In 9 of 16 cells treated with apamin, short pulses that initially evoked simple spikes continued to do so in apamin although their spontaneous activity or response to longer step depolarizations became predominantly complex spiking. However, the simple spikes triggered by short current pulses in the presence of apamin revealed a larger, or more slowly decaying, ADP compared with that in control conditions. The control complex spikes in ii can be seen to consist of 2 groups of delayed complex spikes, one starting with 1 simple spike (short-delay) and the other with 2 simple spikes. D: overlaid complex or simple spikes in control (black) and apm (red). i: complex spikes indicated by arrowheads in A. Note the complex spike in apm is longer in duration but repolarizes less than the one in control conditions. This was also observed in short pulse-evoked complex spikes from a different cell. Dii: Bias, −105 pA in both conditions. Diii: an example of a cell that converted, on short pulses, from a simple spike-responder to a complex spike-responder by apm. Bias, −105 pA in both conditions. Div: enhanced ADP for short pulse-evoked simple spikes by apm. Each superimposed trace is truncated averaged waveform from a representative cell. Bias: −95 (control), −85 pA (apm). Dv: depolarizing shifts in the Vm at 10 ms from the peak of the evoked spike (vertical dashed line in Div) in 9 cells. E: changes in HTSs in TTX, 0.5 μM, by apm, 100 nM, shown for 2 different cells, i and ii. HTSs were broadened with more peaks (asterisks) and slower repolarization (arrows). The cell in ii had a prestep Vm of −75 mV.
unclear whether this was due to incomplete block of BK channels by iberiotoxin, as the sensitivity of BK channels to iberiotoxin may vary with subunit composition (Meera et al. 2000), or due to consequences of whole cell dialysis or extremely low intracellular Ca\(^{2+}\). It is also possible that the eventual narrowing of complex spikes with 20 mM BAPTA may not be related to the block of BK and SK channels because multi-spikelet waveforms persisted during the combined application of apamin and iberiotoxin (extracellular recording, \(n = 3\); not shown).

**Specific block of subtype Ca\(^{2+}\) channels**

**N-TYPE.** Application of \(\omega\)-conotoxin-GVIA (1–3 \(\mu\)M), which specifically blocks N-type channels had no effect on either the firing pattern or on the shape of spikes (\(n = 2\) without TTX, \(n = 3\) with TTX).

**P/Q-TYPE.** The presence of P/Q type Ca\(^{2+}\) channels was probed with \(\omega\)-agatoxin-TK, \(\omega\)-agatoxin-IVA, or \(\omega\)-conotoxin-MVIIIC. \(\omega\)-agatoxin-TK (100–200 nM; \(n = 16\)), \(\omega\)-agatoxin-IVA (200 nM; \(n = 1\)) and \(\omega\)-conotoxin-MVIIIC (2–3 \(\mu\)M; \(n = 5\)) each caused increased complex spiking. Thirteen all-simple-spikeing cells as well as 2 complex-spikeing cells started to fire clusters of complex spikes and higher frequency simple spikes in the presence of these toxins (Fig. 9A). The frequency of spontaneous complex spikes for the two complex-spiking cells and three extracellularly recorded complex-spiking cells was increased in P/Q channel blockers (0.8 ± 0.6 vs. 1.9 ± 0.8 Hz, \(n = 5\), paired \(t\)-test, \(P = 0.043\)). The spontaneous complex spikes in the presence of P/Q channel blockers were often broader with more spikelets than those in control conditions (Fig. 9Aii). Plots of interspikelet intervals versus their duration were generated and analyzed as with apamin-treated cells (Fig. 9B). The duration of spontaneous complex spikes for one of the two complex-spikeing cells and three extracellularly recorded cells, increased from 4.4 ± 0.9 to 10.0 ± 1.2 ms in P/Q channel blockers (\(n = 4\); paired \(t\)-test, \(P = 0.011\)). The number of spikelets included was 2.7 ± 0.5 in control and 3.8 ± 1.2 in the drug (\(n = 4\), paired \(t\)-test, \(P = 0.083\)). The duration of the first interspikelet interval, unlike with apamin, did not decrease (Δ 3.9 ± 5.0 ms, \(n = 4\), paired \(t\)-test, \(P = 0.22\)). The one complex-spikeing cell not included in the preceding text fired only two-spikelet complex spikes after >150 ms of hyperpolarized periods in control condition and continued to do so in P/Q channel blocker but with increased occurrence of delayed complex spikes following the two-spikelet one. For this cell, the interspikelet interval of the two-spikelet complex spikes increased from 4.7 ± 0.6 (\(n = 23\)) to 5.5 ± 0.9 ms (\(n = 20\); \(P = 0.002\)). In the response to depolarizing current pulses, P/Q channel blockers caused faster trains of simple spikes, such that the maximum frequency of simple spike train not giving rise to a late complex spike during a 325-ms current step was increased (78 ± 30 vs. 115 ± 55 Hz, \(n = 15\), paired \(t\)-test, \(P = 0.001\)). Also, the late complex spikes appeared at a lower current levels in the presence of P/Q channel blockers (140 ± 78 vs. 37 ± 33 pA, \(n = 12\), paired \(t\)-test, \(P < 0.001\), suggesting that the excitability of CWCs had increased (Figs. 9C and supplemental Fig. 3C). The late complex spikes were broadened, while the onset spike did not appear so (Figs. 9C and supplemental Fig. 3C). The lowering of the threshold for complex spikes by P/Q channel blockers was reminiscent of the effects of apamin (supplemental Fig. 3, A and B vs. C), suggesting that the Ca\(^{2+}\) influx through P/Q-type Ca\(^{2+}\) channels serves to activate SK channels. However, differences between the effect of P/Q blockers and that of apamin were noted in short pulse-evoked spikes, in induced complex spikes, and in Ca\(^{2+}\) spikes. P/Q blockers caused the pFR of short pulse-evoked spikes to become less negative by 1.8 ± 1.4 mV (\(n = 11\), paired \(t\)-test, \(P = 0.002\)), whereas apamin did not affect the pFR. The less negative pFR led to a more depolarized ADP (Fig. 9D), and 1 of the 10 cells that under control conditions responded to short pulses with only simple spikes became able to fire complex spikes. In one cell, the averaged pFR of spikes evoked by short pulses did not change in the presence of P/Q channel blockers; in this cell, the amplitude and decay of the ADP also were unaffected by P/Q channel blockers. All-simple-spikeing cells were induced to fire spontaneous complex spikes along with slow \(V_m\) oscillations by both the SK and P/Q-type Ca\(^{2+}\) channel blockers. A difference in these cases between the block of these two channels was that the complex spike occurring after a hyperpolarized phase was prompt or just briefly delayed when SK channels were blocked, but only delayed when P/Q channel were blocked (Figs. 8B, left traces vs. 9Ai or supplemental Fig. 3, B vs. C).

The shapes of Ca\(^{2+}\) spikes recorded in the presence of TTX were also affected by P/Q channel blockers (Fig. 9E). \(\omega\)-Agatoxin-TK changed the waveform of HTSs without affecting the LTS; HTSs became broader with slowed repolarization and afterhyperpolarization (peak-to-trough time, 10.8 ± 2.6 vs. 15.9 ± 3.1 ms, \(n = 7\), paired \(t\)-test, \(P = 0.005\)), and reduced peak-to-peak amplitude (15.1 ± 2.3 vs. 12.1 ± 2.5 mV, \(n = 7\), paired \(t\)-test, \(P < 0.001\)). Compared with the effect of SK channel blocker on HTSs, P/Q block caused the peaks of HTSs to become less distinct or attenuated.

**L-TYPE.** Block of L-type Ca\(^{2+}\) channels with the dihydropyridine, nifedipine, (2–10 \(\mu\)M; \(n = 14\)) did not cause notable changes in firing patterns of eight spontaneously spiking cells except for slightly depolarizing the base \(V_m\). Six all-simple-spikeing cells fired at higher (Δ 2–10 Hz) frequencies. The pFR and ADP of spikes evoked by short current pulses were unchanged by the presence of nifedipine (ΔpFR 0.3 ± 0.6 mV, \(n = 6\); paired \(t\)-test, \(P = 0.26\)). However, in responses to longer depolarizing current pulses, half (7 of 14) of the cells became devoid of the late complex spikes, leaving only an onset complex spike followed by a simple spike train (Fig. 10A). In the other half of the cells, nifedipine slightly or moderately depressed the late complex spikes (Fig. 10C, top 2 traces). Increasing the concentration of nifedipine (≤20 \(\mu\)M) did not further attenuate the late complex spikes for these cells. In many cells treated with nifedipine, accommodation of fast spikes and development of a plateau depolarization occurred during responses to current steps of 0.5–0.7 nA, whereas in control solutions, currents in excess of 0.7 nA would be required for a comparable response. This plateau depolarization may result from inhibition of voltage-dependent K\(^{+}\) channels, which has been reported for dihydropyridines including nifedipine (Fagni et al. 1994; Grissmer et al. 1994).

We then examined the effect of nifedipine on Ca\(^{2+}\) spikes in TTX (\(n = 19\)). As with the onset complex spike, the LTS was not affected by nifedipine. By contrast, HTSs were eliminated
FIG. 9. Effect of P/Q-type Ca\(^{2+}\) channel block with ω-agatoxin-TK (agtx). A: change in spontaneous activity induced with agtx, 100 nM. Ai: all-simple-spiking cell developed clusters of simple and complex spikes. Aii: complex-spiking cell fired more complex spikes with more spikelets. Fast spikes are larger in amplitude in the agtx trace because of greater perforation by gramicidin with time. Insets: expansion of spikes under asterisks. Calibration, 10 mV and 10 ms. B: plots of interspikelet interval vs. its duration for complex spikes preceded by >150 ms of silent period. Two extracellularly recorded cells, i and ii. Number of plotted complex spikes: 61 for both conditions, i and 41, 50 for control and agtx, ii. The number of spikelets and the duration of complex spikes increased in agtx for each cell. An example of spike waveform in control and agtx is shown for ii. Calibration, 50 pA and 10 ms. C: increase in excitability in the presence of agtx, 100 nM, as shown by increased tendency to fire complex spikes and more depolarized base \(V_m\) during a 100-pA step. Note the broader late complex spikes in agtx. A different cell from those in A. Bias currents were 23 (control) and 34 pA (agtx). D: small depolarizing shifts of pFR in agtx, 100 nM, as observed from short pulse-evoked spikes. Three superimposed traces of truncated spikes are shown for each condition for two different cells, i and ii. Bias currents: –25, –20, 23, 23 pA from left to right. E: superimposed Ca\(^{2+}\) spikes in 0.5 mM TTX (black) and in TTX plus 200 nM agtx (red) from 3 different cells. agtx caused the HTS to become broader with reduced afterhyperpolarization and less distinct peaks. The LTS (asterisk), was not affected. Spikes were evoked with a 400-pA (left, middle) or 500-pA (right) current steps and the prestep \(V_m\) was ~65, ~71, and ~74 mV from left to right.
or largely depressed when evoked by current steps from a relatively hyperpolarized $V_m$ (more negative than approximately –70 mV) in nifedipine (Fig. 10Bi). However, at depolarized holding potentials, from which evoked HTSs are inherently more robust (Fig. 4A), slower regenerative events ranging in appearance from distinct spikes to small ($\leq 5$ mV peak-to-peak) irregular oscillations could be seen in nifedipine across different cells. These nifedipine-resistant regenerative events or spikes were found to be abolished by 200 nM $\alpha$-agatoxin-TK when the drug was added along with nifedipine and TTX to cells that showed relatively distinct events (peak-to-peak amplitude $>5$ mV; $n = 6$; Fig. 10Bii). We then examined how P/Q blockers affect nifedipine-resistant late complex spikes. One all-simple-spiking cell and one silent cell (Fig. 10C), both of which showed late complex spikes in responses to injection of depolarizing current pulses in nifedipine, developed spike clusters during spontaneous and evoked activity, respectively, when $\alpha$-agatoxin-TK (100–200 nM) was added, as often seen with P/Q blockers applied alone (Fig. 9A). The spike clusters in these cases, however, often included a plateau depolarization that sometimes required hyperpolarizing current injection to reinstate spike activity (Fig. 10C, bottom 2 traces). For these two cells, TTX was added subsequently, and absence of regenerative events was confirmed. In these cells and five other cells for which P/Q blockers were applied first followed by addition of nifedipine, late complex spikes could no longer be seen in response to current steps, replaced with plateau depolarizations. Thus these data indicate that both P/Q- and L-type channels may be required for late complex spikes during a long depolarizing stimulus.

**ROLE OF LOW-VOLTAGE-ACTIVATED CA$^{2+}$ CHANNELS IN GENERATION OF COMPLEX SPIKES.** Several lines of evidence suggesting the presence of a low-threshold inactivating conductance and its contribution to complex spiking in CWCs have been presented in this study so far. 1) The LTS, a presumptive T-type Ca$^{2+}$ spike, could be observed in the presence of TTX in many CWCs. 2) The onset complex spike evoked by step depolarizations depended on a previous hyperpolarized $V_m$ (more negative to approximately –65 mV). And 3) in the context of the spontaneous slow $V_m$ oscillation, complex spikes were more frequently observed after hyperpolarized periods. Another line of evidence in the same context was found when the amplitude of a clearly distinguishable LTS was compared retrospectively between complex-spiking and all-simple-spiking cells. The maximum peak-to-trough amplitude was significantly larger in the complex-spiking cells than in all-simple-spiking cells ($12.8 \pm 5.2$ (n = 22) vs. $8.4 \pm 3.3$ mV (n = 41),

![FIG. 10. Effect of L-type Ca$^{2+}$ channel block. A: example of late complex spike suppression by nifedipine (nif), 2 µM. B: in TTX, 0.5 µM, HTSs evoked by depolarization from resting potential were eliminated by 10 µM nif. Bii, in the same cell, current step from a depolarized holding potential revealed nif-resistant spikes, which were abolished after subsequent addition of $\alpha$-agatoxin-TK (agttx), 200 nM. C: addition of agttx, 200 nM, in addition to nif, 5 µM, (bottom 2) made nif-resistant late complex spikes (2nd from top) disappear, but instead caused broad plateau depolarizations. Bias currents: 0, –25, –40, –40 pA from top to bottom. A–C: 3 different cells. Synaptic blockers were not present in A.](http://jn.physiology.org/).
Mibebradil has been shown to block T-type and some forms of R-type Ca\(^{2+}\) channel more effectively than other Ca\(^{2+}\) channel subtypes (Bezprozvanny and Tsien 1995; Martin et al. 2000). Mibebradil, in the presence of TTX, reduced the maximum peak-to-trough amplitude of LTSSs (protocol as in Fig. 4B) by 76, 82, and 53% for three cells (at 10, 3, and 3 \(\mu\)M, respectively) without an apparent change in the shape of HTSSs (Fig. 11C). Spontaneous or evoked spiking was monitored with 1.5–3 \(\mu\)M mibebradil to reduce mibebradil’s effects on ion channels other than the Ca\(^{2+}\) channels (Bezprozvanny and Tsien 1995; Eller et al. 2000; Martin et al. 2000). We have observed that mibebradil caused small depolarization of \(V_m\) when used in the presence of TTX, and indeed increases in firing frequency were noted in six all-simple-spiking cells in mibebradil. In five complex spiking cells and two silent cells, all of which showed prompt complex spikes in their spontaneous or induced activity in control conditions, the prompt spikes were lost in mibebradil (Fig. 11A). Where prompt complex spikes preferentially occurred in the context of slow \(V_m\) oscillation in control conditions, delayed complex spikes or simple spikes became prominent. In three cells that also fired complex spikes in the middle of spiking phases of slow \(V_m\) oscillation, such complex spikes persisted but with longer trains of preceding simple spikes in mibebradil (Fig. 11Ai). Similarly, in responses to current pulses, late complex spikes did not appear different but the onset complex spikes were variably affected. Among 21 cells treated with mibebradil, 10 cells, including 5 of the 6 all-simple-spiking cells, stopped firing complex spikes at the onset of depolarizations.

T-type and/or R-type Ca\(^{2+}\) channels may contribute to the ADP in CWC. In some cells, it was clear that ADPs were larger for simple spikes at the beginning of a spike cluster that terminated a period of hyperpolarization (supplemental Fig. 2). Spikes evoked by short pulses were inspected for changes in the pFR and ADP caused by mibebradil (3 \(\mu\)M), and the result is plotted in Fig. 11D for 14 cells. Nine cells fired only simple spikes, one cell fired both complex and simple spikes (Fig. 11E), and four cells fired only complex spikes. In the 10 cells that had simple spike responses in control conditions, the peak potential of ADP became more negative by mibebradil (\(\Delta\)ADP peak 2.4 ± 3.3 mV, \(n = 10\), paired \(t\)-test, \(P = 0.047\)). For the four cells that responded to short pulses with complex spikes, two failed to fire complex spikes in mibebradil, but the other two continued to generate three-spikelet complex spikes which had longer first interspikelet intervals than those of control complex spikes (from 3.0 to 3.9, from 2.2 to 2.9 ms, respectively). For pFR, a small but significant negative shift was observed in mibebradil (\(\Delta\)pFR −1.1 ± 1.1 mV, \(n = 14\), paired \(t\)-test, \(P < 0.001\)).

There was a confounding factor in the action of mibebradil on spike activity, aside from the \(V_m\) depolarization, which could be controlled with injection of a negative bias current. Mibebradil caused fast spike thresholds to increase by 1.4 ± 0.9 mV (at 1.5 \(\mu\)M, \(n = 4\)) or 2.9 ± 0.9 mV (at 3 \(\mu\)M, \(n = 8\)). A higher threshold for fast spikes would be expected to decrease the efficiency of generating a complex spike from a simple spike’s ADP. We therefore applied TTX at 10 nM to block Na\(^{+}\) channels partially so that fast spikes would persist with elevated thresholds. Spontaneous fast spikes became smaller in amplitude and their thresholds were raised by >10 mV in 10 nM TTX. Spikes evoked by short pulses in 10 nM TTX were smaller in amplitude and required larger currents than in control conditions (Fig. 11F), but neither pFR nor the ADP peak level was changed significantly (\(\Delta\)ADP peak 0.2 ± 1.5 mV, \(n = 5\), \(P = 0.74\); \(\Delta\)pFR 0.0 ± 0.6 mV, \(n = 6\), \(P = 0.84\); paired \(t\)-test for both). One of the cells that fired three-spikelet complex spikes on depolarization with short pulses in control conditions, fired complex spikes with two-spikelets in 10 nM TTX with the interspikelet interval longer than the first interspikelet interval of control complex spikes (not shown). Under voltage clamp, a reduction in the persistent inward current was observed with 10 nM TTX (\(n = 2\), by 36 and 56 pA, which were 40 and 36% of the mean 500 nM-TTX-sensitive, persistent current). Thus although mibebradil’s depression of prompt or onset complex spikes can arise partly from an elevation of fast spike threshold, the lack of 10 nM TTX’s effect on ADP of isolated spikes evoked from a hyperpolarized \(V_m\) suggests that mibebradil’s primary action on complex spike suppression may be block of a low-voltage-activated Ca\(^{2+}\) current that contributes to the ADP. We suggest therefore that such channels facilitate complex spike generation after hyperpolarized periods by enhancing the ADP.

**Discussion**

**Ionic mechanisms underlying spike waveforms**

Blocking all Ca\(^{2+}\) conductances in CWCs suppressed complex spikes while increasing excitability and weakening simple spike repolarization. Buffering of intracellular Ca\(^{2+}\) also markedly affected spike shape. We explored the basis of these effects by selectively blocking subtypes of Ca\(^{2+}\) channels and Ca\(^{2+}\)-dependent K\(^{-}\) channels. Our results are summarized in Fig. 12. The repolarization of the fast Na\(^{+}\) spike requires BK channels in addition to voltage-dependent K\(^{-}\) channels. BK channels presumably contribute more toward the late part of the spike because BK block, as well as general block of Ca\(^{2+}\) channels, made pFR less negative and broadened the base of the spike. The source of Ca\(^{2+}\) for BK channels is probably mainly from R-type Ca\(^{2+}\) channels and in small part from the P/Q-type channels because among the subtype-specific blockers we have used, only P/Q-type blockers reduced pFR. On the other hand, mibebradil, which blocks T-type and some forms of R-type channels, caused a small negative shift of pFR along with a decrease in ADP for spikes evoked from hyperpolarized \(V_m\). This suggests that the low-voltage-activated Ca\(^{2+}\) current, when available, provides depolarization at the end of a fast spike, which adds to the ADP. The ADP of a simple spike decays to the trough level between spikes; this can be viewed a second afterhyperpolarization (Manis et al. 1994; Zhang and Oertel 1993a). The SK channel may participate in this process as block of this channel made the trajectory of single evoked spikes’ ADP less negative and caused interspike intervals to progressively depolarize during a train of simple spikes. The major supply of Ca\(^{2+}\) for SK channels during interspike intervals at depolarized \(V_m\) is probably from the P/Q-type Ca\(^{2+}\) channel as P/Q-type blockers caused similar depolarization of interspike intervals. For single spikes evoked from a hyperpolarized \(V_m\), SK channel activation during ADP decay may occur by the Ca\(^{2+}\) influx through the T- or R-type channel (Metz et al. 2005; White et al. 1989).

Figure 12 shows a prompt onset spike and a late spike evoked from a hyperpolarized \(V_m\) by a current step. The LTS
FIG. 11. Effect of T-type Ca$^{2+}$ channel block with mibe-fradil (mib). A: prompt complex spikes (asterisk) disappeared in 1.5 μM mib. i, spontaneously complex-spiking cell; ii, silent cell spiking by sustained +100 pA injection. Note in ii that short-delay complex spikes (rectangle) are also lost. B: attenuation of the onset complex spike with slowed rising phase and intact late complex spikes in mib, 3 μM. Bias currents: –45 (control) and –50 pA (mib). C: the LTS, asterisk, in 0.5 μM TTX, was inhibited selectively by mib, 3 μM. D: changes in the $V_m$ at the peak of ADP (ADP peak) and pFR of short pulse-evoked spikes in mib. Red symbols, cells that showed complex spike responses in control condition. E: a cell that had a large ADP and occasionally fired complex spikes with short pulses became unable to do so after its ADP had become smaller in mib, 3 μM. Four traces are overlaid for each condition. –15 pA bias currents for traces in mib. F: for comparison, an example of effect of 10 nM TTX on short pulse-evoked spikes is shown. 10 nM TTX did not affect the ADP amplitude or the pFR, but the partial inhibition of Na$^+$ conductance was evident from the smaller amplitude of evoked spikes and the larger pulse current required to evoke spikes. Four spike traces and 1 passive response (gray) are superimposed for each condition. –20-pA bias currents for both conditions. A, i and ii, B, C, E, and F: six different cells.
and HTS are thought to share their Ca\textsuperscript{2+} conductance with the onset and late complex spike, respectively, so the different susceptibility of these Ca\textsuperscript{2+} spikes were taken into account in deducing the conductance underlying different complex spikes. The onset complex spike, which is a low-threshold response, was delayed at just-suprathreshold current injection in many all-simple-spiking cells and became prompt with larger current injection. The onset spike, whether delayed or not, was unaffected in the presence of N-, L- or P/Q-type Ca\textsuperscript{2+} channel blocker alone or L- and P/Q-type blockers together. However, mibefradil at concentrations exerting partial block of T- or R-type channels eliminated or attenuated the onset spike. Considering that the LTS is mediated by the T-type Ca\textsuperscript{2+} channel, given its resistance to Cd\textsuperscript{2+}, the T-type current may be essential in generating the onset complex spike. The T-type Ca\textsuperscript{2+} current was probably active at the end of a single spike evoked by a short pulse from a hyperpolarized \( V_{\text{th}} \) because mibefradil also attenuated the ADP. The transient subthreshold depolarization seen before the rise of a delayed onset spike (Fig. 2Ai, 80 pA trace) with a long depolarizing current pulse may then be caused by directly evoked T-type current or summated spike aftercurrent. It remains unclear whether T-type channels alone mediate the full spectrum of onset spikes, from delayed to prompt, or whether a higher-threshold Ca\textsuperscript{2+} current, the R-type, adds to the depolarization from T-type current to drive the steep upslope of the underlying envelope (the core motif). This distinction is further obscured as mibefradil has been reported to block the R-type current from expressed Ca\textsubscript{2.3} channels or in cerebellar granule cells with a potency lower than or comparable to that for the T-type (Bezprozvanny and Tsien 1995; Jimenez et al. 2000; Randall and Tsien 1997). The observation that only a vestigial onset complex spike could be seen in Cd\textsuperscript{2+} supports a role for R-type current.

For the late complex spike, L-type and P/Q-type Ca\textsuperscript{2+} currents together may provide the underlying depolarization with a larger contribution from the L-type current. Blockade of L-type channels eliminated late complex spikes in half of the cases, and blocking the L- and P/Q-type channels together abolished all high-threshold regenerative events in TTX. It was not clear, however, whether, after blocking these channels in the absence of TTX, the late complex spike was suppressed or just prevented by an overwhelming plateau depolarization. If one was to view the disappearance of the HTSs as well as late complex spikes in L-plus-P/Q-blockers as mere depolarization block, the alternative interpretation could be that the L-type Ca\textsuperscript{2+} current’s role was largely in providing the slow depolarization course of \( V_{\text{th}} \) leading to the steep rise of the late complex spike or the HTS. In this view, the HTS could be mediated mainly by an R-type Ca\textsuperscript{2+} current with small contribution of L- and P/Q-type current; however, the R-type current in this role would have to be of higher-threshold than the L-type current and could not be of low-threshold. The SK channel is believed to contribute to the termination of the underlying slow depolarization of complex spikes, as apamin broadened most complex spikes. The fact that block of the P/Q-type Ca\textsuperscript{2+} current caused similar broadening of complex spikes indicates that this current flows during complex spikes and activates the SK channel. The apparently unaffected duration of onset complex spikes during block of P/Q-type Ca\textsuperscript{2+} or SK channel may be due to the presence of a voltage-dependent K\textsuperscript{+} current activated at the onset of a current step.

This discussion has so far focused on evoked onset and late complex spikes with obvious low- and high-threshold characteristics. In spontaneous activity, prompt or short-delay complex spikes preceded by clear hyperpolarized periods were suppressed in mibefradil and thus are considered to share the same ionic mechanism with the onset spike. On the other hand, the equivalent of late complex spikes in spontaneous activity, delayed spikes that are preceded by >100 ms of depolarized (\( V_{\text{th}} \) less negative than ~70 mV) activity, were rare, so we could not examine the effect of blockers on them. However, spontaneous delayed spikes of intermediate characteristics, with a hyperpolarization ending 50–100 ms before the complex spike (like some in Fig. 11Aii, control), were not uncommon, and when treated with mibefradil, these complex spikes became less frequent. Two possible interpretations are that these intermediate delayed complex spikes are also mediated by T- or T- plus R-type Ca\textsuperscript{2+} current but were not completely blocked by low concentrations of mibefradil or that spontane-
ous complex spikes are not only generated by either low- or high-threshold Ca\textsuperscript{2+} currents but rather that the two types of Ca\textsuperscript{2+} current can cooperate to generate complex spikes.

Why do some CWC not show spontaneous complex spikes? The increased tendency for complex spiking was found with those blockers that, in single evoked spikes, reduced fAHP (BK blocker) or increased ADP (SK blocker), whereas one that reduced ADP (T-type blocker) decreased complex spikes. These, along with the finding that spontaneously complex-spiking cells had less fAHP in their simple spikes than did all-simple-spiking cells, suggest that more depolarized ADPs facilitate the generation of complex spikes in CWCs, as suggested for other bursting neurons in different brain areas (Brumberg et al. 2000; Franceschetti et al. 1995; Jung et al. 2001; Metz et al. 2005; Nishimura et al. 2001; White et al. 1989; Wong and Prince 1981; Yue et al. 2005; Yue and Yaari 2004). Different balances of spike aftercurrents that regulate ADP size, BK and SK versus T/R-type Ca\textsuperscript{2+} current across different CWCs can be one reason for the variable propensity for complex spiking. Another factor can be the difference in the ability to fire with slow \(V_m\) oscillation, the mechanism of which may involve an interaction of subthreshold conductances (Del Negro et al. 2002; Williams et al. 1997; Wilson 2005). Almost all complex-spiking cells we have observed showed intermittent hyperpolarizations after which a complex spike was often recognized. Similar to CWCs in this relationship of slow \(V_m\) oscillation and low-threshold complex spike are the thalamocortical neurons and thalamic reticular neurons, although in these neurons, the T-type Ca\textsuperscript{2+} spike is the primary event that sustain the bursts (Crnueili et al. 2005; McCormick and Bal 1997). Still other possibilities include a difference in dendritic membrane contributions, such arises from variation in the size or number dendrites (Chagnac-Amitai et al. 1990; Mainen and Sejnowski 1996; Mason and Larkman 1990) provided that dendritic electrogenesis contributes to complex spike generation. In neocortical and hippocampal pyramidal cells, simultaneous dendritic and somatic recordings, as well as computer models, have shown that dendritic depolarization by electrotonically or actively propagated somatic action potential contributes to the somatic ADP, which can trigger a burst when boosted by a more depolarizing event in the dendrite, such as a Ca\textsuperscript{2+} spike and/or excitatory postsynaptic potential (Golding et al. 1999; Larkum et al. 1999; Mainen and Sejnowski 1996; Traub et al. 1991; Williams and Stuart 1999).

**Persistent Na\textsuperscript{+} current and its contribution to firing**

A persistent Na\textsuperscript{+} current was apparent in CWC as a TTX-sensitive, negative slope conductance in steady-state I-V plots. This current was manifest in current-clamp mode as a depolarizing trend in \(V_m\) starting at around –75 mV. The occasional nondecaying subthreshold responses from which a fast spike would arise (e.g., Fig. 11F, control trace) and additional firing of fast spikes during decay of a depolarized ADP (Fig. 5C) may reflect the \(V_m\) being amplified to a critical range by persistent Na\textsuperscript{+} current (Hirsch and Oertel 1988). Thus it is expected that without persistent Na\textsuperscript{+} current, the rheobase for silent cells would be larger, and conversely, spiking cells would require less hyperpolarizing current to be quieted. The persistent Na\textsuperscript{+} current may contribute to ongoing spike discharge by providing a depolarizing ramp before the fast upstroke of each spike (Fig. 12). Indeed it has been shown in a variety of neurons that TTX-sensitive currents flow during interspike intervals in studies employing action potential waveforms as voltage commands (Do and Bean 2003; Raman and Bean 1999; Taddese and Bean 2002). Taddese and Bean (2002) have suggested that the persistent Na\textsuperscript{+} current is generated from the same Na\textsuperscript{+} channel responsible for the transient Na\textsuperscript{+} current and so that all neurons that fire Na\textsuperscript{+} spikes have the “inntrinsic drive” to repetitive discharge, resulting in spontaneous firing as long as the current is not counteracted by a resting K\textsuperscript{+} conductance. For CWCs, the presence of subthreshold conductances active below the threshold of the persistent Na\textsuperscript{+} current is suggested by variation in our \(I-V\) plots: although the persistent Na\textsuperscript{+} current showed a similar activation range across cells, silent cells had their \(I-V\) curve shifted up, whereas in spiking cells, it was shifted down. The inward rectifier K\textsuperscript{+} current and hyperpolarization-activated cation current (\(I_h\)) are present in CWCs, and inhibition of each led to depolarization or hyperpolarization of the \(V_m\), respectively (unpublished observations). The balance of these conductances along with any leak conductance may be important in determining the level of activity for CWCs.

**Comparison with the Purkinje cell**

It is of interest to compare the ionic mechanism of complex spiking in CWC with that of cerebellar Purkinje cells as these are often considered to be molecularly and functionally related cell types (Berrebi and Mignaini 1991; Berrebi et al. 1990; Mignaini and Morgan 1987). We identified a number of differences on comparing Purkinje cell spikes from the literature or recorded by us with those of CWCs. For example, simple spikes of Purkinje cell are narrower in half-width (–0.25 vs. 0.4 ms of CWCs), repolarize faster, and do not show an ADP comparable to that of CWCs (Manis et al. 1994; unpublished observations). The fast repolarization of the Purkinje cell simple spike, attributable to the expression of Kv3 channels, enables tonic high-frequency (>40 Hz) firing (Ake-\=mann and Knopfel 2006; Martina et al. 2003; McKay and Turner 2004), which CWCs cannot reliably do without giving rise to late complex spikes. The inability of CWC to emit long periods of regular activity suggests a different role in information processing when compared with Purkinje cells. With strong current injection, or even spontaneously in some cells, Purkinje cells discharge mixed bursts of Na\textsuperscript{+} and Ca\textsuperscript{2+} spikes (Ca\textsuperscript{2+}-Na\textsuperscript{+} burst), similar to the generation of late complex spikes in CWCs; moreover, both cells produce Ca\textsuperscript{2+} spikes in TTX that occur in similar patterns to their respective burst events, the Ca\textsuperscript{2+}-Na\textsuperscript{+} burst or late complex spikes (Llinas and Sugimori 1980; McKay and Turner 2004; Womack and Khodakhah 2002). However, Ca\textsuperscript{2+} spikes of Purkinje cells are mediated by the P-type channel, and unlike in CWCs, the low-threshold Ca\textsuperscript{2+} spike has been rarely seen in somatic recordings except in immature cells and in cultured cells (Cavelier and Bossu 2003; Llinas et al. 1989; McKay and Turner 2005; Watanabe et al. 1998) even though the T-type current could be isolated at the soma (Isoppe and Murphy 2005; McDonough and Bean 1998). The fast rising CWC complex spike (the prompt spike) and Purkinje cell complex spike seem comparable at first glance. One notable difference was that with Purkinje cells, the second spikelet, arising from a depo-
larized level, was often the smallest in the entire waveform (Khaliq and Raman 2005). By contrast, in CWC spikes, the last (i.e., the 3rd or 4th) spikelet was the shortest; in this respect, the CWC complex spikes may appear more like those of hippocampal or neocortical pyramidal cells (Khaliq and Raman 2005; Mansivais et al. 1994). The shape of the underlying depolarization is likely to be responsible for this difference: a fast rising synaptic potential in Purkinje cells versus a more gradual depolarization triggered from the ADP of the first spikelet in CWCs. This difference may bear on the frequency of information transferred to the axon. Indeed the propagation of complex spikes down the axon has been reported to be correlated with the spikelet amplitude with the shortest one propagating least well (Khaliq and Raman 2005; Monsivais et al. 2005). Thus it is expected that the initial two to three spikelets of a CWC complex spike would be more reliably transmitted than those of Purkinje cell complex spikes down the axon; these would appear as the multi-peaked high-frequency postsynaptic potential transferred to the axon. Indeed the propagation of complex spikes down the axon is expected that the initial two to three spikelets of a CWC complex spike would be more reliably transmitted than those of Purkinje cell complex spikes down the axon; these would appear as the multi-peaked high-frequency postsynaptic potentials that are seen in target cells of CWCs (Golding and Oertel 1997; Tzounopoulos et al. 2004). Therefore, although the CWC and Purkinje cell, share many molecular markers and similar electrophysiological hallmarks, they may utilize a different balance of ion channels, and this difference could bear on the meaning of their spike output.

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