Activation and Deactivation of Voltage-Dependent K⁺ Channels During Synaptically Driven Action Potentials in the MNTB

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Klug, Achim and Laurence O. Trussell. Activation and deactivation of voltage-dependent K⁺ channels during synaptically driven action potentials in the MNTB. J Neurophysiol 96: 1547–1555, 2006. First published June 14, 2006; doi:10.1152/jn.01381.2005. K⁺ channels shape individual action potentials and determine their pattern of firing. In auditory relays, both high- and low-voltage–activated K⁺ channels (HVA and LVA) are critical for preservation of auditory timing cues. We examined how these channels participate in firing in the medial nucleus of the trapezoid body. Principal cells at physiological temperature were voltage clamped using spike waveforms previously recorded in response to calyceal firing. Current components were isolated by digital subtraction of traces recorded in the channel antagonists dendrotoxin-I or tetraethylammonium. During orthodromic spikes delivered at 300 and 600 Hz, both currents activated with a slight delay, peaking just after the crest of the spike. The decay of HVA was sufficiently fast to match the time course of the spike. By contrast, with 300 Hz stimuli, LVA continued to decay after the spikes reached a stable interspike potential. Although LVA currents partially inactivate during prolonged voltage steps, their peak amplitudes remained stable or increased during trains of spikelike stimuli. At 600 Hz, LVA did not fully deactivate between the spikes and therefore generated a leak current. To determine the effect of blocking LVA channels on spiking, prerecorded postsynaptic conductances were injected, with and without dendrotoxin-I. After block of LVA channels, strong synaptic conductances produced broader spikes, with tetraethylammonium also broadened spikes but led to less error in timing. These results reveal multiple roles for LVA channels in spike repolarization and timing during synaptic activity.

INTRODUCTION

The preservation of action potential timing related to acoustic signals is accomplished by diverse morphological and biophysical specializations in cells of auditory brain-stem nuclei (Trussell 1999). In mammalian bushy cells and octopus cells of the ventral cochlear nucleus, and principal cells of the medial nucleus of the trapezoid body (MNTB), small depolarizations from the resting potential lead abruptly to activation of a large outward K⁺ current (Bal and Oertel 2001; Brew and Forsythe 1995; Manis and Marx 1991). A very similar current plays a prominent role in bushy cells of the chick and their downstream targets in the nucleus laminaris (Kuba et al. 2003; Rathouz and Trussell 1998; Reyes et al. 1994, 1996). A consequence of this low-voltage–activated current (LVA) is that neurons may generate only one spike for each stimulus, regardless of the duration of the stimulus. Moreover, the onset of the LVA is just slow enough to reduce temporal summation of staggered synaptic inputs and can endow neurons with the ability to detect the rate of rise of a stimulus (Ferragamo and Oertel 2002; Kuba et al. 2003). Accordingly, many studies have shown that pharmacological or genetic alteration or elimination of the channels that generate LVA leads to supernumerary and ill-timed spiking in some of the aforementioned cell types (Brew et al. 2003; Kopp-Scheinpflug et al. 2003; Leao et al. 2004).

Although these studies highlight the critical role of these channels and their component subunits, it remains unclear how the LVA participates in the electrical activity of the neurons during synaptic activity. The general picture is that a fast synaptic current triggers the spike but then quickly subsides, so that intrinsic membrane properties shape the rest of the spike (Joshi et al. 2004). The spike waveform is subsequently repolarized by a high-voltage–activated current (HVA) sensitive to tetraethylammonium (TEA), whereas the dendrotoxin (DTX)-sensitive LVA acts afterward to prevent secondary spikes. Previous studies depended almost entirely on the response to simple voltage and current pulses to reach these conclusions about the role of K⁺ currents; however, neurons in vivo are driven by excitatory synaptic currents (EPSCs), whose waveforms do not resemble regular square pulses of current. For example, repetitive synaptic activity leads to progressive synaptic depression and development of small depolarizing plateaus (Taschenberger and von Gersdorff 2000; Zhang and Trussell 1994). Moreover, previous studies of the LVA used brain slices maintained at room temperature, which may differentially slow the various current components of the electrical response of neurons (Cao and Oertel 2005).

We have taken a different approach to determining the role of LVA and HVA in the activity of neurons of the rat MNTB. Synaptically driven spike trains were recorded and used as voltage-clamp commands to determine the amplitude and time course of pharmacologically isolated ionic components of the spike train. In addition, glutamatergic synaptic currents were used to generate waveforms for conductance-clamp experiments to determine how loss of the LVA alters postsynaptic responses. In this way, postsynaptic effects of channel blockers could be differentiated from their potential presynaptic effects (Dodson et al. 2003; Ishikawa et al. 2003). We found that the LVA and HVA provide similar peak current during spikes trains, but that, as a result of its slower decay kinetics, LVA is the dominant outward current between spikes and acts as a leak...
current during high-frequency activity. LVA also plays a key role in repolarizing the orthodromic action potential and in minimizing variation in spike latency.

**METHODS**

**Slice preparation**

Coronal slices of brain stem were prepared from postnatal day 13–18 Wistar rats. Animals were briefly anesthetized by isoflurane inhalation (Isoflo, Abbott Laboratories, Abbott Park, IL) and decapitated according to approved methods. The brain stem was dissected out under ice-cold dissection ringer (125 mM NaCl, 2.5 mM KCl, 1 mM MgCl$_2$, 0.1 mM CaCl$_2$, 25 mM glucose, 1.25 mM Na$_2$PO$_4$, 25 mM NaHCO$_3$, 0.4 mM ascorbic acid, 3 mM myo-inositol, and 2 mM pyruvic acid; all chemicals from Sigma, St. Louis, MO). Sections (250 μm) were cut with a vibratome (VT100S, Leica, Deerfield, IL). Slices were transferred to an incubation chamber containing extracellular solution (ECS) (125 mM NaCl, 2.5 mM KCl, 1 mM MgCl$_2$, 2 mM CaCl$_2$, 25 mM glucose, 1.25 mM Na$_2$PO$_4$, 25 mM NaHCO$_3$, 0.4 mM ascorbic acid, 3 mM myo-inositol, and 2 mM pyruvic acid; all chemicals from Sigma) and bubbled with 95% O$_2$–5% CO$_2$. Slices were incubated for 1 h at 37°C, after which the chamber was brought to room temperature for further incubation before recording. Recordings were obtained within 4–5 h of slicing.

**Whole cell recordings**

When ready for use, slices were transferred to a recording chamber and continuously superfused with ECS at 3–4 ml/min through a gravity-fed perfusion system. All recordings were performed at 36–37°C except as indicated. MNTB neurons were viewed through a Leica DMLS 2 microscope equipped with DIC optics and a ×63 water-immersion objective (Leica). Whole cell recordings were made with an Axopatch 200B Amplifier (Axon Instruments, Foster City, CA). Signals were filtered at 5–10 kHz and subsequently digitized at 20–100 kHz using Clampex 9.0 software (Axon Instruments). Current clamp was performed using the *Jpf* mode. Action potentials were similar in size and shape to those recorded in MNTB using other amplifiers (e.g., Scott et al. 2005; Taschenberger and von Gersdorff 2000). Patch pipettes were pulled from 1.2-mm borosilicate thin-walled glass (WPI, Sarasota, FL) using a Sutter P-97 electrode puller (Sutter Instruments, Novato, CA). Pipettes were filled with a solution containing 113 mM K-glucurate, 4.5 mM MgCl$_2$, 9 mM HEPES, 5 mM EGTA, 14 mM Tris-2-phosphocreatine, 4 mM Na$_2$ATP, 0.3 mM Tris-GTP, and 1.5 mM CaCl$_2$ at pH 7.3. Series resistance was between 2 and 8 MΩ (average 4.4 MΩ) and was compensated between 70 and 99% (average 95.1%) with a lag time of 7–15 μs. Cell capacitance values ranged between 8 and 24 pF (mean 15.3). Thus the voltage-clamp time constant should be <25 μs for these recordings. All voltages are corrected for a −12-mV junction potential.

**Pharmacology**

During voltage-clamp recordings, 500 nM tetrodotoxin (TTX, Alomone Labs, Jerusalem, Israel) was added to the ECS to eliminate sodium currents. For recordings in which dendrotoxin-I (DTX, Sigma)–sensitive currents were measured (i.e., the LVA), 5 mM tetraethylammonium (TEA) chloride (Sigma) was added to the ECS to block HVA channels (Wang et al. 1998). DTX was applied by puffing ECS containing 500 nM DTX onto the cell, using a large-tip glass pipette and a Picospritzer II (Parker Instruments, Fairfield, NJ). The success of blocking LVA currents was confirmed either by checking voltage steps before and after the puff (voltage-clamp recordings) or by comparing the cell’s response to current steps before and after the puff (current and conductance clamp recordings; e.g., Fig. 1, A and B). Because DTX washes out very slowly, it was sufficient to puff for only a brief period of time and then record for ≤30 min during which DTX-sensitive currents remained completely blocked. Application of DTX in this manner shifted the zero-current holding potential positive by 2.4 ± 0.5 mV and caused a 2.2 ± 0.9-fold increase in the resting input resistance (measured ±5 mV around the zero-current potential) in three cells tested. Isolation of HVA was performed by measuring currents before and after bath application of 5 mM TEA, after application of DTX to block LVA.

TEA may block both the HVA current mediated by Kv3.1-containing channels and also Ca$^{2+}$-activated K$^+$ channels (K$_{Ca}$). However, Brew and Forsythe (1995) showed that reduction in extracellular Ca$^{2+}$ did not alter outward currents in rat MNTB, suggesting K$_{Ca}$ is not prominent in these cells. Because TEA broadens action potentials in MNTB (Wang et al. 1998), TEA-sensitive channels are activated during the spike and regulate its duration. Consistent with Brew and Forsythe’s conclusions, we found that bath application of 100 μM Cd$^{2+}$ had no effect on spike width (P = 0.7, n = 4 cells). Thus it is unlikely that K$_{Ca}$ contributes to the currents underlying the spike waveform.

**Voltage-clamp stimuli**

Voltage-clamp recordings were performed by clamping the neurons either with spike waveforms or voltage steps using pClamp 9 software (Axon Instruments) (e.g., Fig. 1, C and D). At physiological temperature, trains of action potentials at frequencies of 300 and 600 Hz were tested. These waveforms were recorded previously from MNTB neurons at physiological temperature in current-clamp mode, while stimulating the afferent fibers that form the calyces. Stimulus artifacts were digitally removed from the traces before using them as stimulus waveforms. We observed that between spikes the degree of repolarization back to the rest varied among cells, presumably as a result of the different degrees of clearance of residual transmitter (Zhang and Trussell 1994). We thus decided to use the 300-Hz waveform data from a cell in which the potential did not come fully back to rest between spikes (with residual depolarization ~6 mV), to test the effect on deactivation of LVA. For 600-Hz traces, where deactivation of current would be expected to be incomplete from spike to spike, we chose a cell in which the spikes fully repolarized, to test the maximal residual activation of LVA between spikes. Excitatory postsynaptic potentials (EPSPs) typically depressed at high stimulus rates (Taschenberger and von Gersdorff 2000), which was apparent in the 600-Hz trains as a delay in spike onset and a reduction in spike amplitude (Fig. 1, E and F). The decline in amplitude presumably reflects both cumulative Na$^+$ channel inactivation and inactivation during the rising phase of the EPSP. EPSPs delivered at high frequency at room temperature often fell subthreshold (Taschenberger and von Gersdorff 2000). To test the activation of currents at room temperature, we therefore had to record orthodromic spikes at low frequency and concatenate the resulting waveforms to generate a 300-Hz train. This was then used as a stimulus waveform for voltage clamp.

**Voltage-clamp analysis**

K$^+$ currents were determined by recording the total currents in response to the spike waveforms before and after applying TEA or DTX and digitally subtracting the responses from each other. Additionally, immediately after responses to a given spike waveform were recorded, an inverted and 20X-reduced, but otherwise identical, control waveform was injected. These smaller voltage commands were used to evaluate leakage (ohmic) currents and linear capacitative currents and were performed for both the predrug and postdrug recordings. Responses to these inverted “mini” waveforms were multiplied by 20 and digitally added to the respective responses to the actual waveforms before subtracting the predrug–postdrug responses.
from each other. Thus the reported current waveforms were calculated with the following equation:

\[
\text{Reported current} = [\text{Response to Waveform X in control}
\]
\[+ (20 \times \text{Response to Mini Waveform X in control})]
\[- [\text{Response to Waveform X in blockers}
\]
\[+ (20 \times \text{Response to Mini Waveform X in blockers})]
\]

This procedure is illustrated for a representative cell in Fig. 2.

**Conductance-clamp experiments**

Excitatory postsynaptic conductances (EPSGs) were simulated with a SM-1 amplifier (CambridgeConductance, Cambridge, UK). The 10–90% rise of the current output in response to a voltage change for this amplifier is given by the manufacturer as 290 ns. Reversal potentials were set to 0 mV for the EPSGs. The conductance waveforms used were previously recorded as EPSCs in voltage-clamp mode. Waveforms used for experiments performed at physiological temperature were also recorded previously at physiological temperature. After blanking the artifacts, EPSGs were normalized such that the peak in the waveform corresponded to a conductance of 200 or 80 nS. These peak conductances were chosen to simulate the range of published values for excitatory conductances at the mature calyx of Held (e.g., Taschenberger and von Gersdorff 2000). Synaptic conductances had decay time constants ranging from 0.23 to 0.26 ms for the 300-Hz train and 0.25 to 0.33 ms for the 600-Hz train. Sample conductance waveforms (300 and 600 Hz at physiological temperature) are illustrated in Fig. 1, G and H.

**RESULTS**

**Voltage-clamp experiments**

The activation and deactivation of LVA and HVA were assessed by delivering voltage waveforms modeled after recordings of orthodromic action potentials (see METHODS). The

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**FIG. 1.** Firing of medial nucleus of the trapezoid body (MNTB) neurons and stimulus waveforms. A: response to 1-nA current step in control solution. B: same cell after a puff of 500 nM dendrotoxin (DTX), 1 min before recording data. C and D: 300- and the 600-Hz spike waveforms used for the voltage-clamp experiments. E and F: 10 spike waveforms in each train were overlaid to illustrate changes in timing and amplitude. Excitatory postsynaptic potential (EPSP) is apparent (*) late in the 600-Hz trains due to synaptic depression. G and H: 300- and 600-Hz conductance waveforms used in the conductance-clamp experiments.
drug-sensitive component of this current was then obtained by
digital subtraction. Figure 3 shows current responses from
representative cells to the spikelike voltage waveforms driven
at either 300 Hz (Fig. 3, A and C) or 600 Hz (Fig. 3, B and D).
These frequencies were chosen to span the range of moderate
to very high level of activity that MNTB may experience in
vivo (Kopp-Scheinflug et al. 2003; Spirou et al. 1990). Be-
cause of the subtractive procedure, this approach removes
background levels of LVA current activation, and so the traces
begin at zero current. Currents (shown in black) are overlaid
with their respective voltage commands (shown in red) and
scaled to make apparent the relative changes in the sizes of the
peak waveforms during the train. Action potentials declined in
amplitude during the course of the trains, presumably resulting

FIG. 2. Method for extracting low-voltage–activated (LVA) current during a 300-Hz spike train. Bath contained
500 nM tetrodotoxin (TTX) and 5 mM tetraethylammo-
nium (TEA). A: raw current response to the 300-Hz spike
waveform. Trace reflects ionic, leak, and capacitative
currents. B: raw leak current response to the inverted spike
waveform at 1/20 reduction. C: sum of currents in A and
B after multiplying the current in B by 20. D: 1/20
waveform after applying DTX. This was 10–15 min after
recording in A. E: sum of the current in D (×20) and the
response to a full-size spike waveform (not shown). Re-
sulting current is small, indicating that almost no voltage-
gated current remains in DTX, TTX, and TEA. F: current
after subtracting C – E. These are the subtracted traces of
pure DTX-sensitive current. A similar procedure was
made to record TEA-sensitive currents, in which case
DTX was applied initially, and currents were recorded
before and after bath application of TEA.

FIG. 3. High-voltage–activated (HVA) and LVA
currents during orthodromic spike waveforms. A and
B: HVA responses to 300- and 600-Hz spike stimuli
in one cell. Black lines show current from a repre-
sentative cell; red line is the voltage waveform. As-
terisks (*) mark peaks of currents for selected stimuli.
C and D: LVA responses to 300- and 600-Hz spike
stimuli in a different cell. Black lines show current
from a representative cell; red line is the voltage
waveform. Asterisks (*) mark peaks of voltages for
selected stimuli. Peaks of current and voltage in A–D
are scaled to the peaks of the first response. Arrow in
D marks level of LVA current between spikes. E:
voltage and HVA and LVA currents (from represen-
tative cells) for the 1st and 7th spike in a train are
scaled to illustrate the difference in timing between
these waveforms. Red line is voltage; black is HVA;
and green is LVA.
Means shown are * indicates peak of spike waveform, with corresponding axis on the right. Gray line shows average currents for the two frequencies and shows that, although the HVA produces more current than LVA during the first spike, by the end of the train the two components are not statistically different. Normalizing the currents in Fig. 4, C and D, indicates that LVA decayed with time constants nearly twice those of HVA.

A consequence of this slower decay is apparent in the traces of Fig. 3D, showing that, for 600-Hz stimuli, the LVA current did not decay back fully to prestimulus levels between each spike (see arrow in figure). Indeed, on average 200 ± 60 and 27 ± 20 pA current remained between spikes 9 and 10 for 600- and 300-Hz stimuli, respectively (n = 10). By contrast, the average HVA current was 79 ± 24 and 34 ± 20 pA for 600- and 300-Hz trains, respectively (n = 6). Thus LVA does not deactivate completely during the highest frequency activity and therefore contributes a rate-dependent shunt that may suppress continued excitation.

Additional experiments were conducted to assess the current amplitudes and waveforms at room temperature. Orthodromic spikes were recorded at room temperature and concatenated to generate a 300-Hz train (see METHODS); these spikes were 0.7 ms in half-width, as compared with 0.4 ms for spikes recorded at physiological temperature. Figure 6 summarizes the results for isolation of LVA and HVA current responses. Representative traces are shown in Fig. 6A, which illustrates that currents rose with a slight delay relative to the spike, and for the first spike the LVA peaked after the HVA. Deactivation was incomplete between spikes so that peak currents grew during the initial part of the train. Several differences were apparent when compared with currents at physiological temperature. Currents from the cumulative inactivation of Na⁺ current (see also Jung et al. 1997). During the decline in spike height, the peak HVA current also declined (* in Fig. 3, A and B). By contrast, LVA changed little in amplitude or increased in amplitude. Thus despite the apparent inactivation of LVA observed during prolonged voltage steps (Brew and Forsythe 1995; Rathouz and Trussell 1998), the current is relatively stable during normal spiking activity. Current amplitudes and their change during trains is quantified in Fig. 4, for HVA (n = 6; filled circles) and LVA (n = 10; open circles). Figure 4, A and C shows average currents for the two frequencies and shows that, although the HVA produces more current than LVA during the first spike, by the end of the train the two components are not statistically different. Normalizing the currents in Fig. 4, B and D and scaling to the amplitude of the spikes (gray line) shows again that the HVA declines sharply as the spike height falls, whereas LVA shows a transient increase. The increase in LVA was significant for the second spike at 600 Hz (P < 0.025) and spikes 2–4 at 300 Hz (P < 0.01).

Figure 3E shows the waveform for the first and seventh spikes in a 300-Hz train, superimposed on the respective LVA and HVA currents from two cells. These reveal that both currents activate with a slight delay with respect to the voltage (in red), peaking just after the crest of the spike. HVA (in black) decayed quickly essentially following the time course of the spike. By contrast the LVA (in green) decayed more slowly, such that substantial current remained after the spike had decayed. These kinetics are quantified in Fig. 5 for currents at both frequencies. The time courses of the currents were measured in two ways. In Fig. 5, A and B the half-widths of currents (HVA: open symbol; LVA: filled), from foot to peak, were measured and compared with the half-widths of spike (gray line). These data show that the current half-widths are similar to those of the spikes, but that LVA may be slightly slower at the lower frequencies. However, examination of records as in Fig. 3E suggests that greater differences in current waveforms are apparent after they have decayed >50%. We therefore also fit exponential decay curves to the individual current responses to each spike during the trains. These results, shown in Fig. 5, C and D, indicate that LVA decayed with time constants nearly twice those of HVA.
of both types were about one third as large, and the durations were about twice as long, as at the higher temperature. In Fig. 6B amplitudes are shown for the first and tenth responses in the trains. These data show that even by the end of the train the HVA current was still about twice as large as the LVA current. The similarity in half-widths and fast decay constants in the two currents was surprising; we attribute it partly to the fact that some of the LVA must be active before the rise of spikes (especially the later spikes; see following text), and therefore follows the spike waveform more closely. This could account for why the LVA peaks earlier as the train proceeds in Fig. 6A.

Recordings at room temperature revealed that both HVA and LVA persisted to some extent between the 300-Hz spikes. Measurements of current just before the tenth spike in the train gave 80 ± 13 pA of LVA (n = 5) and 128 ± 19 pA of HVA (n = 4) current. Thus LVA current between spikes was similar to that observed at physiological temperatures (79 pA; see above), whereas the HVA current was much larger (compare with 34 pA). Examination of the current after the train provided an explanation (Fig. 6A, arrow) for these changes. At room temperature, a slower exponential component of decay became apparent, as summarized in Fig. 6B. Both currents had fast decay constants of about 0.5 ms, but LVA also had a slow component of 3 ms (25% of the fit) and HVA had a slow component of 16 ms (9% of the fit). Given the larger peak amplitude of HVA, this slow component must summate to produce the larger residual current between the spikes. Note that very little, if any, such current was seen at 300 Hz at physiological temperature (Fig. 3, A and C). Slow LVA and HVA time constants similar to these have been reported for bushy cells in the ventral cochlear nucleus (Rothman and Manis 2003b) at room temperature.

**Conductance-clamp experiments**

To examine the consequences of loss of postsynaptic LVA for orthodromic transmission, conductance-clamp waveforms were injected based on trains of synaptic currents recorded at physiological temperature (see METHODS). These were delivered at two peak conductance levels, 200 and 80 nS, to explore the effects of either very strong or more modest calyceal transmission. For comparison, these conductances would correspond to EPSCs of 14 and 5.6 nA, respectively, at a V_held of −70 mV, similar to values obtained previously in rat (Ishikawa et al. 2003; Taschenberger and von Gersdorff 2000). In Fig. 7, A and B, the voltage responses to 300- and 600-Hz conductance-clamp waveforms are shown before and after application of DTX. In general, the results differed from what might be predicted based on the effects of DTX on the response to a single, long current step (Fig. 1, A and B). After application of DTX, spikes decayed more slowly (Fig. 7, C and D) and occurred with a more variable latency from the onset of each synaptic conductance waveform in a train (Fig. 7, E and F). We expect that from, trial to trial, spikes in the presence of DTX are triggered irregularly and thus some of the earliest-occurring responses in the train may have been only EPSPs unaccompanied by spikes. This could account for the enhanced jitter in the responses. Repolarization tended to be incomplete and led to large plateau depolarizations and shorter amplitude spikes. These effects derive at least in part from the persistence of synaptic current between stimuli; although this current is small, it apparently has significant depolarizing power in the absence of the LVA. Despite this level of depolarization, the cells still fired on each stimulus early in 300-Hz trains of stimuli. For the 300-Hz waveforms, the number of spikes fired versus the
number of stimuli was only slightly increased by DTX. This effect on spiking was quantified by counting in 10 sweeps the number of spikes observed associated with the first five stimuli in each sweep (50 total). In 15 control cells, there were 49 ± 1 spikes for 200-nS stimuli and 46 ± 3 for 80-nS stimuli, consistent with the weaker EPSPs. In DTX, the 200- and 80-nS sweeps produced 62 ± 3 and 52 ± 2 spikes, respectively, among 14 cells. One cell in the presence of DTX became unable to generate discrete spikes to 300-Hz stimuli. For 600-Hz EPSGs, the summating effect of residual synaptic current in DTX was so great that in some cells no obvious spikes occurred after the first stimulus (Fig. 7B). Thus loss of LVA resulted in an increase in spike number, duration, and jitter for low-frequency stimuli and an apparent failure to spike at higher stimulus frequencies. We then repeated the conductance-clamp experiments in the presence of 5 mM TEA and examined the width and timing of spikes in a 300-Hz train of orthodromic conductance waveforms. Figure 8 shows that spikes were significantly broader throughout the train (Fig. 8, A and B), but that the timing of the spikes relative to the peak of the conductance waveform was unaltered (Fig. 8, C and D). Thus the HVA has a significant role in spike repolarization, although its effect on timing is less apparent than that of the LVA.

**Discussion**

In this study, the activation and deactivation of the LVA was shown to have kinetics distinct from that of HVA. Both currents activated with a slight delay and peaked just after the peak of the spike, similar to Hodgkin–Huxley models of delayed rectifier activation during the action potential (Hille 2001). However, HVA at physiological temperature deactivated so fast that its later falling phase seemed to follow the time course of the spike. By contrast, the LVA had slower decay kinetics and, after each spike reached its stable interpulse spike potential, the LVA continued to decline. Assuming LVA and HVA are indeed the primary conductances opposing inward Na+ and synaptic current, LVA must therefore be the only stabilizing current active between trains of orthodromic spikes at these frequencies. This persistent LVA current must add to LVA active at the resting potential, as evidenced by the depolarization observed on addition of DTX (see METHODS). LVA was maintained or even grew slightly in peak amplitude during the first few spikes in a train, despite the fact that it inactivates during steady depolarizations (Brew and Forsythe 2001). However, HVA at physiological temperature deactivates so fast that its later falling phase seemed to follow the time course of the spike. By contrast, the LVA had slower decay kinetics and, after each spike reached its stable interpulse spike potential, the LVA continued to decline. Assuming LVA and HVA are indeed the primary conductances opposing inward Na+ and synaptic current, LVA must therefore be the only stabilizing current active between trains of orthodromic spikes at these frequencies. This persistent LVA current must add to LVA active at the resting potential, as evidenced by the depolarization observed on addition of DTX (see METHODS). LVA was maintained or even grew slightly in peak amplitude during the first few spikes in a train, despite the fact that it inactivates during steady depolarizations (Brew and Forsythe 2001).
1995; Rathouz and Trussell 1998). This growth of current during spike trains was even more apparent in room-temperature recordings, where current decays were slower. The rise in current (e.g., spikes 1–5 in Fig. 4D) may reflect a failure to fully deactivate between spikes; during synaptically driven activity at physiological temperature, some LVA conductance remains active and adds to the current activated by succeeding spikes.

The consequences of incomplete deactivation were especially apparent when very high frequency spike activity was tested, such that LVA current fell no lower than 200 pA between spikes. This result suggests that during a train part of the LVA became a K+ “leak” current; to the extent that this fraction of LVA increases during trains of spikes, the spike threshold should gradually rise. This residual LVA current is similar in absolute magnitude to the baseline level of synaptic current preceding each EPSC in a train. For the synaptic conductances we injected, this amounts to a few percent of the peak conductance, corresponding to several hundred picamperes. Thus the residual K+ current seems necessary to oppose residual synaptic current. Although not relevant to the MNTB, such an ongoing K+ current might have consequences for related neurons such as globular bushy cells or octopus cells, which feature summing EPSPs. In those cases, it has been proposed that the kinetics of LVA endows the cells with sensitivity to rate of rise of voltage, thus filtering synaptic potentials that summate more slowly (Ferragamo and Oertel 2002). In a background of high-frequency synaptic activity, we suggest that this sensitivity would be obscured by a tonically activated LVA.

Conductance-clamp experiments revealed specific roles for the LVA. Although the LVA is generally associated with spike adaptation, we find that it also contributes significantly to spike repolarization, such that DTX resulted in a 42% increase in spike width and an enhanced jitter in the timing of the spike’s peak. Moreover, in DTX, spikes could not fall as deeply toward rest between synaptic stimuli because residual synaptic current exerted a powerful depolarizing influence. These results point toward different ways in which LVA is used: a phasic role in control of spike shape and a tonic role in shunting synaptic current. The voltage activation range of the LVA, which has a \( V_{1/2} \) of about −43 mV (Brew and Forsythe 1995; A Klug, unpublished observations), is thus optimized to serve two functions during synaptic activity.

Several differences were observed in our results compared with those of previous studies, which we attribute both to recording at physiological temperatures and to using more realistic stimuli for current activation. In other studies of either rat or mouse MNTB, block of LVA had little effect on the width of the action potential, suggesting that HVA plays the dominant role in repolarization (Brew and Forsythe 1995; Brew et al. 2003; Dodson et al. 2002). However, at the second spike in a 300-Hz train, DTX broadens the spike by 69 and 88% for the 200- and 80-nS synaptic stimuli, respectively (Fig. 7, C and D). After blocking the HVA with TEA, the spike was broadened to a similar extent (last spike in train: 76%; Fig. 8B). However, comparison of Figs. 7A and 8A reveals quite different effects of the channel blocking agents: loss of the LVA slows the initial decay of the spike but also completely prevents full repolarization arising from residual synaptic current between stimuli. Spike broadening after LVA block may thus reflect a loss of repolarizing current and secondary effects of protracted depolarization. An additional difference in our recordings is in the relative magnitude of LVA and HVA. Previous studies found that HVA is the dominant current in MNTB or bushy cells (Brew and Forsythe 1995; Rathouz and Trussell 1998; Wang et al. 1998; but see Rothman and Manis 2003a). However, using spike stimuli at physiological temperatures suggests that HVA does not activate as fully as LVA and is more sensitive to declining spike amplitudes; thus by the end of a train of stimuli (Fig. 4) the peak currents are not significantly different. Their similar amplitudes and time course of activation with physiological temperature and stimuli could account for why they have similar effects on spike half-widths.

The experiments we have described were designed specifically to evaluate LVA and HVA function in the soma and proximal axon in MNTB neurons, to avoid complications of studying synaptic responses when both pre- and postsynaptic channels are blocked pharmacologically. In fact, it is now clear that both channel components play roles in shaping presynaptic function in the MNTB. Electrophysiological and immunohistochemical studies show that Kv1.1 and 1.2 subunits, components of LVA, are expressed in the preterminal axon of the calyx, whereas the calyceal terminal membrane harbors Kv3.1, a primary component of HVA (Dodson et al. 2003; Ishikawa et al. 2003). Blockade of HVA broadened presynaptic spikes and increased exocytosis (Ishikawa et al. 2003). By contrast, no effect was seen on calyceal spike width or transmitter release when LVA was blocked. Nevertheless, after blocking LVA there was an enhanced sensitivity to the spike’s afterdepolarization, which led to supernumerary spikes (Dodson et al. 2003; Ishikawa et al. 2003). We found that postsynaptic spikes at physiological temperature are broadened after LVA block and that LVA may indeed activate rapidly enough to participate in repolarization. Given the profound sensitivity of the transmitter release to spike size and shape (Bollmann and Sakmann 2005; Borst and Sakmann 1999), it may be that LVA couldmodify synaptic strength under some physiological conditions.

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REFERENCES


