In mammals, principal neurons of the medial superior olive (MSO) exhibit biophysical specializations that enable them to detect sound-localization cues with microsecond precision. In the present study, we used whole-cell patch recordings to examine the development of the intrinsic electrical properties of these neurons in brainstem slices from postnatal day 14 (P14) to P38 gerbils. In the week after hearing onset (P14–P21), we observed dramatic reductions in somatic EPSP duration, input resistance, and membrane time constant. Surprisingly, somatically recorded action potentials also dramatically declined in amplitude over a similar period (38 ± 3 to 17 ± 2 mV; τ = 5.2 d). Simultaneous somatic and dendritic patch recordings revealed that these action potentials were initiated in the axon, which primarily emerged from the soma. In older gerbils, the rapid speed of membrane voltage changes and the attenuation of action potential amplitudes were mediated extensively by low voltage-activated potassium channels containing the Kv1.1 subunit. In addition, whole-cell voltage-clamp recordings revealed that these potassium channels increase nearly fourfold from P14 to P23 and are thus a major component of developmental changes in excitability. Finally, the electrophysiological features of principal neurons of the medial nucleus of the trapezoid body did not change after P14, indicating that posthearing regulation of intrinsic membrane properties is not a general feature of all time-coding auditory neurons. We suggest that the striking electrical segregation of the axon from the soma and dendrites of MSO principal neurons minimizes spike-induced distortion of synaptic potentials and thus preserves the accuracy of binaural comparisons.

**Key words:** MSO; auditory brainstem; dendrotoxin; dendrite; MNTB; Kv1.1

### Introduction

The medial superior olive (MSO) is one of several brainstem auditory nuclei that comprise the first stage for processing sound localization cues. MSO neurons compute the horizontal location of low-frequency sounds using the difference in the time required for a sound to propagate to each ear. These interaural time delays (ITDs) are submillisecond cues, the physiological range of which is dependent on the diameter of the animal’s head. The principal neurons of the MSO are able to extract these brief ITDs from converging binaural inputs that include an excitatory component from the ipsilateral and contralateral ventral cochlear nucleus (VCN) (Cant and Casseday, 1986; Smith et al., 1993; Beckius et al., 1999) and an inhibitory component from both the medial nucleus (MNTB) and lateral nucleus (LNTB) of the trapezoid body (Helfert et al., 1989; Adams and Mugnaini, 1990; Cant and Hyson, 1992; Vater, 1995). Although the mechanism for extracting ITDs from these excitatory and inhibitory inputs has yet to be fully elucidated (for review, see Joris et al., 1988; Grothe, 2003; Palmer, 2004), ITD information is conveyed to higher auditory centers through a rate code (Goldberg and Brown, 1969; Yin and Chan, 1990; Spitzer and Semple, 1995; Brand et al., 2002). To detect these rapid time-varying cues, time-coding auditory neurons in the MSO and elsewhere in the brainstem exhibit biophysical specializations, such as low voltage-activated potassium currents (I_{K(LVA)}), which allow them to signal with high temporal fidelity (Svirskis et al., 2004) (for review, see Trussell, 1999).

Although the auditory canal opens at approximately postnatal day 12 (P12) in gerbils, rats, and mice, auditory function is not mature from the outset. In gerbils, the sound intensity required to produce responses by the cochlea and VCN neurons declines during the first week of hearing (Woolf and Ryan, 1984, 1985; Echteler et al., 1989). Additionally, the maximum firing rates of VCN and superior olivary neurons increase and tuning matures in these neurons as well as the cochlea (Harris and Dallos, 1984; Woolf and Ryan, 1985; Sanes and Rubel, 1988; Echteler et al., 1989). In humans and other mammals, the ability to localize sound sources on the horizontal plane also matures after hearing onset (Kelly et al., 1987; Ashmead et al., 1991).

Less attention has focused on the development of the intrinsic properties of MSO neurons. In fact, few intracellular recordings have been reported in the MSO beyond P20 (but see Smith, 1995). In the present study, we describe developmental changes in the properties of MSO principal neurons after hearing onset (P14–P38). During the first week of hearing, the membrane time constant decreases and EPSPs narrow. Action potentials, initiated in the axon, also decrease in amplitude. These developmental changes are mediated primarily by an increase in I_{K(LVA)}; although MNTB principal neurons also express I_{K(LVA)} (Banks and...
Smith, 1992; Brew and Forsythe, 1995; Dodson et al., 2002), their intrinsic properties do not change after hearing onset. Thus, the developmental refinement of electrical properties is not a general feature of all time-coding neurons.

Materials and Methods

Slice preparation. Mongolian gerbils (Meriones unguiculatus) were obtained from Charles River Laboratories (Wilmington, MA) or bred at the Animal Resource Center of the University of Texas at Austin. Gerbils (P14–P38) were anesthetized with halothane, and the brain was removed while submerged in oxygenated artificial CSF (ACSF) (in mM: 125 NaCl, 2.5 KCl, 2 CaCl2, 25 NaHCO3, 1 MgSO4, 1.25 NaH2PO4, and 25 glucose), pH 7.4, with NaOH. Horizontal sections (200 μm) were cut at 32°C using an oscillating tissue slicer (VT-1000S; Leica, Solms, Germany) and were examined (see below). To determine the effect of recorded cells was made on the basis of their location in the slice, cell were placed both medial and lateral to the MSO. The identification of ulator (Digitimer, Welwyn Garden City, UK). Stimulation electrodes (100 synaptically inputs to MSO and MNTB cells was achieved by delivering brief recordings, biocytin (0.1%) was also included in the internal solution for 0.5 EGTA, 4 MgATP, 0.3 NaGTP, pH 7.3, with KOH. For current-clamp experiments. The series resistance (4 –10 MΩ) was later added to test for somatic recordings and 40 – 80 nM dendrotoxin-K (DTX-K; K(LVA) on the intrinsic properties do not change after hearing onset. Thus, the developmental increase in synaptic inputs to MSO and MNTB neurons was visualized using infrared differential interference contrast microscopy (Axioskop 2FS Plus; Zeiss, Oberkochen, Germany) in combination with a Newvicon tube camera (Dage-MTI, Michigan City, IN). Recordings were made using heat-polished borosilicate patch pipettes (1.65 mm outer diameter; World Precision Instruments, Sarasota, FL) with open tip resistances of 2–4 MΩ (somatic electrodes) and 6–11 MΩ (dendritic electrodes). The internal solution in patch pipettes contained the following (in mM): 115 potassium gluconate, 20 KCl, 10 sodium phosphocreatine, 25 NaHCO3, 1.25 NaH2PO4, and 20 glucose), pH 7.4, with KOH. For current-clamp recordings, biocytin (0.1%) was also included in the internal solution for subsequent morphological analyses of the recorded cells. Somatic and dendritic recordings were made with Dagan (Minneapolis, MN) BVC-700A amplifiers in current-clamp mode. Activation of synaptic inputs to MSO and MNTB cells was achieved by delivering brief (100 μs) electrical pulses to the slice through patch pipettes broken to tip diameters of ~10 μm. Pulses were generated by a constant current stimulator (Digitimer, Welwyn Garden City, UK). Stimulation electrodes were placed both medial and lateral to the MSO. The identification of recorded cells was made on the basis of their location in the slice, cell body shape, and responses to depolarizing current steps. Additionally, many cells were successfully labeled with biocytin and their morphology examined (see below). To determine the effect of I(KLVA) on the intrinsic properties of MSO neurons, 40–80 nM dendrotoxin-K (DTX-K; Alomone Labs, Jerusalem, Israel) was bath applied. To test the impact of the developmental increase in I(KLVA) on temporal summation, two simulated EPSCs (biexponential waveform, 0.2 ms rise, and 0.2 ms decay, 3.5 ms duration) separated by 0–2.5 ms (0.1 ms increments) were injected at the soma. Recordings were included if the series resistance was <15 MΩ for somatic recordings and <50 MΩ for dendritic recordings. Data were low-pass filtered at 5 kHz and acquired at 50 kHz using custom macros programmed in IgorPro Carbon (WaveMetrics, Lake Oswego, OR).

Somatic whole-cell voltage-clamp recordings were made with an Axopatch 200B amplifier (Molecular Devices, Union City, CA). Pipettes were wrapped with Parafilm (Pechinix Plastic Packaging, Chicago, IL) to reduce electrode capacitance. In addition to cell location and soma shape, MSO principal neurons were identified by their transient responses to opening of the auditory canal, EPSP width averaged 0.78 ms (Fig. 2), measured at half-maximal amplitude. Although the rate of rise of EPSPs remained relatively stable between P14 and P38 (Fig. 2A,B), in the week after hearing onset, the duration of EPSPs declined exponentially with a time constant of 4.3 ms (Fig. 2C). The input resistance and membrane time constant also declined over the same time window, reaching asymptotic values of ~7 MΩ and 0.3 ms by ~3 weeks after birth (Fig. 2D). This developmental shortening of the time constant constrains the duration of the EPSP to more accurately reflect the time course of the underlying EPSC.

The developmental decline in EPSP duration, input resistance, and membrane time constant were significantly different when comparing means for P14–P16 and P19–P21 (EPSP duration, p < 0.01; input resistance, p < 0.001; membrane time constant, p < 0.001; n = 7–18). There was no additional change in these parameters between P19–P21 and P28–P30. Also, the EPSP 7888 • I. Neurosci., August 31, 2005 • 25(33):7887–7895 Scott et al. • Development of Temporal Processing in the MSO
Developmental changes in action potential amplitude could be caused by a shift in the balance of sodium and potassium currents after hearing onset. Alternatively, the site of action potential initiation could shift farther from the soma. To distinguish between these two possibilities, we examined action potential initiation through morphological and electrophysiological analyses. First, we determined where the axon emerged from the cell by analyzing the morphology and axon location of 129 biocytin-labeled neurons (Fig. 4A). In most cells, the axon emerged directly from the soma (73%), whereas the remaining axons typically emerged from the proximal dendrite, <30 µm from the soma (8% medial, 19% lateral) (Fig. 4B). This pattern was observed at all ages included in our study.

To address whether action potential initiation occurs in the axons of MSO principal neurons, we made dual current-clamp recordings at the soma and proximal dendrite, <50 µm from the soma in P16–P19 gerbils. Action potentials were initiated with either somatic current injection or synaptic stimulation. When the axon emerged from the soma (n = 5 of 6), action potentials evoked by somatic current injection were always detected first at the soma and subsequently in the dendrite in an attenuated form (Fig. 4C, top left). This was also true for synaptically evoked action potentials, although in one case, the peak timing was ambiguous because of the broader shape in combination with a shorter recording distance (Fig. 4C, bottom left). However, when the axon emerged from the lateral dendrite within 5 µm of the dendritic recording site (50 µm from the soma; n = 1 of 6), the action potential was detected first in the dendrite (Fig. 4C, right column). The range of action potential amplitudes in these dual recordings (17–56 mV at the soma) suggests that both small and large action potentials initiate near or in the axon. Together, the anatomical and physiological results are consistent with an axonal site of action potential initiation, independent of electrophysiological maturity. Thus, developmental changes in action potential amplitude and shape are more likely attributable to changes in the balance of voltage-dependent conductances.

To test the role of $I_{K(LVA)}$ in action potential generation, we examined the effects of the Kv1.1-containing channel blocker DTX-K on MSO principal neurons exhibiting mature electrophysiological properties (P20–P21 gerbils). Although the transient firing pattern of five neurons was not altered in the presence of 40 or 80 nM DTX-K (Fig. 5A), the toxin induced dramatic changes in other response properties. The membrane time constant and input resistance increased approximately threefold, and action potential amplitude increased from 15 ± 3.6 to 37 ± 4.9 mV (Fig. 5B–F). DTX-K reduced the threshold current for action potential initiation from 2.7 ± 0.19 to 0.9 ± 0.09 nA (Fig. 5D,F). These results show that Kv1.1-containing channels contribute strongly to the low input resistance and fast membrane time constant, enabling these channels to play a key role in the rapid signaling of MSO principal neurons.

Because $I_{K(LVA)}$ shapes the membrane time constant and action potential amplitude, this current is a likely candidate for the change in electrical properties during the first week of hearing. We tested the idea that $I_{K(LVA)}$ is developmentally regulated by pharmacologically isolating this current during whole-cell voltage-clamp recordings of 49 MSO principal neurons (see Materials and Methods). Depolarizing voltage steps elicited fast-activating and slow-inactivating outward currents (Fig. 6A, top). Peak $I_{K(LVA)}$ increased almost fourfold between P14 and P23 (Fig. 6C), with no significant change in voltage sensitivity of activation.
The physiological properties of MSO principal neurons undergo developmental changes after hearing onset. Action potentials were evoked in 81 cells via suprathreshold 100 ms square current steps. At P14, action potentials were large, exceeding 0 mV. Over the next few days, action potential amplitudes and rates of rise decreased markedly. Current steps are shown below the action potential responses. A, Maximum rates of rise decreased with a time constant of 3.1 d. B, The rate of rise for EPSPs remained relatively stable after hearing onset (n = 67). C, The time constant for the activation of this DTX-sensitive current decreased with age (P14, τ = 1.47 ± 0.17 ms; P21, τ = 0.95 ± 0.10 ms, p < 0.05; steps from −80 to −45 mV). This faster activation kinetics at P21 than P14 could contribute to the developmental change in action potential amplitude by decreasing the ratio of inward-to-outward current during action potential generation. However, the action potentials themselves were initiated earlier at P21 versus P14 (0.70 ms and 1.03 ms, respectively), which would, in part, offset these effects. A precise quantification of the influence of channel kinetics on action potential amplitude cannot be made based on these experiments. Together, the developmental changes in the density and possibly also the kinetics of IK(LVA) are critical factors underlying the developmental maturation of intrinsic properties and action potential signaling in early posthearing development.

To gain insight into how the developmental upregulation of Kv1.1-mediated currents shapes the temporal resolution of excitatory synaptic integration, two artificial EPSCs separated by a variable delay (0–2.5 ms, in 0.1 ms steps) were combined and injected somatically into cells from P14 and P21 animals. The amplitude of a single EPSC was adjusted to evoke an ~3 mV EPSP, and the peak voltage of paired responses were normalized to this value. In normal saline, the window for temporal integration (defined as the time taken for averaged EPSPs to repolarize to within 10% of baseline) was twice as narrow at P21 versus P14 (0.87 ± 0.04 ms vs 1.64 ± 0.23 ms; p < 0.05) (Fig. 7B, C). This difference reflects the shorter membrane time constant in older animals. To isolate the contribution of IK(LVA) upregulation to the developmental change in EPSP summation, we used DTX-K on cells from P21 animals to block IK(LVA) to a similar level found in cells from P14 animals (in separate voltage-clamp experiments, 31 nM DTX-K blocked 73.5 ± 1.8% of IK(LVA)) (Fig. 7A). In the presence of 31 nM DTX-K, the window for temporal summation increased to a value comparable with that observed in cells from P14 animals (P21 in DTX-K, 1.72 ± 0.09 ms; P14, 1.64 ± 0.23 ms) (Fig. 7B, C). Together, these findings show that the age-related increase in IK(LVA) significantly sharpens the window for temporal integration.

Kv1.1-mediated currents are found in nearly all brainstem auditory neurons concerned with temporal coding and have been studied most extensively in MNTB principal neurons (Brew and Forsythe, 1995; Grigg et al., 2000; Dodson et al., 2002; Brew et al., 2003; Barnes-Davies et al., 2004; Leao et al., 2004). To address whether the properties of MNTB neurons change after hearing...
onset, we made whole-cell current-clamp recordings from 20 principal neurons between the ages of P14 and P37. Although the morphology of MNTB principal neurons is distinct from MSO neurons (Fig. 8A), they exhibit many of the same general electrophysiological features, including a phasic firing pattern and strong outward rectification (Fig. 8B) (Banks and Smith, 1992; Brew and Forsythe, 1995). Our recordings from MNTB principal neurons in gerbils generally were similar to previous studies but revealed significant electrophysiological differences from their MSO counterparts present in the same slices. MNTB principal cells fired large action potentials that were greater in amplitude and required far more modest currents (average across all ages, 0.47 nA) for initiation relative to principal neurons of the MSO (Fig. 8D,E). The input resistance and membrane time constant of MNTB principal cells also remained stable after hearing onset (P14–P16, 131 ± 33.2 MΩ, 3.9 ± 0.75 ms; P19–P20, 115 ± 20.0 MΩ, 4.3 ± 0.60 ms; P28–P29, 86 ± 13 MΩ, 3.6 ± 0.72 ms; p > 0.4; n = 6–7). Furthermore, the values of these membrane characteristics were larger than those of the MSO neurons (input resistance, p < 0.001, n = 6–18; membrane time constant, p < 0.001, n = 6–18). (Banks and Smith, 1992; Brew and Forsythe, 1995)

Discussion

Principal neurons of the medial superior olive detect interaural time differences introduced by sounds in the environment and encode these cues in their action potential firing rate. The resolution of this computation relies in part on the speed and brevity of excitatory synaptic transmission. In the present study, we describe the temporal refinement of action potential signaling and subthreshold electrical properties of MSO neurons in gerbils. The week after hearing onset, action potential amplitudes decrease considerably, and a decline in EPSP duration mirrors a reduction in membrane time constant. These changes are driven primarily by an increase in I_{K(LVA)}, which in turn is mediated by channels containing the Kv1.1 subunit. Finally, these developmental changes in the MSO are not universal to all brainstem auditory neurons concerned with temporal coding, because MNTB principal neurons exhibit stable intrinsic electrical properties and action potential signaling after the onset of hearing.

Properties and developmental regulation of I_{K(LVA)} in MSO principal neurons

We show that, in principal neurons of the MSO, a developmentally regulated expression of low-voltage potassium channels plays an important role in the signaling of microsecond differences in synaptic timing. From 2 to 3 weeks of age, a decrease in average EPSP halfwidth from 0.78 to 0.55 ms is temporally correlated with a nearly fourfold increase in I_{K(LVA)}. One possible mechanism underlying this change in EPSP duration is an acceleration in AMPA receptor kinetics, which has been described in studies of other auditory brainstem synapses (Bellingham et al., 1998; Lawrence and Trussell, 2000; Taschenberger and von Gersdorff, 2000; Joshi et al., 2004; MacLeod and Carr, 2005). However, this issue has not yet been addressed in the MSO. The developmental increase in I_{K(LVA)} decreases EPSP duration in two ways. First, tonic I_{K(LVA)} activation near rest decreases input resistance, thereby increasing the intrinsic speed of membrane volt-
Figure 5. The application of DTX-K altered the electrophysiological properties of MSO principal neurons exhibiting mature features (P20–P21). A, The response of a typical MSO neuron to current pulses (−2 to 8 nA, 2.1 nA steps) before and after the application of 80 nM DTX-K shows that a transient firing pattern persisted in the presence of the toxin. B, In contrast, the slope of the peak V–I plot below rest reveals that the input resistance of this cell increased >2.5-fold. C, Normalized inward voltage responses show that the neuron’s membrane time constant also increased threefold from 0.4 to 1.3 ms after toxin application. D, Action potentials in this neuron increased in amplitude and the threshold current for action potential initiation decreased in response to DTX-K. E, F, Average membrane time constant, peak input resistance, threshold current for action potential initiation and action potential amplitude before (open bars) and after (filled bars) DTX-K application (n = 5). All parameters were significantly different in DTX-K (***p < 0.001). AP, Action potential. Error bars represent SEM.

Figure 6. Low voltage-activated potassium current (I_{K(LVA)}) increases after hearing onset in MSO principal neurons. I_{K(LVA)} was isolated pharmacologically (see Materials and Methods) and evoked by a family of 1 s depolarizations (−70 to −30 mV; 5 mV steps; from a prepulse potential of −80 mV). A, In a typical neuron from P21, I_{K(LVA)} (top) was essentially blocked by the addition of 80 nM DTX-K (middle). DTX-K-sensitive currents are shown in the bottom panel. Traces are not leak-subtracted. B, Peak I–V curves for leak-subtracted I_{K(LVA)} before (circles) and after (triangles) the addition of 80 nM DTX-K (the same neuron as A). For this neuron, I_{K(LVA)} was blocked by 80–90% across the range of voltages shown. C, Peak I_{K(LVA)} (leak-subtracted) as a function of age. This current increases 390% between P14 and P23. The histogram displays peak current responses for voltage steps to −45 mV, grouped in 2 d bins. The superimposed filled circles represent currents from individual cells. The asterisks indicate significant difference from the P14–P15 group; ***p < 0.001. D, Whole-cell capacitance did not change significantly between P14 and P23, indicating that changes in cell size/membrane area do not account for the developmental increase in I_{K(LVA)}. E, DTX-K (80 nM) blocks the majority of I_{K(LVA)} in response to steps to −45 mV. The percentage block increases from 76 to 91% between P14 and P21 (**p = 0.06; n = 4–6). Error bars represent SEM.

dence of binaural integration in the face of high-frequency synaptic activity. Increased I_{K(LVA)} also imparts a threefold higher current threshold for action potential initiation, increasing the requirement for synchronicity of binaural excitation.

A similar increase in I_{K(LVA)} has been reported in neurons of the nucleus laminaris (Kuba et al., 2002), the avian homolog of the MSO, suggesting that developmental upregulation of I_{K(LVA)} is a common feature of binaural coincidence detectors in birds and mammals. However, unlike in chick nucleus laminaris (Smith and Rubel, 1979; Kuba et al., 2005), we did not find electrophysiological or morphological changes along the putative tonotopic axis in the MSO. The reason that electrical maturity is
The developmental increase in $I_{K(LVA)}$ narrows the window for temporal summation. $A$, $I_{K(LVA)}$ measured in whole-cell voltage-clamp recordings increases approximately fourfold between P14 and P21 (amplitude shown for steps to $-45$ mV from a $-80$ mV prepulse). Bath application of 31 nM DTX-K partially blocked $I_{K(LVA)}$, by $73.5 \pm 1.8\%$ rendering the amplitude of $I_{K(LVA)}$ at P21 similar to the amplitude at P14. $B$, Current-clamp responses to single (thick lines) and paired simulated EPSC injections (pairs separated by 0–2 ms, 0.4 ms steps, thin lines) at P14 and P21. Partial block of DTX-K-sensitive current (31 nM DTX-K) widened responses at P21 such that they were similar to those at P14. Single EPSPs were adjusted to $\sim 3$ mV. $C$, Peak voltage responses to paired EPSCs. Summed responses were normalized to the peak amplitudes of single EPSPs. The window for temporal summation was larger at P14 than at P21 ($p < 0.01$). The reduction of DTX-K-sensitive current with 31 nM DTX-K broadened the window for temporal integration at P21 ($p < 0.001$). The window of temporal integration was defined as the time taken for averaged EPSP amplitudes to fall within 10% of baseline. CTL, Control.

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Delayed until a week after hearing onset in MSO principal neurons is not clear. One possibility for this delay is that channel expression may not be preprogrammed, but rather can be influenced by sound-induced afferent activity. Consistent with this idea, in cultured hippocampal pyramidal neurons, Kv1.1- and Kv1.2-containing potassium channels are upregulated during growth in culture through a process that is sensitive to synaptic activity (Grosse et al., 2000). Few other studies have examined the postnatal expression of Kv1-containing channels in detail. However, the robust upregulation in channel expression observed in the MSO is not a general feature of neural development, because Kv1-containing channels in chick vestibular neurons appear to undergo a decrease in expression after hatching (Gamkrelidze et al., 2000; Popratiloff et al., 2003). Also, in the present study, there was no significant change in Kv1.1-related electrical properties in MNTB principal neurons between P14 and P38.

In MSO principal neurons, the majority of $I_{K(VLVA)}$ was blocked by 80 nM DTX-K, indicating that Kv1.1-containing channels are a primary constituent of this outward current. The voltage sensitivity, kinetics, and dendrotoxin sensitivity of $I_{K(VLVA)}$ in MSO principal cells in many respects resembles that of other brainstem auditory neurons concerned with maintaining the precise timing of excitatory inputs, such as bushy and octopus cells of the VCN, and principal cells of the MNTB (Manis and Marx, 1991; Rathouz and Trussell, 1998; Bal and Oertel, 2001; Dodson et al., 2002; Rothman and Manis, 2003). In situ hybridization and immunochemical analyses also reveal that the Kv1.1 subunit is prominently expressed in these brainstem auditory nuclei (Grigg et al., 2000; Dodson et al., 2002; Lu et al., 2004). The reduction of $I_{K(VLVA)}$ in MNTB principal cells as a result of channel expression may not be preprogrammed, but rather can be influenced by sound-induced afferent activity. Consistent with this idea, in cultured hippocampal pyramidal neurons, Kv1.1- and Kv1.2-containing potassium channels are upregulated during growth in culture through a process that is sensitive to synaptic activity (Grosse et al., 2000). Few other studies have examined the postnatal expression of Kv1-containing channels in detail. However, the robust upregulation in channel expression observed in the MSO is not a general feature of neural development, because Kv1-containing channels in chick vestibular neurons appear to undergo a decrease in expression after hatching (Gamkrelidze et al., 2000; Popratiloff et al., 2003). Also, in the present study, there was no significant change in Kv1.1-related electrical properties in MNTB principal neurons between P14 and P38.

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**Action potential initiation in MSO principal neurons**

Morphological analyses revealed that axons emerge primarily from the soma, and secondarily from the proximal dendrites of gerbil MSO principal neurons [see also the study by Smith (1995) in guinea pigs]. Our data also indicate that the site of action potential initiation is closely correlated with axon location in these neurons. During simultaneous somatic and dendritic recordings, action potentials were always detected first at the retinal.
cording site closest to the axon. These results are in agreement with evidence from multiple types of central neurons indicating that action potentials initiate in the axon (Colbert and Pan, 2002) and subsequently propagate down the axon as well as back into the soma and dendrites (for review, see Häusser et al., 2000). However, unlike other CNS neurons, action potentials in electrophysiologically mature MSO principal neurons are unusually small at the soma (<15 mV). These action potentials increased 2.5-fold in amplitude in the presence of DTX-K, showing that Kv1.1-mediated currents strongly shape action potential amplitude in MSO neurons. Our findings raise the possibility that low voltage-activated potassium channels in the axon itself attenuate conventional, large-amplitude action potentials as they back-propagate from the axon to the soma.

MSO principal neurons fire a single, well timed action potential in response to step depolarizations. Similar to our findings, Grothe and Sanes (1993) found that action potential amplitudes did not exceed 20–25 mV in MSO neurons from P17–P25 gerbils (measured from the inflection point). In contrast, Smith (1995) found large action potentials ~50 mV in amplitude in 3- to 5-week-old guinea pigs. This latter discrepancy may reflect species differences in either the rate of development or mature firing properties. Previous studies of the MSO have highlighted the importance of voltage-gated potassium currents, particularly those active at low voltages, in shaping action potential signaling. For example, reducing \( I_{K(LVA)} \) in MSO principal neurons from P12–P14 gerbils with blockers of Kv1 subunits, DTX-K and dendrotoxin-I, lowered the current threshold for action potential initiation and caused repetitive firing (Svirskis et al., 2002, 2004). Similar effects were found in older animals with a less-specific blocker, 1 mM 4-aminopyridine (Smith, 1995). However, in agreement with Svirskis et al. (2002), we found that DTX-K application to MSO principal neurons from gerbils older than P14 lowered the current threshold for action potential initiation without altering firing patterns. Thus, \( I_{K(LVA)} \) is not the only mechanism shaping the transient firing pattern in mature MSO principal neurons.

DTX-sensitive potassium currents in hippocampal, neocortical, and cerebellar Purkinje neurons impact the threshold and timing of both sodium- and calcium-dependent spikes; however, unlike MSO neurons, these currents have little effect on spike amplitude and shape. Variations in the role of DTX-sensitive currents presumably reflect differences in the subunit composition of native Kv1 potassium channels and/or differences in relative channel density (Storm, 1988; Golding et al., 1999; Bekkers and Delaney, 2001; McKay et al., 2005).

Two classes of time-coding auditory neurons? In contrast to the robust changes in intrinsic electrical properties of MSO neurons during the first week of hearing, the electrical properties of principal neurons of the MNTB were stable throughout the entire range of animal ages we used (P14–P37), and were also substantially different in magnitude. In older MNTB neurons (P28–P30), action potential amplitudes were sevenfold larger, and input resistance and membrane time constants were 12-fold larger than in MSO neurons, suggesting there are differences in the relative density and/or composition of conductances between these neuron types. Based on these observations, we suggest that principal neurons of the MSO and MNTB comprise two related but functionally distinct classes of time-coding auditory brainstem neurons.

In MSO principal neurons, \( I_{K(LVA)} \) electrically insulates the soma and dendrites, in which synaptic integration takes place, from the voltage and temporal distortions of axonal action potentials. This functional compartmentalization may be shared by other auditory brainstem neurons integrating precisely timed inputs in dendrites. For example, octopus cells of the VCN detect the synchrony of auditory nerve fiber inputs in their large-caliber dendrites (Oertel et al., 2000). In these cells, the membrane time constant and somatic action potential amplitude undergo a substantial decrease during the first week of hearing in mice (R. Bal and D. Oertel, personal communication). It will be intriguing to see whether this electrophysiological motif is observed in other auditory neurons concerned with submillisecond dendritic integration.

References


