Membrane Properties and Firing Patterns of Inferior Colliculus Neurons: An In Vivo Patch-Clamp Study in Rodents

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Tan ML, Theeuwes HP, Feenstra L, Borst JG. Membrane properties and firing patterns of inferior colliculus neurons: an in vivo patch-clamp study in rodents. J Neurophysiol 98: 443–453, 2007. First published May 16, 2007; doi:10.1152/jn.01273.2006. The inferior colliculus (IC) is a large auditory nucleus in the midbrain, which is a nearly obligatory relay center for ascending auditory projections. We therefore suggest a separate functional role for Ih.

Ih may also contribute to the different firing patterns. Onset and adapting neurons have larger Ih currents than sustained neurons in the IC (Koch and Grothe 2003), presumably because these cells express the HC1 subunit (Koch et al. 2004; Notomi and Shigemoto 2004). Ih may also contribute to rebound firing and to an afterhyperpolarization (AHP) following long depolarizations (Koch and Grothe 2003).

In earlier intracellular or patch-clamp studies from the IC of cats, bats, or guinea pigs (Casseday et al. 1994; Covey et al. 1996; Kuwada et al. 1997; Nelson and Erulkar 1963; Pedemonte et al. 1997), the responses to current injection were not studied; thus it is not yet known whether the firing patterns observed in rodent slices can be observed in vivo as well. We therefore investigated firing patterns in both young-adult rats and mice using in vivo patch-clamp recordings.

METHODOLOGY

Surgical preparation

Animal procedures were in accordance with guidelines provided by the animal committee of the Erasmus MC.

A total of 18 male Wistar rats [postnatal day (P) 19 to P44] and 46 C57/B16 mice (P21–P37) were anesthetized intraperitoneally (ip) with ketamine 60 mg/kg and medetomidine 0.25 mg/kg, administered subcutaneously, or ketamine/xylazine (65/10 mg/kg) ip. Additional anesthesia was given when pinching the toes resulted in a withdrawal reflex. Rectal temperature was maintained between 36.5 and 37.5°C with a homeothermic blanket system (Stoelting). The head was immobilized using a metal pedestal fastened to the skull. After a craniotomy directly caudal from the transversal sinus, any cerebellar tissue overlying the colliculi was aspirated and the pia was removed. Craniotomy was performed using a surgical drill with a 2 or 3 mm diameter burr under constant saline irrigation. The dura mater was removed from the IC and the region overlying the IC was aspirated. Any cerebellar tissue overlying the colliculi was aspirated and the pia was removed. Craniotomy was performed using a surgical drill with a 2 or 3 mm diameter burr under constant saline irrigation. The dura mater was removed from the IC and the region overlying the IC was aspirated. Brain pulsations were reduced by application of agar (agarose 2% in 0.1 M phosphate buffer). All experiments were performed in a single-walled sound-attenuated chamber (Gretch-Ken Industries, attenuation ≥40 dB at 4–32 kHz).

Patch-clamp recordings

Thick-walled borosilicate glass micropipettes with filament (4–6 MΩ) were filled with (in mM) K-glucuronate 125, KCl 20, Na2phosphocreatine 10, Na2GTP 0.3, MgATP 4, EGTA 0.5, HEPES.
10 (pH 7.2). The IC was approached dorsocaudally at a 30–60° angle with the horizontal under high positive pressure, which was lowered to approximately 30 mbar at about 500 μm below the surface in rats and about 200 μm in mice. Gigahm seals and whole cell recordings were established using standard techniques (Margrie et al. 2002). Series resistance was on average 63 ± 2 MΩ (n = 99).

Data were acquired with a MultiClamp 700A patch-clamp amplifier and pCLAMP 9.2 software (Axon Instruments). Potentials were filtered at 10 kHz (eight-pole Bessel filter) and sampled at an interval of 50 μs with a 16-bit A/D converter (Digidata 1322A).

**Stimulation protocols**

Cells were subjected to constant-current injections with 50-pA steps between −200 and +500 pA of different durations (2, 50, 100, and 1,000 ms). To test whether the firing patterns change at more negative membrane potentials (Bal et al. 2002), the 1,000-ms protocol was repeated, except it was preceded by 1,000-ms injection of −200 pA by a hyperpolarizing current. Many cells were stimulated with tones after completion of the current injection protocols, as described in the companion paper (Tan and Borst 2007).

**Histology**

A subset of cells was filled with biocytin. In these experiments the pipette solution contained 0.5% biocytin and the K-glucuronate concentration was reduced to 115 mM. Biocytin-filled cells were visualized as described by Horikawa and Armstrong (1988), with minor modifications. Their location within the IC was obtained by comparing the slices with slices from the reconstructed IC of a 25-day-old C57/Bl6 mouse, for which the vesicular glutamate transporter 1 (vGLU7) was used as a marker for the central nucleus (Tan, Theeuws, Feenstra, and Borst, unpublished results).

**Electrophysiological data analysis**

Data were analyzed using Clampfit 9.2, or using custom-written macros that used the NeuroMatic environment (version 1.91, kindly provided by Dr. J. Rothman, University College London) within Igor Pro 5 (Wavemetrics, Lake Oswego, OR).

Membrane potentials were corrected for a −11-mV junction potential.

Cells often showed a slow depolarization during the recording period. Cells were discarded from analysis when resting membrane potential depolarized beyond −45 mV, without spontaneous recovery to initial Vm; current injection protocols were incomplete; action potentials were not observed during current injection; action potentials had <10-mV peak amplitude arising from high access resistance; or when localization outside the inferior colliculus was histologically confirmed.

**Membrane properties**

If the cell fired spontaneously, the action potential threshold (in millivolts) was estimated from hyperpolarizing current steps or else from depolarizing current injections around the threshold. Input resistance Rm and membrane time constant τ were both calculated by extrapolation of an exponential fit through the average response to hyperpolarizing current steps with durations of 50–100 ms.

The presence of the hyperpolarization-activated current (Ih) was assessed from the response to 100- to 300-pA, 1-s hyperpolarizing current steps. Ih was considered to be present when a depolarizing “sag” was observed in the averaged response exceeding 10% of the maximal hyperpolarization.

Activation kinetics of Ih was estimated from a fit with a single- or a double-exponential function to the depolarizing sag (Fig. 9 B), according to: $I = A_1 \times \exp(-t/\tau_1) + C$, and $Y = A_2 \times \exp(-t/\tau_2) + \frac{A_3 \times \exp(-t/\tau_3)}{1 + \exp(-t/\tau_4)} + C$, where $\tau_1$ and $\tau_2$ represent the fast and slow time constants of $I_h$ activation, respectively, and $t$ represents the time relative to the most hyperpolarized point. The data points were weighted with the reciprocal of their SDs. A double-exponential fit was favored over a single-exponential fit when the residuals of the single-exponential fit were clearly not randomly distributed, the slow time constant was at least threefold larger than the fast time constant, and the amplitude contribution of both time constants was ≥10%. The amplitude contribution was calculated in the range between the peak depolarization and the end of the 1-s trace.

**Firing patterns**

Firing patterns were determined at 100 pA above the smallest suprathreshold current injection. Based on their firing patterns, we classified cells largely following criteria established by Sivaramakrishnan and Oliver (2001), yielding a total of six different firing types: sustained, accommodating, build-up, accelerating, and burst (onset and sustained). We first looked at whether a cell fired a cluster of two or more action potentials. When this occurred only at the onset of depolarization, the cell was classified as burst-onset; if it fired bursts during the entire 1-s depolarization it was classified as burst-sustained. We then looked at the latency of the first spike during depolarization and prehyperpolarization. Buildup neurons showed a typical delay in firing the first action potential, which became more pronounced when the depolarizing current was preceded by a hyperpolarization, as described by Sivaramakrishnan and Oliver (2001). Accelerating neurons showed a clear increase in their firing rate during the depolarizing step. Within this group, either the first interspike interval (ISI) was consistently larger than the second or cells showed a more gradual increase in firing rate, which was followed by accommodation. In contrast to buildup, accelerating, burst-onset, or burst-sustained cells, in the remaining cells spike rates decreased exponentially and in these cells the relation between spike rate (calculated from the ISIs) and spike interval number was fitted with the equation $S = A \times \exp(-x/\tau) + B$, where x is spike interval number, S is spike rate, B is spike rate at x = 0, and A + B is the spike rate for the first interval (x = 0). From this fit we calculated an accommodation index (AI): $AI = \frac{\{(S_{x=0} - S_{x=1})/S_{x=1}\} \times 100\%}$, where $S_{x=0}$ is the value of the fit at x = 0. Because we used spike rates instead of spike intervals (Sivaramakrishnan and Oliver 2001), a low AI represented a cell with little spike accommodation. The AI is thus an estimate for the percentage increase in the ISI between the first and the second ISI. We use the term “accommodation” instead of “adaptation” to avoid confusion with the synaptic adaptation processes studied in the companion paper (Tan and Borst 2006). Cells with AI <4% were sustained; otherwise, they were classified as accommodating.

Neurons were classified as having rebound spikes when these were observed at least twice after hyperpolarization. In addition, we tested for the presence of AHPs >1 mV below baseline after depolarizing current injections or “humps” >1 mV above baseline after hyperpolarizing current injections.

**Spontaneous postsynaptic potentials**

Spontaneous excitatory postsynaptic potentials (spEPSPs) were identified using the built-in template method of Clampfit 9.2. The amplitudes and 20–80% rise times of a minimum of 80 spEPSPs occurring in a baseline period of ≈5 s were measured after digital filtering to 1 kHz.

We also investigated the relation between the occurrence of spEPSPs and firing irregularity. As a measure for the fluctuations in the membrane potential resulting from spontaneous inputs we calculated the average SD of the membrane potential during 3 s, after digital filtering to 1 kHz and after correcting for a linear trend in the average membrane potential. As an estimate for firing irregularity, we used the coefficient...
of variation (CV) of the deviations from an exponential decrease of the spike rates at 100 pA above threshold. This was calculated by dividing the SD of the residuals of the single-exponential fit of the spike rates by the average spike rate. Only accommodating and sustained cells that fired ≥10 spikes during a 1-s depolarizing current step at 100 pA above threshold were included in this analysis.

Statistics

Data are presented as means ± SE. Two-tailed Student’s t-test, Pearson’s χ² test, or ANOVA with post hoc Tukey HSD test were calculated using SPSS 11. Values of P < 0.05 were judged as statistically significant.

RESULTS

Localization of cells within IC

We made in vivo patch-clamp recordings of neurons from the rat or mouse inferior colliculus (IC), which can be subdivided into a central nucleus, dorsal cortex, and external cortex (reviewed in Oliver 2005). To investigate the location within the IC of the cells we recorded from, a subset of 13 cells from 10 different mice were filled with biocytin and histologically retrieved. Figure 1 shows an example of a neuron, with its firing pattern in response to current injection (Fig. 1A) and a reconstruction of its soma and dendritic tree (Fig. 1B). Because thionine staining alone is insufficient to determine the exact borders of the central nucleus, we used a reconstruction of the mouse IC using vGLUT1 as a marker for the central nucleus (unpublished results) to find the location of the 13 neurons that were retrieved. The estimated location of the retrieved cells within this reconstruction is displayed in Fig. 1, C and D from a caudal and a dorsal view, respectively. About half of the cells (6/13) were localized in the central nucleus, five cells were localized in the dorsal cortex, and two in the external cortex of the IC. Their soma size was on average 9 × 16 μm (range 6–28 μm). The cells that were localized in the central nucleus obeyed a tonotopic gradient, with low frequencies in dorsolateral regions and high frequencies in ventromedial regions (range 13.9–27.8 kHz; not shown), in agreement with earlier reports (Stiebler and Ehret 1985; Willott and Urban 1978).

In addition, 86 neurons were acoustically stimulated. One cell did not show any sound-evoked activity in response to pure tones at 80 dB SPL, whereas two cells fired spontaneously, but did not change their firing rate when a sound stimulus was given. This means that probably fewer than five of the total 136 neurons (3.5%) were not auditory.

Relation between spontaneous synaptic potentials and action potential generation

We investigated to what extent the spontaneous inputs influenced action potential generation. Spontaneous EPSPs were observed in all cells, whereas spontaneous inhibitory postsynaptic potentials (spIPSPs) were observed much more frequently and large (>5 mV) spIPSPs were observed in only one cell. An example of spEPSPs is shown in the bottom trace of Fig. 2A. A role for spontaneous inputs in action potential generation was obvious in 14 of the 16 neurons (of a total of 103 neurons) that fired spontaneously in mice because in these 14 cells spontaneous action potentials were typically triggered on top of spEPSPs. In seven of these 14 cells, the spontaneous action potentials were triggered by single, relatively large (>5 mV) spEPSPs. In the other seven cells, the spontaneous action potentials were typically triggered on top of bursts of spEPSPs. In the remaining two spontaneously active cells there was no obvious role for spEPSPs in the timing of action potential generation. One cell had a very negative threshold for action potential generation and the other cell showed an oscillating membrane potential, with action potentials triggered on top of the oscillations. Spontaneously active cells on average had a

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**FIG. 1.** Localization of biocytin-filled cells within the mouse inferior colliculus (IC). A: response to 100-ms, 200-pA hyperpolarizing and depolarizing constant-current injections of the neuron whose morphology is shown in B. B: reconstruction of a single neuron (marked in C and D by a crossed circle). C: caudal view of a reconstruction of the inferior colliculus. Inferior collicus is indicated in blue, except for the central nucleus, which is indicated in yellow. Structures outside of the inferior colliculus (periaqueductal gray) are indicated in turquoise. Location of biocytin-filled cells is indicated as white circles. Circles with a black outline were localized within the central nucleus. Abbreviations: L, left; R, right; ag, cerebral aqueduct. D: like C, except dorsal view.
FIG. 2. Spontaneous inputs affect firing regularity. A: current-clamp recording at resting membrane potential shows spontaneous excitatory postsynaptic potentials (spEPSPs; bottom trace) of variable amplitude. spEPSPs contribute to irregular firing during 1-s, +300-pA depolarizing current step (top trace). Traces have been offset for display purposes. Current injection protocol is indicated below the 2 traces. B: relation between the irregularity of firing during constant-current injection (calculated as described in METHODS) and the SD of the baseline noise. Filled circle is the experiment displayed in A. Solid line is the regression line ($r = 0.30$).

more depolarized membrane potential ($-55.5 \pm 1.1$ mV, $n = 16$ vs. $-61.7 \pm 0.6$ mV, $n = 87$; $P < 0.001$), whereas their action potential threshold did not differ significantly from the other cells ($-49.6 \pm 0.5$ mV, $n = 16$ vs. $-46.8 \pm 0.5$ mV, $n = 87$; $P = 0.25$). The resting membrane potential is therefore an important factor in determining whether a cell fires spontaneously. In rats, resting membrane potential was also more depolarized in active cells compared with cells that did not fire spontaneously ($-57.2 \pm 1.2$ mV, $n = 12$ vs. $-60.7 \pm 1.1$ mV, $n = 21$; $P = 0.036$). In addition, their action potential threshold was significantly lower ($-48.2 \pm 1.3$ mV, $n = 12$ vs. $-42.9 \pm 1.4$ mV, $n = 21$; $P = 0.009$).

To investigate a possible role for spEPSPs in the cells that were not spontaneously active, we compared the depolarization needed to elicit an action potential with the size of spontaneous inputs. The larger the size of the spontaneous inputs and the closer the action potential threshold is to the resting potential, the larger their effect on action potential generation is expected to be. In 11 cells in which the spEPSPs could be relatively easily distinguished from baseline noise, spEPSPs with an average amplitude of $1.1 \pm 0.2$ mV and a rise time of $2.0 \pm 0.2$ ms were observed at an average frequency of around 30 Hz. In these cells, the average ratio between the depolarization needed to reach action potential threshold and the size of the spEPSPs was $21.3 \pm 6.5$ (range 3.5–75). Although the relation between the number of active inputs and membrane potential is far from linear and although the amplitudes of spEPSPs were typically variable and skewed toward larger amplitudes ($CV 0.78 \pm 0.09$, skewness $2.0 \pm 0.3$; $n = 11$), our results nevertheless suggest that the number of simultaneously active inputs needed to elicit a spike varies considerably between cells.

Even if the spontaneous inputs are not sufficiently large to directly trigger action potentials, they could still be large enough to affect firing regularity. For example, in the cell displayed in Fig. 2A a clear acceleration in the spike rate may be observed (top trace) during a burst of spEPSPs. To quantify the relation between firing regularity and the presence of spEPSPs more systematically, we plotted the relation between the CV of the firing rate, which was corrected for accommodation as detailed in METHODS, and the SD of the baseline (Fig. 2B). The two were significantly related ($r = 0.30; P = 0.026$).

In conclusion, in many neurons in the IC, spontaneous inputs could influence the timing and frequency of action potentials. This was most obvious in spontaneously active cells, where action potentials were typically triggered by single, large spEPSPs or bursts of spEPSPs. In many other cells, the depolarization needed to trigger an action potential was less than ten times the average size of the spEPSPs and as a result the spEPSPs are expected to have a significant effect on the ability of sound-evoked EPSPs to trigger a spike, as suggested by the effect of spEPSPs on firing regularity during constant-current injection.

**Spikelets**

A different type of spontaneous potential changes was additionally observed. Spikelets were identified as small (<5 mV), brief, sometimes biphasic potentials (Fig. 3). They typi-
cally resembled very small action potentials. They were seen both in rats [six of 33 cells (18%)] and in mice [13 of 103 cells (13%)]. In the cells in which spikelets were observed, they could occur at rest (Fig. 3A), on top of a spontaneous EPSP (Fig. 3B), or during the rising phase of a spontaneous action potential (Fig. 3C). In mice, cells with spikelets had a significantly lower membrane resistance ($82 \pm 5 \text{ M}\Omega$, $n = 13$ vs. $110 \pm 6 \text{ M}\Omega$, $n = 90$, $P < 0.001$). Time constant, resting membrane potential, action potential threshold, the presence of $I_h$, or the age of the animal was not significantly different between cells both with and without spikelets. In rats, membrane resistance, resting membrane potential, and threshold were similar in cells with and without spikelets, although the time constant appeared significantly higher in cells with spikelets ($15.4 \pm 1.7 \text{ ms}$, $n = 6$ vs. $8.3 \pm 0.9 \text{ ms}$, $n = 27$, $P = 0.015$). If we assume that the spikelets reflect electrotonic coupling between neurons (Söhl et al. 2005), the lower membrane resistance or the longer time constant may be a consequence of the coupling, although the number of cells with spikelets was small, the differences were not large and they were not consistent across both species.

Classification of spike patterns

All cells responded with spikes to depolarizing current injections of sufficient strength. Based on the intervals between the spikes during a depolarizing step, we classified them into six different groups: sustained, accommodating, burst-onset, burst-sustained, buildup, and accelerating. To classify the neurons, we first looked at whether they fired in bursts, or showed buildup or accelerating-type firing.

Burst-onset was observed in 5% (five of 103) of cells (Fig. 4, A and B) and burst-sustained in 10% of cells (10 of 103) in mice. Burst-sustained cells had a typical fast hyperpolarization between the clusters of action potentials, which was not observed in accommodating cells (Fig. 4, C and D). In rats, both burst-onset and burst-sustained were observed in 9% (three of 33) of cells.

Eleven neurons (11%) in mice were classified as accelerating. Eight of these neurons showed an acceleration in spike rate followed by a deceleration (Fig. 5, A–C). In the other three neurons, the first ISI was consistently larger than the next ISI (Fig. 5, D–F). These characteristic firing patterns remained present at larger current injections. In contrast to buildup neurons, the firing patterns of accelerating neurons were not affected by a 1-s prehyperpolarization. In rats, only one cell (3%) showed an accelerating type of firing pattern.

A buildup response during a depolarizing current injection after prehyperpolarization was observed in 14 neurons (14%; Fig. 6). In these neurons, the delay between the start of the depolarizing current injection and the first spike increased from a value of $64 \pm 28 \text{ ms}$ (range 6–416 ms) for depolarizations from the resting potential of $-63 \pm 2 \text{ mV}$ to a delay of $138 \pm 40 \text{ ms}$ (range 30–476 ms) when the membrane potential was hyperpolarized to $-87 \pm 3 \text{ mV}$ before the depolarizing current injection. After the prehyperpolarization, in all cells the delays remained long, even when the current injection was increased well above the standard level of 100 pA above threshold. In comparison, accelerating, sustained, and accommodating cells showed a much smaller delay than buildup neurons, which showed little increase following a prehyperpolarization. In these cells, the delay increased from a value of $8 \pm 1 \text{ ms}$ (range 1–24 ms) at the resting membrane potential of $-60 \pm 1 \text{ mV}$ to a delay of $11 \pm 1 \text{ ms}$ (range 1–43 ms) when the membrane potential was hyperpolarized to $-75 \pm 1 \text{ mV}$. In rats we observed buildup neurons in 12% of cells (four of 33).

In the remainder of the cells (61% in mice, 67% in rats) the relation between the firing rates during the current injections and the ISI could be well described by an exponential function (Fig. 7, A–C). The amount of accommodation differed greatly between cells, but also depended on the amount of current that was injected (Fig. 7D). In general, cells that had a low accommodation index (AI) when tested just above firing threshold also had a low AI when tested with larger depolarizing currents. However, cells that showed clear accommodation just

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**FIG. 4.** Two types of burst firing in mouse IC neurons. A: burst-onset firing pattern was characterized by clustered action potentials at the beginning of depolarizing current injection. Broken line indicates resting membrane potential. Hyperpolarizing current injection showed a depolarizing sag, indicated with arrow. B: raster plot of all burst-onset cells ($n = 5$) at 100 pA above threshold; arrow indicates cell shown in A. C: burst-sustained pattern was observed with clustered action potentials throughout the depolarizing current step. D: raster plot of all burst-sustained cells ($n = 10$) at 100 pA above threshold; arrow indicates trace shown in C.
above threshold showed a more sustained firing pattern when tested with larger currents (Fig. 7). At 100 pA above the first level that resulted in one or more spikes, 23% (24 of 103) of cells had an AI < 4% and were classified as sustained (Fig. 8, A–C), the other 38% (39 of 103) as accommodating (Fig. 8, D–F). A subset of these cells (seven sustained, nine accommodating) showed rebound (anode-break) type firing following hyperpolarizing steps (Fig. 9). Rebound spiking was also observed in one buildup and four burst-sustained cells. In rats, 27% (nine of 33) of cells showed sustained firing. An accommodating firing type was observed in 40% of cells (13 of 33 cells). Two accommodating cells fired a rebound spike after hyperpolarization. The presence of spikelets was not associated with a specific firing type.

Membrane properties of IC neurons

A summary of the membrane properties of the mouse IC neurons belonging to the six different classes is presented in Table 1. Mean input resistance and membrane time constant differed significantly between cell types (ANOVA, \( P = 0.001 \) and \( P = 0.025 \), respectively). Buildup neurons had a significantly larger input resistance than that of accelerating, accommodating, burst-sustained, and burst-onset cells (\( P < 0.05 \); Tukey’s HSD test). In addition, buildup neurons had a longer time constant than that of accelerating cells (\( P = 0.028 \); Tukey’s HSD test). Mean resting membrane potential and absolute spike threshold did not differ significantly between groups. Input resistance did not significantly change between 3 and 5 wk of age and there were no obvious changes in the relative frequency of firing patterns across this age range.

In rat neurons, the average membrane potential was ~59 ± 0.8 mV (range −47 to −68 mV; \( n = 33 \)) and spike threshold was 15 ± 1 mV above the resting membrane potential (range 3–29 mV; \( n = 33 \)).

No significant differences between cell groups were found in rat IC neurons for resting membrane potential \( V_m \) (\( P = 0.09 \)) or threshold (\( P = 0.44 \)). Input resistance, time constant, and the
presence of a depolarizing sag were not measured because of the lack of proper hyperpolarization protocols.

Hyperpolarization-activated current ($I_h$)

In mice, in 52 of 103 cells a depolarizing sag (of >10%) was observed during hyperpolarizing current injection, suggesting that these cells had the hyperpolarization-activated mixed cation current $I_h$. These cells had a more depolarized resting membrane potential ($-59.4 \pm 0.6$ mV, $n = 52$ vs. $-62.1 \pm 1.0$ mV, $n = 51$, $P = 0.03$) and a lower action potential threshold ($-49.3 \pm 0.7$ mV, $n = 52$ vs. $-45.1 \pm 0.8$ mV, $n = 51$, $P < 0.001$). Membrane resistance was not significantly different between cells either with or without $I_h$ ($98 \pm 6$ MΩ, $n = 52$ vs. $115 \pm 7$ MΩ, $n = 51$, $P = 0.11$).

We measured the activation of $I_h$ by fitting a single- or a double-exponential function on the depolarizing sag during hyperpolarizing current injections (Fig. 9B). In 14 cells, a single-exponential function was sufficient to describe $I_h$ activation. The average time constant was $47 \pm 26$ ms. In the other 38 cells, a double-exponential function was needed with a fast time constant of $42 \pm 5$ ms, which contributed 68 ± 3% to the total amplitude and a slow time constant, which ranged from 30 ms to more than a few seconds and had a median value of 380 ms.

The fraction of cells with $I_h$ differed greatly between cells with different firing patterns ($\chi^2$ test, $P < 0.001$). All cells that were highly accommodating (i.e., burst-onset cells or cells with an AI >40%) had $I_h$ and in each case showed a rapid depolarizing sag. The time constant of single exponential or the fast exponential of a biexponential fit was in each case <40 ms. The presence of rapidly gating $I_h$ in these cells suggests that the depolarizing sag, which is attributed to deactivation of $I_h$ during depolarizing current injection, contributes to their firing pattern. An example of a brief depolarizing sag of a burst-onset cell is shown in Fig. 5A.

All cells with $I_h$ showed an AHP of >1 mV at the end of the depolarizing current injections, whereas only 51% of cells without $I_h$ showed an AHP.

All cells with $I_h$ showed a depolarizing overshoot after hyperpolarization ended. This so-called hump most likely reflects the gradual deactivation of $I_h$ back to the resting open probability after the activation of $I_h$ during the hyperpolarizing step (Koch and Grothe 2003). Only five of 51 cells without $I_h$ showed a hump, but in these cases the hump had a different time course than that of the AHP, except in one cell, which did show a marginal presence of $I_h$ (8% depolarizing sag). Presumably, by its contribution to this hump, $I_h$ was important for rebound spiking since of the 21 cells with rebound spiking, 18 had a clear depolarizing sag (>10%) and in three it was marginal (4–7%).

We conclude that cells with $I_h$ had a more depolarized resting potential, were more likely to fire bursts (13 of 15 bursting cells had $I_h$), were more likely to show an AHP following depolarizing current injection, were more likely to
fire rebound action potentials, and were more likely to accommodate. Cells with strong accommodation showed rapid $I_h$ gating, suggesting that rapid $I_h$ deactivation during depolarizing current injections contributed to the accommodation in these cells.

**DISCUSSION**

Herein we describe basic properties of the neurons in the inferior colliculus (IC) of the rat and the mouse. Although these two species are the most commonly used animals in neuroscience research, in vivo intracellular recordings from the rodent IC have not yet been reported. In contrast to previous reports in slices, we did not observe single-spike onset firing, whereas we did observe an accelerating firing pattern. Two other new findings that were not apparent in previous slice (or in vivo) recordings were the occurrence of spikelets and the effect that spontaneous EPSPs had on triggering action potentials and thereby on the regularity of firing in many cells.

**Spikelets are present in the rodent inferior colliculus**

Spikelets resemble very small action potentials. They are widely considered to be the electrophysiological correlate of electrotonic coupling through gap junctions (Söhl et al. 2005), but we cannot exclude that they reflect dendritic action potentials. Although they were not previously reported to be present in the IC, we observed them in both rats and mice, in 13–18% of the cells, respectively. Their location within the IC remains to be investigated. These cells did not show obvious differences in their passive properties and the presence of spikelets was not associated with a specific type of firing pattern. The gap junction proteins Connexin 36 and Pannexin 1 and 2 are expressed in the adult IC, albeit at low levels (Bruzzone et al. 2003; Condorelli et al. 2000; Zappala et al. 2006). As in other brain regions, a primary role of the gap junctions may be to synchronize cells and as such a possible role in audiogenic seizures (Faingold 2002) deserves further attention.

**Spontaneous firing rates were much lower than in extracellular studies**

Cells rarely fired spontaneously, in contrast to what was previously observed using extracellular recordings in rodents (Ehret and Moffat 1985; Palombi and Caspary 1996; Willott and Urban 1978). In other brain areas there is evidence that spike frequencies are overestimated by extracellular recordings (Margrie et al. 2002). Although we cannot exclude that anesthesia may have contributed to the low observed spike rates...
We used the changes in the interspike intervals during long depolarizing steps for classifying the cells. In most cells the relation between spike rates and ISI number could be adequately described by a single-exponential function. Cells in which the fit was poor fired in bursts or showed accelerating responses. Buildup neurons also were distinguished as a separate group. An advantage of the use of the exponential fit over most other methods was that it was more robust against irregular firing, which was observed in many cells, in agreement with extracellular recordings (Rees et al. 1997). Irregularity of firing during current injection was often attributed to spontaneous EPSPs, which were sufficiently large to cause brief increases in firing rates. Some cells needed summation of only a few spontaneous inputs to fire, whereas in others many more simultaneously active inputs would be needed to trigger a spike. It would be interesting to test how this difference translates into a difference in auditory function.

In cells in which the exponential fit was adequate, the increase in the spike interval between the first and the second spike was used to classify the cells as sustained or accommodating. This classification was to some extent arbitrary for two reasons. First, all cells showed some degree of accommodation. Second, although quantification for accommodation was carried out consequently at the same level above threshold to allow a comparison between cells, at higher current levels accommodating cells became more regular, as was also observed by Sivaramakrishnan and Oliver (2001). A similar phenomenon can be expected to occur during auditory stimulation: if the excitatory synaptic conductances become larger, an onset response may become more sustained (Rees et al. 1997).

A clear difference between our data and results obtained from slice recordings was the absence of onset neurons that fired only a single spike even with current injections well above the firing threshold. In recordings from rat slices, onset firing has been described in 8–24% of neurons (Koch and Grothe 2003; Li et al. 1998; Sivaramakrishnan and Oliver 2001; Wagner 1994), except we did not observe single-spike onset firing, the buildup cells did not have a pauser response, and we observed only a few spontaneous inputs for a spike to occur, whereas in others many more simultaneously active inputs would be needed to trigger a spike. It would be interesting to test how this difference translates into a difference in auditory function.

Classification of firing patterns

The neurons of the inferior colliculus are heterogeneous with respect to their firing patterns. We have described six firing patterns in vivo. Their relative frequency was similar between rats and mice. Many of our results correspond to results obtained in slice recordings (Basta and Vater 2003; Koch and Grothe 2003; Li et al. 1998; Peruzzi et al. 2000; Reetz and Ehret 1999; Sivaramakrishnan and Oliver 2001; Wagner 1994), except we did not observe single-spike onset firing, the buildup cells did not have a pauser response, and we observed cells with an accelerating firing type that had previously not been reported in slice recordings from the IC.

![FIG. 9. Rebound spiking in a cell with a depolarizing sag and a hump. A: response to 1-s, 200-pA hyperpolarizing current injection. This neuron showed a depolarizing “sag” during hyperpolarization and a “hump” with rebound spiking after the current injection. B: average of 20 hyperpolarizing current injections in the same cell (1 s, −200 pA) is shown in the bottom trace (thick black line). Thin white line shows the biexponential fit (τ1 = 48 ms, τ2 = 317 ms; relative contribution of τ2; 72% for the first 1 s). Top trace: residuals of the fit.](http://jn.physiology.org/)

(see Nuding et al. 1999 and references therein), most previous studies were also done under anesthesia.

**Table 1.** Membrane properties of neurons of the C57/Bl6 mouse inferior colliculus

<table>
<thead>
<tr>
<th>Percentage of cells</th>
<th>Buildup</th>
<th>Accelerating</th>
<th>Sustained</th>
<th>Accommodating</th>
<th>Burst-Sustained</th>
<th>Burst-Onset</th>
<th>Average of All Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{\infty}$, Ω</td>
<td>14 (14)</td>
<td>11 (11)</td>
<td>23 (24)</td>
<td>38 (39)</td>
<td>10 (10)</td>
<td>5 (5)</td>
<td>100 (103)</td>
</tr>
<tr>
<td>τ, ms</td>
<td>145 ± 18 (14)</td>
<td>81 ± 6 (11)</td>
<td>126 ± 10 (24)</td>
<td>98 ± 8 (39)</td>
<td>87 ± 12 (10)</td>
<td>64 ± 9 (5)</td>
<td>106 ± 5 (P = 0.001)</td>
</tr>
<tr>
<td>$V_{\infty}$, mV</td>
<td>-63.0 ± 2.0 (14)</td>
<td>-64.5 ± 1.6 (11)</td>
<td>-60.6 ± 1.3 (24)</td>
<td>-59.5 ± 1.1 (39)</td>
<td>-57.8 ± 0.8 (10)</td>
<td>-62.6 ± 1.4 (5)</td>
<td>-60.7 ± 0.6 (P = 0.073)</td>
</tr>
<tr>
<td>Absolute firing threshold, mV</td>
<td>-44.4 ± 1.4 (14)</td>
<td>-45.4 ± 1.9 (11)</td>
<td>-48.5 ± 1.5 (24)</td>
<td>-47.7 ± 0.8 (39)</td>
<td>-49.8 ± 1.3 (10)</td>
<td>-44.0 ± 1.4 (5)</td>
<td>-47.2 ± 0.6 (P = 0.089)</td>
</tr>
</tbody>
</table>

Values are means ± SE, with n values in parentheses. $R_{\infty}$ input resistance; τ, time constant; $V_{\infty}$, resting membrane potential. *Significant difference between groups.
We found the classification of buildup and accelerating neurons to be relatively difficult because both types shared some properties with the buildup-pauser neurons previously described in slice recordings, although both groups also differed in other respects. Cells in both groups typically lacked a depolarizing sag and did not show rebound firing. The buildup neurons had a long delay for the first spike, which increased after prehyperpolarization, as previously reported for buildup-pauser cells in slice recordings (Peruzzi et al. 2000; Sivaramakrishnan and Oliver 2001). However, in contrast to the earlier reports, we did not observe a rapid initial spike at large current injections. In mice, these cells had a relatively large membrane resistance, arguing against a much larger conductance being active at rest. We therefore speculate that the lack of this pauser or “hump-and-sag” response could be explained by a more rapid activation of the A-type current than in the slice recordings (Fujino et al. 1997; Kanold and Manis 2001).

The cells that we classified as accelerating also shared some properties with the buildup-pauser firing type previously observed in slice recordings. They robustly showed a decrease in interspike intervals, similar to a pauser response. However, the firing was not appreciably altered by prehyperpolarization, in contrast to earlier reports (Peruzzi et al. 2000; Sivaramakrishnan and Oliver 2001). Neurons with an accelerating firing type have been observed in frog tectum (Gutmaniene et al. 2003) and spinal cord (Jiang et al. 1995; Smith and Perrier 2006; Viana et al. 1995), although in these cases typically no accommodation later during the train was observed. The accelerating firing type may depend on calcium influx (Derjean et al. 2005; Purvis and Butera 2005; Smith and Perrier 2006) or on the presence of a persistent sodium current (Li and Bennett 2003). Voltage-clamp studies will be needed to more firmly establish the accelerating and buildup groups and to investigate the ionic mechanisms underlying these firing types.

Burst firing, which we observed in 15–18% of the cells, was seen in slice recordings infrequently in one report (3% of cells; Bal et al. 2002) and not at all in others (Koch and Grothe 2003; Peruzzi et al. 2000; Sivaramakrishnan and Oliver 2001). This firing type may be more prominent in the dorsal cortex, which was a target in our recordings, and calcium-dependent potassium channels may contribute to the AHPs observed between bursts (Li et al. 1998).

Role of I_h

About half of the neurons in the mouse IC showed a clear depolarizing sag during hyperpolarizing current injections. A similar depolarizing sag has also been observed in vivo recordings from guinea pig (Pedemonte et al. 1997) and this sag most likely signifies the activation of the cation current I_h, although we cannot exclude contributions from other currents. In slice recordings from rat, this sag was blocked by the specific I_h blocker ZD7288 (Koch and Grothe 2003) and IC neurons are known to express different I_h subunits (Koch et al. 2004; Notomi and Shigemoto 2004). The kinetics of the depolarizing sag we observed largely matched the kinetics of I_h activation observed in slice recordings, although because we estimated I_h activation during current-clamp recordings, the precise gating kinetics are somewhat uncertain. In most cells, activation was biphasic, with presumably HCN1 subunits contributing to fast activation and HCN2 homomers responsible for the slowly activating conductance (reviewed in Robinson and Siegelbaum 2003). I_h seemed to be active already at the resting membrane potential, suggesting that I_h activates at relatively positive potentials in IC neurons, similar to octopus cells, for example (Bal and Oertel 2000).

Cells with evidence for the presence of I_h clearly differed from cells without a clear depolarizing sag. They were more likely to fire rebound action potentials and, conversely, all cells with rebound firing had a depolarizing sag. This suggests that activation of I_h during the hyperpolarization contributed to the rebound spikes (Koch and Grothe 2003). We did not investigate the role of calcium channels and calcium-dependent potassium channels in rebound firing (Sivaramakrishnan and Oliver 2001; Smith 1992). In addition, cells with I_h had a more depolarized resting potential, were more likely to fire bursts (13 of 15 bursting cells had I_h), and generally showed more rapid accommodation. Cells with strong accommodation showed rapid I_h gating, suggesting that rapid I_h deactivation during depolarizing current injections contributed to the accommodation in these cells (Koch and Grothe 2003).

In addition, all cells with I_h showed an afterhyperpolarization following long depolarizations (vs. 51% in cells without I_h), as previously observed in slice recordings (Koch and Grothe 2003). In vivo application of specific I_h blockers would be helpful in testing the effect on auditory processing of the contribution of I_h to the resting membrane potential, rebound firing, spike accommodation, or the afterhyperpolarization. In the companion paper (Tan and Borst 2006) we provide further evidence for a special role of I_h in auditory processing.

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REFERENCES


Derjean D, Bertrand S, Nagy F, Shefchyk SJ. Plateau potentials and membrane oscillations in parasympathetic preganglionic neurones and in...


