Intrinsic firing properties in the avian auditory brain stem allow both integration and encoding of temporally modulated noisy inputs in vitro

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Kreeger LJ, Arshed A, MacLeod KM. Intrinsic firing properties in the avian auditory brain stem allow both integration and encoding of temporally modulated noisy inputs in vitro. J Neurophysiol 108: 2794–2809, 2012. First published August 22, 2012; doi:10.1152/jn.00092.2012.—The intrinsic properties of tonically firing neurons in the cochlear nucleus contribute to representing average sound intensity by favoring synaptic integration across auditory nerve inputs, reducing phase locking to fine temporal acoustic structure and enhancing envelope locking. To determine whether tonically firing neurons of the avian cochlear nucleus angularis (NA) resemble ideal integrators, we investigated their firing responses to noisy current injections during whole cell patch-clamp recordings in brain slices. One subclass of neurons (36% of tonically firing neurons, mainly subtype tonic III) showed no significant changes in firing rate with noise fluctuations, acting like pure integrators. In contrast, many tonically firing neurons (>60%, mainly subtype tonic I or II) showed a robust sensitivity to noisy current fluctuations, increasing their firing rates with increased fluctuation amplitudes. For noise-sensitive tonic neurons, the firing rate vs. average current curves with noise had larger maximal firing rates, lower gains, and wider dynamic ranges compared with FI curves for current steps without noise. All NA neurons showed fluctuation-driven patterning of spikes with a high degree of temporal reliability and millisecond spike time precision. Single-spiking neurons in NA also responded to noisy currents with higher firing rates and reliable spike trains, although less precisely than nucleus magnocellularis neurons. Thus some NA neurons function as integrators by encoding average input levels over wide dynamic ranges regardless of current fluctuations, others detect the degree of coherence in the inputs, and most encode the temporal patterns contained in their inputs with a high degree of precision.

nucleus angularis; interaural level difference; frequency-current relationship; noise; cochlear nucleus

THE INTRINSIC FIRING PROPERTIES of neurons are highly varied and are useful in classifying different neuronal cell types within networks. These properties determine the basic input-output functions of the different cell classes, but the consequences for neural coding are not always well understood. Neurons can be placed along a functional continuum on the basis of how their intrinsic properties regulate the integration of their synaptic inputs. At one end of the spectrum are coincidence detector neurons that respond vigorously only when the array of inputs arrive near simultaneously. Their intrinsic properties render the neuron sensitive to large fluctuations in the current drive, which only occurs when inputs are highly correlated. On the other end of the spectrum are neurons that behave more closely to pure integrators, that is, firing action potentials in direct proportion to their average current drive integrated over time regardless of fluctuations around the mean.

In the auditory system, the neurons responsible for the computation of interaural time differences for the purpose of sound localization are the best understood representative of the coincidence detector neuron: neurons of nucleus laminaris (NL) in birds and medial superior olive (MSO) in mammals (Carr and Konishi 1990; Goldberg and Brown 1969; Jeffress 1948; Yin and Chan 1990). The coincidence detection depends on a combination of synaptic and intrinsic characteristics, but one important component is the expression of a low-threshold potassium conductance ($I_{KLT}$) responsible for the low input resistance, fast membrane time constant, and high-pass filter characteristics (Agmon-Snir et al. 1998; Khurana et al. 2011; Kuba 2007; Kuba et al. 2003, 2005; Mathews et al. 2010; Reyes et al. 1996; Svirskis 2002; Svirskis et al. 2003). Similar intrinsic properties are also found in the avian cochlear nucleus magnocellularis (NM) and bushy and octopus cells in the mammalian cochlear nucleus and medial nucleus of the trapezoid body (Bal and Oertel 2001; Brew and Forsythe 1995; Cao et al. 2007; Goulding et al. 1995; Manis and Marx 1991; Rathouz and Trussell 1998). These properties enhance precise phase locking of action potentials to the stimulus waveform and the transmission of fine timing information (for reviews, see Burger and Rubel 2008; Grothe 2000; MacLeod and Carr 2011; Trussell 1999).

A second general cell type found in the cochlear nucleus of both birds and mammals has a tendency to fire multiple spikes repetitively throughout the step-current stimulus, termed “tonic firing” in the avian brain stem and “type I/stellate” (based on physiology/morphology) in the mammalian brain stem (Fukui and Ohmori 2003; Manis and Marx 1991; McGinley and Oertel 2006; Oertel et al. 1990, 2011; Soares et al. 2002; Wu and Oertel 1984). This repetitive firing, in contrast to the phasic firing in the timing pathways, is attributable in part to a relative lack of $I_{KLT}$ (Cao et al. 2007; Ferragamo and Oertel 2002; Fukui and Ohmori 2003; Manis and Marx 1991; Oertel et al. 2008; Rothman and Manis 2003a, 2003b). In birds, tonically firing neurons are found in the cochlear nucleus angularis (NA) (Fukui and Ohmori 2003; Kuo et al. 2009; Soares et al. 2002), which initiates the ascending pathway for intensity information that underlies the computation of interaural level differences (Carr and Soares 2002; Krutzfeldt et al. 2010; Manley et al. 1988; Moiseff and Konishi 1983; Sullivan and Konishi 1984; Takahashi et al. 1984; Takahashi and Konishi 1988a). In vitro experiments show that tonically firing neurons in NA have linear increases in firing rates with current injection, slower membrane time constants, and longer integration times (Fukui and Ohmori 2003; MacLeod and Carr 2005; Soares et al.
In vivo, NA neurons have weak phase locking to carrier frequencies, indicating a loss of fine timing information (Köppl and Carr 2003; Sullivan and Konishi 1984). The regenerative firing and integrative nature of these neurons is thought to be responsible for the loss of phase locking. In mammalian ventral cochlear nucleus, stellate cells in vivo have been correlated with “chopper” responses to sound, a repetitive firing response that produces reliable peaks in the poststimulus time histogram (Blackburn and Sachs 1989; Pfeiffer 1966; Rhode et al. 1983). Chopper responses are also observed in NA in vivo but have yet to be definitively linked to a particular in vitro cell type (Köppl and Carr 2003; Sullivan 1985; Warchol and Dallos 1990). Repetitive firing and tonic activity in the chopper neurons are especially suited for generating a rate code of average sound intensity for spectral analysis or binaural calculations of interaural level difference.

To assess where the NA neurons fall on the coincidence detector-integrator spectrum, we made patch-clamp recordings from brain stem slices in vitro and used direct injection of noisy current stimuli. These noisy currents more closely correspond to natural inputs than the flat current injections typically used to characterize neuronal intrinsic properties. These stimuli simulate the arrival of a population of inputs where the average amplitude relates to the overall number of inputs and the current fluctuations relate to the degree of correlation across inputs. We have shown that many NA neurons not only responded to the average current levels but also were sensitive to the current fluctuations, responding with increased firing rates and reliable patterning of spike timing to fluctuation events. We conclude that although some NA neurons may function as integrators by encoding average input levels over a wide dynamic range, many are highly capable of precisely encoding temporal envelope cues and detecting the degree of coherent firing across inputs.

MATERIALS AND METHODS
Brain Slice Preparation
All animal procedures were performed in accordance with University of Maryland guidelines on animal welfare and a protocol approved by the university Institutional Animal Care and Use Committee. Chicken embryos (embryonic day 17–18) were cooled and rapidly decapitated, and the head section containing the brain stem was submerged in chilled and oxygenated low-sodium artificial cerebral spinal fluid (ACSF) (in mM: 97.5 NaCl, 3 KCl, 2.5 MgCl₂, 26 NaHCO₃, 2 CaCl₂, 1.25 NaH₂PO₄, 10 dextrose, 3 HEPES, and 230 sucrose). The brain stem was dissected out of the embryo, and transverse slices (250 μm thick) containing NA were made on a vibrating tissue slicer (Leica Microsystems, Wetzler, Germany). During slicing, the brain stem was submerged in chilled, oxygenated low-sodium ACSF, mounted with cyanoacrylate glue, and supported by a 5% agarose gel solution. Slices were incubated in oxygenated ACSF (in mM: 130 NaCl, 3 KCl, 2 MgCl₂, 26 NaHCO₃, 2 CaCl₂, 1.25 NaH₂PO₄, 10 dextrose, and 3 HEPES) for 30 min at 34°C and then held in solution at room temperature until the time of recordings. During recording, slices were submerged in a recording chamber and continuously perfused with warmed (29.6 ± 1.4°C), oxygenated ACSF (1–2 ml/min). No synaptic channel blockers were used.

Patch-Clamp Electrophysiology
Whole cell patch-clamp recordings were made from visually identified NA cells using infrared-differential interference contrast video microscopy. Initial micropipette resistances were 4–8 MΩ with an internal solution consisting of (in mM) 110 potassium gluconate, 20 KCl, 1 EGTA, 2 MgCl₂, 10 HEPES, 2 Na₃ATP, 0.3 Na₃GTP, 10 phosphocreatine, and 0.1% biocytin. Electrical recordings were made with either an AxoPatch 200B patch-clamp amplifier (Molecular Devices, Sunnyvale, CA) in fast current-clamp mode or an Axoclamp-2 amplifier in bridge mode. Stimulation and recordings were controlled via an analog-to-digital board (National Instruments, Austin, TX) and a personal computer running custom software written using the programming software IGOR Pro (Wavemetrics, Lake Oswego, OR). Slices containing biocytin-filled NA neurons were processed as previously described (MacLeod and Carr 2007), using avidin-conjugated AlexaFluor 488 (Invitrogen) to verify the recording site and visualize the neuronal morphology. Morphology and physiology could be unambiguously matched for two tonic II and two tonic III neurons. Most often, however, multiple recordings from nearby sites resulted in ambiguity in matching morphology to specific recordings, and therefore we relied on physiological criteria to classify the neurons.

Current Stimulation and Data Collection Protocols
Flat current steps and noisy current steps were produced using custom routines written in IGOR Pro. The noise currents were generated by convolving Gaussian white noise with an exponential function (time constant 3 ms). The noise stimulus was chosen to simulate the arrival of many small, stochastic, and statistically independent synaptic currents, both excitatory and inhibitory. The procedure we used was a simplified variation of a more precise simulation of synaptic inputs replicated by an Ornstein-Uhlenbeck process, which results in a Gaussian distributed output (Rauch et al. 2003). The time constant of the exponential filter reflects the rapidity of synaptic responses in the auditory brain stem. Measurements of the time course of AMPA receptor-mediated synaptic currents in NA neurons showed rapid decay time constants (~1 ms), whereas inhibitory currents were slightly slower (2- to 4-ms fast time constant) (Kuo et al. 2009; MacLeod and Carr 2005). NA neurons receive excitatory inputs directly from auditory nerve fibers and inhibitory inputs from the superior olivary nucleus or other descending projections (Burger et al. 2005; MacLeod and Carr 2007). Stimulation created by adding current fluctuations on a direct current (DC) pedestal bears a close resemblance to measurements of intracellular membrane potentials recorded in cochlear nucleus neurons in vivo, where tone stimuli evoked slower depolarizing potentials with rapidly fluctuating synaptic events superimposed (unpublished observations in chicken; MacLeod KM, Carr CE, Soares D; see also Rhode and Smith 1986). In investigations of cortical neurons, the use of similar currents has been shown to generate firing that resembles in vivo firing patterns (Destexhe et al. 2001; Mainen and Sejnowski 1995).

Stimuli were generated and data collected with a resolution of 20 kHz. To calibrate the noise currents to accommodate cell-to-cell differences in input resistance, a Gaussian exponential-filtered noise stimulus was generated with zero mean amplitude and the standard deviation of the noise current was adjusted until it produced a 4-mV voltage fluctuation in the target neuron; this standard deviation was designated “1σ” (following the notation of Higgs et al. 2006). The amount of voltage fluctuation was varied by multiplying the standard 1σ noise stimulus by a factor of 0 (i.e., a flat current step), 2, or 4 (i.e., 8- and 16-mV SD voltage fluctuation, respectively). The noise was then superimposed on the flat DC rectangular current step. A complete series of stimuli in the parameter space [mean DC, noise level] was systematically generated by varying the DC amplitude and noise level independently.

All voltages were corrected for a measured junction potential of ~6 mV during analysis. Neurons with resting potentials more depolarized than ~50 mV were rejected. A constant current was injected as necessary to maintain a holding voltage of ~66 mV. This turned out
to be low relative to the average resting potential of 53.9 ± 7.0 mV. However, we performed experiments measuring FI curves (both with and without noise) from different baseline voltages and found the curves were identical, except for a leftward shift in the step levels to compensate for the holding voltage, thus indicating that the holding voltage did not influence the results. To classify the cell type, flat current injections (200 – 400 ms long) ranging from −0.1 up to 1 nA were applied in 50- to 100-pA steps. To acquire FI curves to compare responses with and without noise, 2-s-long stimuli were delivered for 3–5 trials per noise level-current level combination with an inter-stimulus interval of 8–10 s. To measure spike time reliability, a single DC level and noise level were chosen, and 50–100 trials were collected. If a cell was tested with multiple DC or noise levels, trials were collected in blocks. Experiments that showed changes in firing rate unrelated to stimulus parameters (i.e., due to recording non-stationarity) or increased in input resistance (>20%; mean input resistance across all NA neurons 198.4 ± 79.3 MΩ) over the recording session were excluded from analysis.

Analysis

Offline analysis was performed using custom procedures and functions using IGOR Pro, Matlab (The MathWorks, Natick, MA), and Microsoft Excel.

Cell classification. Classification was determined using physiological firing criteria. All classification protocols were carried out using 400- to 500-ms steps and 50- to 100-pA step amplitude intervals, adjusted and repeated until sufficient data were collected to make a cell type decision. A decision tree was used to simplify categorization of cell type based on previous detailed electrophysiological characterization and cluster analysis of firing response criteria and morphological correlates (Soares et al. 2002). Specifically, we considered the latency of the onset spike, number of spikes elicited (single or tonic firing), spike height adaptation, the pattern with which additional spikes were elicited with increasing current step levels, and the time course of the afterhyperpolarization (AHP). Single spikers fired only a single (or rarely, a doublet) action potential at stimulus onset for all current step amplitudes. Damped neurons were classified as such if the voltage response had a >30% decline in action potential amplitude at current steps ≥300 pA and oscillatory ringing at higher current steps. These criteria allowed us to distinguish damped neurons from neurons that were tonic but showed some degree of depolarization block at high-amplitude current steps. Tonic neurons fired repetitively and could be subdivided into three classes. Tonic I neurons had an onset burst whose duration lengthened with increasing step amplitude. Tonic II neurons were most numerous in our sample and had a characteristic delayed firing pattern (with or without an initial spike at onset) at low step amplitudes. Both tonic I and II neurons had fast, deep AHPs. Tonic III neurons had slower, shallower AHPs and fired action potentials distributed in time over the duration of the step, unlike the bursting behavior of tonic I and II neurons.

Analysis of the input-output function. Firing responses to flat current steps and noisy current steps were analyzed by measuring the spike rates and spike times using hard threshold discrimination (usually at 0 mV but adjusted as needed) and displayed as an “FI curve,” plotting firing rate vs. mean DC current level. Firing rates are reported as mean rates ± 1 standard deviation (SD) calculated over the entire 2-s stimulation; this included responses that, for example, had short bursts at the onset followed by a longer duration “silent” interval. Some of the increase in firing rate reported with noise was certainly due to the fact that noise elicited spikes throughout the duration of stimulus instead of only during a short burst. However, burst extension was not the only cause of increased firing rates; confining analysis to epochs that span only the length of the initial bursts yielded qualitatively similar results. FI curves taken under different noise conditions were compared. Our criteria for labeling neural responses as “differentiating” or “integrating” are as follows: if the curves were separated by more than 2 SD of the data (1 SD from each data curve) over a current range of step levels greater than or equal to 200 pA, the neuron was classified as a differentiator. Typically, the curves were separated by gaps far larger than this criterion. If the curves differentiated for most of the dynamic range but converged at the highest current steps, they were considered “convergent” (see Fig. 2B); if the curves remained separated at the highest current steps, they were considered “divergent” (see Fig. 2A), and maximum firing rates significantly differed between the no-noise and 4σ curves (see Fig. 4). If the no-noise, 2σ, and 4σ curves overlapped within 2 SD of each respective curve for nearly all of the dynamic range, except for 1–2 points around rheobase, the neuron was classified as an integrator (see Fig. 2C).

To analyze how the input-output function is altered with noise, we characterized the FI curves by fitting them with a logistic (sigmoidal) function of the form

\[ FR(x) = FR_0 + \frac{FR_{max}}{1 + e^{x/rate}} \]

where \( FR(x) \) is the firing rate output and \( x \) is the average (DC) current level. During fitting, \( FR_0 \), the baseline rate, was set to zero and free parameters \( FR_{max} \) (saturation rate), \( x_{mid} \) (midpoint), and rate were found using IGOR Pro’s curve-fitting algorithm. Rheobase was calculated from the logistic curve as the current value at which the firing rate reached 10% of the maximum rate. Dynamic range (DR) was calculated as the current range corresponding to 10–90% of the maximum firing rate. Slope was computed as the derivative of the curve taken at the midpoint. Changes in each of these parameters between no noise (0σ) and 4σ within each class are shown in Figs. 3 and 4:

\[
\begin{align*}
\Delta \text{gain} & = 100 \cdot \frac{\text{slope}_{4\sigma} - \text{slope}_{0\sigma}}{\text{slope}_{0\sigma}} \\
\Delta \text{FR}_{max} & = \text{FR}_{max,4\sigma} - \text{FR}_{max,0\sigma} \\
\Delta \text{rate} & = \frac{\text{rheobase}_{4\sigma} - \text{rheobase}_{0\sigma}}{\text{rheobase}_{4\sigma} - \text{rheobase}_{0\sigma}} \\
\Delta \text{midpoint} & = \frac{\text{midpoint}_{4\sigma} - \text{midpoint}_{0\sigma}}{\text{midpoint}_{4\sigma} - \text{midpoint}_{0\sigma}} \\
\Delta \text{DR} & = \frac{\text{DR}_{4\sigma} - \text{DR}_{0\sigma}}{\text{DR}_{0\sigma}}
\end{align*}
\]

Negative changes in gain indicate a decrease in slope with noise; negative changes in rheobase and midpoint indicate a leftward shift in these parameters with noise. Asterisks in Figs. 3 and 4 indicate which of these changes between no noise and 4σ were statistically significant using Student’s t-test. Comparisons were also calculated to determine whether there were statistically significant differences in the FI curves between response types of tonic neurons (integrators, differentiators that diverged, and differentiators that converged) and between cell type classifications as reported in the text.

Nearly all tonic firing neurons could be well fit with a logistic function. Many single-spike and damped neurons, however, were only poorly fit because the responses did not saturate over the current levels tested or were not monotonic; in these cases, the maximum firing rate, midpoint, and dynamic range were left undefined. For single-spike and damped neurons, the slope was estimated with a linear fit to the rising portion of the FI curve.

Regularity analysis. The regularity of the spiking responses was estimated from the coefficient of variation (CV) of the interspike intervals over the 2-s stimulation such that the lower the CV, the more regular the firing. CV was calculated as the ratio of the standard deviation of the intervals over the mean of the intervals:

\[ CV = \sigma_{\text{intervals}} / \mu_{\text{intervals}} \]

Timing analysis. To investigate the timing reliability of the neuronal responses, we used a single frozen noise stimulus (or flat current stimulus) presented repeatedly for 50–100 trials. Raster plots of the spike times showed vertical lines during the noise stimulation, pro-
viding a visual indication of the reliability of spiking at consistent times across trials. To quantify reliability, we calculated a correlation index using a shuffled autocorrelation (SAC) analysis. The SAC analysis is an all-intervals histogram across all trials, excluding intervals taken from within the same trials, and normalized by the normalizing factor NF:

\[
NF = N \cdot (N - 1) \cdot r^2 \Delta r D
\]

where \(N\) is the number of trials, \(r\) is the mean firing rate, \(\Delta r\) is the bin width of the correlation function (typically set to 0.2 ms), and \(D\) is duration of the stimulus epoch (2 s) (Joris et al. 2006; Street and Manis 2007). The central peak maximum is reported as the correlation index. The precision of the firing across trials is represented in the width of the central peak. We calculate the window width as full width at half-maximum (FWHM), which is

\[
\text{FWHM} = 2 \sqrt{2 \ln 2} \cdot \sigma_{\text{SAC}}
\]

where \(\sigma_{\text{SAC}}\) is the standard deviation of the Gaussian fit of the shuffled autocorrelation function.

To determine the current signal that drove the response, the spike-triggered average was calculated. The current stimulus just preceding each spike was extracted and averaged for all spikes within a set of trials from 0.1 to 1.8 s of the stimulus epoch, thereby excluding the onset and offset.

**RESULTS**

**Noisy Current Stimulation Abolishes Hallmark Intrinsic Firing Patterns**

The firing patterns evoked with flat current steps are typically used in many studies to distinguish cell types. The relationship between these firing patterns and responses to natural, in vivo stimulation, however, is often ambiguous. To investigate the intrinsic responses of NA neurons to stimuli that more closely approximate a naturalistic stimulus, we added varying amounts of noise to the standard rectangular current steps. We made whole cell patch-clamp recordings from a total of 53 NA neurons, as well as from four NM neurons. Each neuron was first probed with a series of flat current steps immediately after whole cell rupture and classified on the basis of the central peak maximum reported as the correlation index. The precision of the firing across trials is represented in the width of the central peak. We calculate the window width as full width at half-maximum (FWHM), which is

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added to the current steps, tonic I and II neurons fired throughout the step, losing their bursting behavior. Single-spiking neurons also fired throughout the step, as did NM neurons, in agreement with previous experiments in the timing nuclei (Higgs et al. 2006; Kuznetsova et al. 2008; Reyes et al. 1996). In damped neurons, the decline in spike amplitudes was far less or completely eliminated during noisy current stimulation. In addition, the firing was visibly more irregular with noise than without. Thus the more naturalistic noisy currents obscured many of the differences so readily observable with the use of flat current stimulation.

Noisy Currents Altered the Input-Output Function of Most NA Tonic Neurons

In this section, we consider the effects of noisy, and presumably more naturalistic, input stimulation on the input-output functions of the tonically firing NA neurons. Single-spiking and damped NA neuron responses to noisy stimulation are considered separately below.

The tonically firing neurons of the cochlear nuclei in different animals have long membrane time constants (relative to other auditory neurons; Ferragamo and Oertel 2002; MacLeod and Carr 2005; Oertel et al. 1990; Soares et al. 2002:) and “infinite” integration time windows with ramped currents (McGinley and Oertel 2006), and they generate approximately linear input-output functions (Oertel et al. 1990; Fukui and Ohmori 2003), where the output (firing) is proportional to the mean input (current) level. These studies all indicated that these neurons should behave like integrators, smoothing over input fluctuations due to membrane filtering and regenerative spiking mechanisms, in contrast to coincidence detectors in the timing pathways, such as NM and NL neurons, which require large voltage fluctuations to elicit firing. However, noisy stimulation currents have been found to alter the input-output functions of many repetitive spiking neurons, such as pyramidal neurons and some fast-spiking interneurons in cortex (Arsiero et al. 2007; Chance et al. 2002; Higgs et al. 2006; La Camera et al. 2008; Rauch et al. 2003). To determine whether tonically firing NA neurons behave like integrators, we measured the input-output function [measured as the firing rate to (mean) current input relation, or FI curve] while varying the noise levels imposed on the step current. We measured FI curves in recordings from 22 neurons classified as tonic firing: 6 tonic I, 10 tonic II, and 6 tonic III.

In the absence of noise, tonically firing NA neurons had sigmoidal or linear FI curves (Fig. 2) (see also Fukui and Ohmori 2003). The addition of noise to the current steps had a range of effects on the FI curves across the tonic neuron population. A minority of tonically firing NA neurons (8/22, or 36%; Table 1) did in fact behave like integrators, where noise had little to no effect on the input-output function (i.e., the FI curves overlapped; Fig. 2C). In contrast, more than 60% (16/22) of the tonically firing NA neurons showed changes in their FI curves, increasing their firing over all or part of the step range (Table 1). Neurons that showed firing rate sensitivity to the current fluctuations were labeled differentiators (see MATERIALS AND METHODS for classification criteria; after Higgs et al. 2006). Differentiators were subdivided into two types. Divergent differentiators showed firing rate increases and differences in the FI curves that persisted into the maximum or saturation firing rates at the highest current steps (Fig. 2A). Convergent differentiators showed firing rate increases throughout most of their dynamic range but converged at the highest current steps and had similar maximal firing rates (Fig. 2B).

Tonic I Neurons

Each subtype of tonic neurons showed variation in the changes in their FI curves, but there were some clear trends as shown in Table 1. Tonic I (onset bursting) neurons were all differentiators and most (4 of 6) were divergent. To quantify the effects of noise on the input-output functions, we fit the data for each neuron with sigmoidal curves and used these to define five parameters: rheobase (mean current threshold for firing), midpoint, gain/slope, dynamic range, and maximum firing rate (see MATERIALS AND METHODS; Fig. 3, A and B). Adding noise to current steps had an overall effect of shifting the FI curve to the left, with significant decreases to the rheobase and midpoint (Fig. 3D). In the absence of noise, tonic I neuron curves tended to have steep slopes (i.e., high gains) and narrow dynamic ranges. Noisy currents generated FI curves that had shallower slopes (i.e., lower gains), higher maximal firing rates, and wider dynamic ranges (Fig. 3, B, C, and E).

Tonic II Neurons

Tonic II (delayed firing) neurons were most diverse in their FI curve changes with and without noise. Most (7 of 10) were differentiators, but a minority (3 of 10) were integrators. Of the differentiators, most (5 of 7) were convergent (1 example is shown in Fig. 2B and a second example in Fig. 3A, bottom). Overall effects were similar to those of tonic I neurons, with a general leftward shift of the curve toward lower current steps, shallower gains, and wider dynamic ranges. On average, however, the effects were lesser due to the inclusion of integrators and greater number of convergent differentiators (Fig. 3, B–E) (see also the analysis grouped by effects on FI curves discussed below).

Tonic III Neurons

Most notably, nearly all the tonic III neurons were integrators (5 of 6; Fig. 2C), with one neuron narrowly qualifying as a convergent differentiator. Tonic III neurons showed no significant changes in any of the FI curve parameters (Fig. 3, B–E). Interestingly, in the absence of noise, their FI curves tended to have lower rheobase and shallower slope values than those for tonic I or II neurons (Table 2). To illustrate these differences, we constructed idealized sigmoidal input-output curves for each cell type, using the mean values of the parameters in Table 2, for the no-noise and 4σ-noise conditions (Fig. 3F, top and bottom). Without noise, the idealized FI curves for the neuron tonic subtypes were different, but with noise the curves showed heavy overlap. In summary, the changes that occurred with noisy stimuli tended to make the tonic I and II FI curves more tonic III-like. Overall, more naturalistic currents resulted in input-output curves with wider dynamic ranges than would be predicted from flat currents alone.
Quantifying Noise Responses Grouped as Integrators vs. Differentiators

Each cell type (tonic I, II, or III) showed one of three responses changes (integrator, differentiator divergent, or differentiator convergent) in their FI curves with noisy stimulation, but none of them were monolithic (Table 1). We regrouped the data by FI curve response, regardless of tonic subtype, to determine whether the effects observed were significant (Fig. 4). We found that the divergent differentiators (consisting of tonic I and II neurons) showed the largest changes not only in maximum firing rate (which was one of the criteria used for labeling them divergent) but also in rheobase, midpoint, and dynamic range. Gain changes, however, were similar for both divergent and convergent differentiators. Convergent differentiators (consisting of tonic I, II, and III neurons) showed a small but significant increase in firing rate, as well. This was due to small but consistent elevations in firing rate that did not meet the criteria to be labeled “divergent” on an individual cell basis (a difference between the no-noise and 4σ curves of twice the SD of the data). Integrators (consisting of tonic II and III neurons) showed no significant changes in any of the parameters. Thus these three response types are categorically distinct, suggesting that distinct mechanisms may underlie each type.

Table 1. Summary of FI curve response changes to noise stimulation by NA cell type

<table>
<thead>
<tr>
<th>Total</th>
<th>Differentiation (Converged)</th>
<th>Integration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tonic (all)</td>
<td>22</td>
<td>14 (8)</td>
</tr>
<tr>
<td>Tonic I</td>
<td>6</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Tonic II</td>
<td>10</td>
<td>7 (5)</td>
</tr>
<tr>
<td>Tonic III</td>
<td>6</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Damped*</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Single</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

Values are total no. of each type of nucleus angularis (NA) neuron as well as no. of neurons showing differentiation (with no. of neurons showing convergence in parentheses) or integration. *One damped neuron was ambiguous. FI, firing rate vs. average current.

Fig. 2. Effects of noise added to current steps on the input-output functions. In A–F, top graph shows a firing rate-current (FI) curve. Mean firing rates (±SD) were measured over the 2-s-long stimulus and plotted against the mean direct current (DC) current step amplitudes (current injection). Each line shows the response under a different noise condition: blue circles, no noise; green triangles, noise = 2σ; red diamonds, noise = 4σ. A–C, top, show the range of response types for NA tonically firing neurons: differentiator divergent (A), differentiator convergent (B), and integrator (C). These 3 examples happen to coincide with the 3 tonic types: a tonic I neuron (A), a tonic II neuron (B), and a tonic III neuron (C). A–C, bottom, show graphs that plot the coefficient of variation (CV) of the spike intervals vs. DC current step amplitudes for the 3 noise conditions and correspond to the spiking responses in the FI curves above them. D–F: FI curves (top) and CV plots (bottom) for a single-spiking neuron (D) and a damped neuron from NA (E) and for a NM neuron (F).
Regenerative Spiking Mechanisms in Tonic Neurons During Noisy Current Drive

In the absence of noise, the timing of repetitive firing is governed by the intrinsic properties that drive regenerative spiking, including the passive membrane time constant as well as multiple voltage-gated conductances that control spike initiation and afterhyperpolarization. The addition of noise, however, clearly disrupted the regularity of firing (Fig. 1). To determine the degree to which regenerative spiking mechanisms influenced the firing of tonic neurons in the presence of noise, we performed a regularity analysis. The mean CV of the interspike intervals was calculated for each current step and noise level combination and plotted against the current step level (bottom plot in each panel in Fig. 2). For tonic neurons in the absence of noise, firing was highly regular with CV tending to decline with increasing current and reaching a minimum level of 0.1 to 0.2. The addition of noise resulted in a higher CV, a reflection of irregular firing. The minimum CV in the presence of noise was rarely as low as that without noise, even at the highest current step levels. This indicates that there is no current level above which regenerative spiking overcomes the patterning imposed by the noise signals, even among the integrator neurons. Compared across the group of integrators and differentiators, the integrators did tend to have the most regular firing (lowest minimal CV) in the absence of noise, but the differences were not statistically significant (data not shown). In the presence of noise, minimal CVs were similar for integrators and convergent differentiators and highest for div-

Table 2. Comparison of FI curves across tonic neuron subtypes, with and without noise (4σ)

<table>
<thead>
<tr>
<th>Tonic I</th>
<th>Tonic II</th>
<th>Tonic III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheobase, pA</td>
<td>No noise</td>
<td>Noise</td>
</tr>
<tr>
<td></td>
<td>227 ± 135*</td>
<td>-86 ± 97†</td>
</tr>
<tr>
<td>Midpoint, pA</td>
<td>325 ± 174†</td>
<td>106 ± 61</td>
</tr>
<tr>
<td>Gain, sp/nA</td>
<td>391 ± 304</td>
<td>222 ± 173</td>
</tr>
<tr>
<td>Maximum firing rate, Hz</td>
<td>52.2 ± 31.6</td>
<td>68.1 ± 31.7</td>
</tr>
<tr>
<td>Dynamic range, pA</td>
<td>193 ± 117</td>
<td>384 ± 135†</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tonic II</th>
<th>No noise</th>
<th>Noise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheobase, pA</td>
<td>111 ± 49*</td>
<td>19 ± 35†</td>
</tr>
<tr>
<td>Midpoint, pA</td>
<td>189 ± 62†</td>
<td>130 ± 46</td>
</tr>
<tr>
<td>Gain, sp/nA</td>
<td>569 ± 212Ì</td>
<td>422 ± 161Ì</td>
</tr>
<tr>
<td>Maximum firing rate, Hz</td>
<td>84.5 ± 50.9</td>
<td>94.7 ± 50.9</td>
</tr>
<tr>
<td>Dynamic range, pA</td>
<td>157 ± 53Ì</td>
<td>422.4 ± 160Ì</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tonic III</th>
<th>No noise</th>
<th>Noise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheobase, pA</td>
<td>20 ± 22*</td>
<td>-23 ± 34</td>
</tr>
<tr>
<td>Midpoint, pA</td>
<td>157 ± 43</td>
<td>132 ± 73</td>
</tr>
<tr>
<td>Gain, sp/nA</td>
<td>162 ± 39†</td>
<td>154 ± 49‡</td>
</tr>
<tr>
<td>Maximum firing rate, Hz</td>
<td>44.8 ± 25.3</td>
<td>53.3 ± 33.5</td>
</tr>
<tr>
<td>Dynamic range, pA</td>
<td>286 ± 130‡</td>
<td>347 ± 113</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.01, tonic I, II, and III neurons mutually significantly different. †P < 0.05, tonic I and II neurons significantly different. ‡P < 0.01, tonic II and III neurons significantly different.
vergent differentiators. This pattern suggests that the ability to maintain regularity by regenerative spiking mechanisms has a weak influence on the integration of inputs. When grouped by cell type, there were also no significant differences among the tonic I, II, and III neurons, although tonic III neurons tended to show the most regularity in their firing in the presence of noise. Interestingly, even the integrator/tonic III neurons showed differences in the CV between noise levels. This is because even though the average firing rates do not change, the timing of the spikes followed the noise stimulus envelope, as discussed below. Tonic III neurons are notable for having slower AHPs than tonic I and II neurons (Soares et al. 2002; for example, see Fig. 1C), and regenerative spiking mechanisms may be more dominant in these neurons.

Single-Spiking NA Neurons

Noisy currents have been shown to effectively drive neurons in NM and NL (Higgs et al. 2006; Kuznetsova et al. 2008; Reyes et al. 1996). Single-spiking neurons in NA resemble these timing nuclei neurons in their intrinsic properties: they generate a single onset spike and show outward rectification in their voltage responses to depolarizing current steps, fire repetitively in the presence of dendrotoxin, and have lower input resistances and faster membrane time constants than the tonic neurons (Fukui and Oertel 2003; Soares et al. 2002), all properties associated with a low-threshold potassium current in timing nuclei neurons (Brew and Forsythe 1995; Fukui and Oertel 2004; Gao and Lu 2008; Kuba et al. 2002; Rathouz and Trussell 1998). To determine whether they responded to noisy currents in a similar way to NM and NL neurons, we measured the FI curves for nine single-spiking neurons with and without noise. Without noise, by definition, only a single spike was evoked at the onset of the step. With noise, all of the single spiking neurons showed increased firing rates with increasing average DC level (Fig. 2D). The FI curves of most single-spiking neurons (7/9) increased linearly without saturation over the measured current step range (as shown in Fig. 2D), whereas the others (2 of 9) showed a distinct peak followed by declining firing rates (data not shown), due to depolarization block. Because these responses could not be fit by sigmoidal curves, a linear fit was made to the initial slope to measure the effect of noise on the gain and compared between 2 and 4σ noise levels. There were two clear effects: a large leftward shift in the rheobase (by $-74 \text{ pA}$, $P < 0.05$) and a twofold increase in
gain (116%, P < 0.05; n = 9). Thus noise had an opposite effect on the FI curve gain for single-spiking neurons than for differentiator tonic neurons (Fig. 3B). Similar changes were also observed in NM neurons in our study, with a significant leftward shift in rheobase (by −56 pA, P < 0.05; n = 4) and a severalfold increase in gain between 2σ and 4σ noise levels (270%, P < 0.05). Thus the changes seen in the input-output functions of single-spiking NA neurons differed from those of tonic neurons and more closely resembled those of NM neurons.

Damped Neurons

Our sample of NA neurons classified as damped were highly heterogeneous in their damping properties and in their responses to noise stimulation. In the absence of noise, some damped neurons fired tonically at lower current steps, only damping at larger current injections, whereas others exhibited damping at all steps, including the first step that evoked spiking. The majority of damped neurons (7/11) were noise sensitive and had increased firing rates across the FI curve (Fig. 2E), whereas a minority (3/11) behaved like integrators throughout most of their nondamped range (Table 1). The FI curve shapes were also variable such that only four damped neurons could be fit with a sigmoid. The remainder were characterized by using linear fits of the initial slope and estimating rheobase as the lowest current step that evoked an action potential. Similar to other NA neurons, the addition of noise caused a leftward shift in the rheobase with noise level 4σ (by −76 pA, P < 0.01; n = 11). In cases in which sigmoidal fits allowed estimation of the midpoint of the FI curve, there was a leftward trend from no noise to noise level 4σ (by −83 pA; not significant, P = 0.086; n = 4). A significant increase in the maximum firing rates occurred, from 24.4 ± 7.9 Hz for no noise to 31.9 ± 7.0 Hz with noise level 4σ (P < 0.01; n = 11), although firing rates were low overall. The effect of noise on the FI curve gain was highly varied. Damped neurons showed both increases and decreases in the gain, averaging out in aggregate (Fig. 3B). The damped neurons that showed increases in gain tended to fire only weakly in the absence of noise, more closely resembling single-spiking responses. The damped neurons that showed decreases in gain tended to fire tonically at lower current steps and more closely resembled tonic neurons.

Temporal Locking to the Noise Stimulus by NA Neuronal Spike Trains

To investigate how well the NA neurons locked to the current stimulus, we measured their responses to frozen noise over repeated trials to analyze the spike timing. We recorded timing data from 27 NA neurons: 13 tonic, 9 single, and 5 damped; among the tonic neurons were 2 tonic I, 8 tonic II, and 3 tonic III neurons. A single DC current step amplitude (typically 100–400 pA) was presented with or without noise. The mean current amplitudes were generally chosen to provide a firing rate of several tens of Hz (mostly between 10–60 Hz).

All of the NA neurons responded to noisy stimuli with highly reliable spike timing patterns evident in raster plots, as shown for one tonic III neuron in Fig. 5. During the flat current step, the neuron fires repetitively, but small amounts of jitter in the timing of the spikes reduce patterning across trials (Fig. 5A). With the addition of noise the spikes show strong synchronization across trials, visible in the vertical patterns of the spike times (Fig. 5B). The reliability of the temporal locking was analyzed using the SAC technique (see MATERIALS AND METHODS). The SAC function is an all-to-all interspike interval histogram, excluding spike pairs within the same trials. The
normalized peak of the SAC at zero delay is denoted the correlation index (CI) (Joris et al. 2006). We also measured the width of the SAC at the half-maximum of the peak. CI and width of the SAC function provided an estimate of the reliability and precision of the time locking, respectively. Without noise, the SAC is flat; with noise level 4σ, a large, narrow peak is observed (Fig. 5D). For an intermediate noise level of 2σ, a smaller, slightly wider peak is observed.

A summary of SAC analyses for all NA neurons showed that increased noise resulted in greater reliability and precision and with virtually no correlation during flat current steps (Fig. 6, A–D). To determine that the patterning of the firing was being driven by the specific fluctuations in the noise current stimulus, we calculated the spike-triggered average (STA) of the stimulus. The STA showed a large positive peak just prior to spiking with a delay of 2.3 ms (Fig. 5C), indicating the firing was largely fluctuation driven.

Larger noise levels increased firing rates in many neurons and also increased the CI, suggesting most of the additional spikes were specifically timed to the fluctuations in the noise stimulus. The increased firing may be due to sensitivity to more rapid rises in membrane voltage or to the membrane voltage spending more time above spike threshold and the timing being incidental. To distinguish between these possibilities, we investigated whether simply increasing the DC level, while maintaining the same noise amplitude, would lead to an increase or decrease in the CI. An increase in the DC level and the attendant increase in firing rate resulted in a decrease in the CI (Fig. 7). Elevating the mean voltage above the spike threshold, without changing the rate of rise of the voltage, led to greater jitter in the timing, as can be observed in the raster plots shown in Fig. 7A. The lowest DC level evoked the fewest spikes, but these were tightly synchronized. Increasing the DC level led to higher firing rates and more spiking events, but with greater jitter and a larger number of likely “incidental” spikes not correlated with the fluctuations, decreasing the CI and broadening the peak width. These data suggest that the increase in CI with larger noise levels (while maintaining the same DC current amplitude) is most likely driven by a sensitivity to the rate of rise, or derivative, of the voltage, conferring NA neurons with a high degree of sensitivity to the temporal patterning of their inputs.

To determine whether there were differences in the time locking of different NA cell types, we plotted the CI vs. correlation peak width (Fig. 6F) and firing rate (Fig. 6E), all taken using 2σ noise levels. In both plots the points tend to cluster. Single-spiking neurons had high CIs and narrow peak widths compared with the tonic and damped neurons. Tonic I/II neurons had lower CI but similar window widths. Damped neurons had the widest peak windows but CIs similar to tonic neurons, suggesting the damped neurons were less precise than the tonic I/II neurons. Tonic III neuron CIs and windows fell on the low end of the range of tonic I/II neurons.

Because all neurons were tested at different DC levels and firing rates, we analyzed the trends in the CI vs. firing rate. There was a clear negative trend for CI vs. firing rate for damped neurons (damped: y = −1.5x + 46, R² = 0.749; tonic I/II: y = −0.62x + 46, R² = 0.23). Regression fits suggest that the differences between damped and tonic I/II groups were not due to firing rate differences. Firing rates were similar across tonic I/II and tonic III neurons. Single-spiking firing rates were lower than tonic I/II rates, but it was unclear whether elevating the firing rates in single-spiking neurons to equal those in tonic I/II would result in similar CI values. Single-spiking and tonic I/II neurons were relatively good at locking to temporal fluctuations in the current stimulus, whereas tonic III and damped neurons performed more poorly.

![Fig. 6. Correlation index (CI) and peak widths by cell type and noise level. A–D: markers connected by lines represent data from the same cell and at the same DC current step. Representative data were chosen when some cells were tested with multiple DC steps. A: tonic I/II neurons. B: tonic III neurons. C: damped neurons. D: single-spiking neurons. The CI is negatively correlated with the firing rate (E) and with the correlation window width (F). Data are shown for 2σ noise level only. Scatter plots show the different cell types clustered by their timing responses.](http://jn.physiology.org/)
We asked whether single-spiking neurons and NM neurons had similarly reliable firing. Because the NM neurons required larger noise fluctuations to reach firing rates necessary for an analysis of spike timing, we stimulated them with 4 noise levels and compared them with responses to 4 noise levels in the single-spiking NA neurons. The shuffled correlograms for NM neurons showed higher peaks (CI 145.9 ± 98.3; mean ± SD, n = 3 NM neurons tested at 7 step levels) and narrower widths (0.29 ± 0.09 ms) compared with the single-spiking neurons (CI 70.7 ± 29.0 Hz, width 0.54 ± 0.20, n = 9 single-spiking neurons tested at 11 step levels; significantly different from NM with P < 0.05 and P < 0.01, respectively), despite having higher firing rates (61.4 ± 46.4 Hz for NM; 21.2 ± 10.7 Hz for single-spiking neurons). In both data sets, there was a negative correlation of CI with firing rate; the regression lines had the same slope but were shifted toward higher CI values for NM neurons (single spiking: y = −1.86x + 111.3, R² = 0.47; NM, y = −1.87x + 260.5, R² = 0.78). NM neurons therefore responded to noisy currents significantly more reliably and precisely than single-spiking NA neurons (as well as more precisely than tonically firing neurons, data not shown).

DISCUSSION

The intrinsic properties of a neuron regulate its firing response and excitability and provide clues to its functional role in vivo. In the avian cochlear nucleus angularis (NA), a broad range of intrinsic properties have been shown using standard flat current steps during intracellular recordings from chick brain stem slices in vitro. These neuronal firing properties distinguish several types of single-spiking and tonic firing neurons that are correlated with morphology, resulting in four to five neuronal classes (Carr and Soares 2002; Fukui and Ohmori 2003; Häusler et al. 1999; Kuo et al. 2009; Soares and Carr 2001; Soares et al. 2002). In this study, we used more naturalistic current injections that simulate a barrage of incoming synaptic inputs to better reveal the relationship between the intrinsic properties of the diverse neurons of the avian cochlear nuclei and their putative functional roles in the neural coding of auditory stimuli.

Our first major finding in this study is the demonstration that different types of tonic firing neurons show different degrees of sensitivity to rapid input fluctuations as measured in their firing rate. More than one-half of the tonically firing neurons showed increased firing rates to fluctuating currents over flat currents with the same mean amplitude. The tonic I neurons showed the largest increases in firing rate and changes in their input-output functions. A smaller group of tonic firing neurons (mainly the tonic III neurons), however, behaved like near-pure integrators, that is, their firing rates were unchanged even when presented with large fluctuations in their inputs. In addition, the single-spiking neurons in NA respond to noisy currents very much like the coincidence detectors in the timing pathway NM and NL (Higgs et al. 2006; Kuznetsova et al. 2008; Reyes et al. 1996). An elaboration of the ionic conductances that define how these cell types respond to noisy stimuli is needed.

A second major finding of this study is that all NA neurons demonstrated highly reliable firing to the patterns of current fluctuations, suggesting that these neurons are more capable of temporal coding than previously appreciated. All of the toni-
cally firing NA neurons had regular firing when stimulated with flat current steps, as shown by within-trial analyses of the interspike intervals, but small jitter in the timing of spikes eliminated any temporal correlation across trials. In contrast, noise imposed on the current step dominated the timing of the spikes with a high degree of precision (frequently \(<1–2\) ms), leading to a reliable response that could be observed on repeated presentations. The degree of reliability and precision in the temporal response was varied in the different cell types, with tonic III neurons (putative integrators) at the least reliable end of the range. Together, these results demonstrate that in the NA there exist 1) a broad range of potential responses to the temporal correlations in the inputs, from almost pure integration to coincidence detection, and 2) a greater inherent capability for encoding temporal information contained in the inputs than has been generally ascribed to the “intensity” pathway. Interestingly, a recent in vivo study in barn owls that investigated the firing responses of NA neurons to acoustic noise stimuli showed that these neurons are driven by the temporal fluctuations in the sound with a high degree of reliability and precision, discussed in more detail below (Steinberg and Pena 2011). The spike timing precision we report in vitro is comparable to that found in a similar study of the repetitively firing pyramidal neurons of the rat dorsal cochlear nucleus (Street and Manis 2007; 1–2 ms precision). Studies with noisy currents applied to the stellate cells of the mammalian ventral cochlear nucleus, the neurons most closely analogous to tonically firing NA neurons, have not been reported.

In our study, the types of changes observed in the input-output functions of tonic NA neurons categorized as differentiators were quite consistent: a decrease in the rheobase and a decrease in the gain (slope), and a concomitant increase in the dynamic range. In some cases an increase in the maximum firing rates was also observed (divergent differentiators). These changes differed from those seen in single-spiking NA neurons (this study) and in NL neurons (Higgs et al. 2006); in those cells, the gain of the input-output functions increased with increasing noise levels. Noisy currents have been applied in other auditory brain stem regions, primarily to “phasic” neurons in the timing pathway but also to repetitively firing pyramidal neurons of the dorsal cochlear nucleus, but these studies focused on other aspects of coding and did not report comparable analyses of gain changes (Gai et al. 2009; Kuznetsova et al. 2008; Street and Manis 2007). Gain modulation has been observed with noisy stimuli in several studies of cortical neurons, with the direction of gain change depending on specific cell type, cortical region, and method of noise application (current clamp vs. dynamic clamp applications) (Arsiero et al. 2007; Chance et al. 2002; Fellous et al. 2003; Higgs et al. 2006; Rauch et al. 2003).

**Noisy Currents and the Encoding of Sound Intensity**

Noisy, depolarizing pedestal voltage potentials have been observed during intracellular in vivo recordings from cochlear nucleus neurons (personal observations in chicken NA, MacLeod KM, Soares D, and Carr CE; see also Rhode and Smith 1986 for examples in mammalian cochlear nucleus). Similar fluctuating inputs under current or conductance clamp have been used to generate firing responses in vitro that more closely resemble in vivo firing patterns (Chance et al. 2002; Destexhe et al. 2001; Mainen and Sejnowski 1995; Reyes et al. 1996; Street and Manis 2007).

Unlike NM neurons, which receive few large inputs (Trussell 1999), NA neurons receive many smaller inputs (MacLeod and Carr 2005). We assume that mean current levels correspond to the overall number of active inputs, which in turn is roughly proportional to the mean sound intensity in the frequency band to which the afferents are tuned.

If these inputs are largely uncorrelated, their arrival times spread out and sum as a relatively flat current; if the same number of inputs are highly correlated, their arrival times synchronize and produce large, fast current fluctuations, but with the same mean amplitude as uncorrelated inputs (Chance et al. 2002; Fellous et al. 2003; Reyes et al. 1996). The NA integrator neurons in our study were relatively insensitive (in terms of firing rate) to the noise fluctuations and would appear to be well suited for encoding the mean input amplitude regardless of the correlations in the inputs. This subpopulation of tonic neurons (mainly tonic III) would be more likely to generate a reliable rate-coded output for mean sound level and is therefore a suitable candidate for providing the output used for interaural level coding.

In contrast, many tonically firing neurons in NA showed a robust firing rate sensitivity to fluctuations in the current stimulus. In these differentiators, larger noise fluctuations also led to larger increases in the firing rate, indicating a sensitivity also to the degree of fluctuation. These neurons may be less useful for encoding average sound levels as a rate code due to the decoding confound between the firing rate produced by a large number of uncorrelated inputs and a similar firing rate produced by a smaller number of well-correlated inputs. A population of differentiator neurons receiving correlated synaptic inputs would have a strong temporal signature as well as elevated firing rate and would potentially signal a coherent, fluctuating stimulus. We speculate that this subpopulation of NA neurons could detect and encode coherent temporal envelope fluctuations of acoustic stimuli, a cue that could be used in auditory scene analysis (Shamma et al. 2011).

**In Vivo Responses in the Intensity Pathway to Temporal Modulation of Auditory Stimuli**

Currently, few studies address the encoding of temporal aspects of sound stimuli in the intensity pathway of birds. Temporal coding of fine timing (on the order of microseconds) for the computation of interaural time differences has been extensively studied in the so-called “timing pathway,” which ascends along the pathway comprising the cochlear NM and its target, the NL (Burger and Rubel 2008; Carr et al. 2001; Knudsen et al. 1977; Konishi et al. 1985; Köppl and Carr 2003; Sullivan 1985; Sullivan and Konishi 1984; Takahashi et al. 1984; Trussell 1999). Cochlear NA is best known in its role in encoding intensity information for the computation of interaural level differences (ILD) for sound localization (Manley et al. 1988; Mogdans and Knudsen 1994; Sullivan and Konishi 1984; Takahashi et al. 1984), but its heterogeneity along with the specialization of the timing pathway suggests that a broader range of non-ILD-related functions reside with NA, such as sound recognition and discrimination (Carr and Soares 2002; Fukui and Ohmori 2003; Hotta 1971; Köppl and Carr 2003; MacLeod and Carr 2007; Sachs and Sinnott 1978; Soares and...
Rheobase, pA 44 decreases with noise amplitude. These differential effects on our results in NA tonically firing neurons that showed gain NM (our data) and NL neurons (Higgs et al. 2006) and unlike multiplicative gain changes are similar to the effect of noise on respond to noisy currents with FI curves whose gain increases velocities (Ferragamo and Oertel 2002; McGinley and Oertel Oertel 2011; Cao et al. 2007) have steep spike threshold I Reyes et al. 1994). Similarly, bushy cells and octopus cells in required to elicit an action potential (Fukui and Ohmori 2003; presses repetitive firing and a threshold voltage velocity is I Christianson and Pena 2007). Together, these studies suggest that the timing pathways are not necessary for dynamic sound envelope coding, which can instead ascend via the intensity pathway.

Mechanisms of Sensitivity to Current Noise Fluctuations

$I_{KLT}$. The heterogeneity in the way different neurons change their input-output functions in response to noisy fluctuations suggests that differences in the intrinsic properties contribute in distinct ways. In single-spiking NA neurons, as well as neurons in NL and NM, the presence of a low-threshold potassium channel current ($I_{KLT}$; a dendrotoxin-sensitive current) suppresses repetitive firing and a threshold voltage velocity is required to elicit an action potential (Fukui and Ohmori 2003; Higgs et al. 2006; Kuznetsova et al. 2008; Reyes et al. 1996; Reyes et al. 1994). Similarly, bushy cells and octopus cells in mammalian cochlear nucleus that are also defined by a large $I_{KLT}$ (Bal and Oertel 2001; Cao and Oertel 2005; Cao and Oertel 2011; Cao et al. 2007) have steep spike threshold velocities (Ferragamo and Oertel 2002; McGinley and Oertel 2006). Our results show that single-spiking neurons in NA respond to noisy currents with FI curves whose gain increases with increasing noise fluctuation amplitudes. These positive multiplicative gain changes are similar to the effect of noise on NM (our data) and NL neurons (Higgs et al. 2006) and unlike our results in NA tonically firing neurons that showed gain decreases with noise amplitude. These differential effects on gain suggest different mechanisms underlie noise sensitivity in these two broad classes of neurons.

The role of $I_{KLT}$ in coincidence detection has been extensively studied, especially in the context of MSO and NL (Golding et al. 1995; Khurana et al. 2011, 2012; Mathews et al. 2010; Svirskis et al. 2002, 2004). Whereas the subthreshold and sustained action of $I_{KLT}$ is important for the fast membrane time constant at rest, its dynamic activation is also critical for coincidence detection (Day et al. 2008). Sensitivity to (high frequency) noise can also enhance the ability of coincidence detector neurons to encode slower signals (Gai et al. 2009).

Conventionally, the repetitive firing by NA tonic neurons is thought to be due to the relative absence of $I_{KLT}$. Single-spiking NA neurons fire multiple spikes when dendrotoxin is applied, whereas small dendrotoxin effects were observed for tonically firing neurons (Fukui and Ohmori 2003), suggesting the presence of a low-threshold potassium conductance in some tonic neurons at a low level or with activation kinetics shifted to a more depolarized voltage regime, or both. Notably, the small dendrotoxin effects parallel some of the effects on noise in many tonic neurons, namely, a leftward shift in the rheobase and a decrease in the gain of the FI curve. Voltage clamp and pharmacological analysis of identified T-stellate neurons in slice indicates little to no contribution of a classical, dendrotoxin-sensitive low threshold (i.e., approximately −60 mV and partially activated at resting potential) K+ conductance (Cao and Oertel 2011; Ferragamo and Oertel 2002; Manis and Marx 1991), although a ventral cochlear nucleus subpopulation of unclear cell type was found to express a smaller amplitude dendrotoxin-sensitive conductance that was activated at more depolarized voltage levels (Rothman and Manis 2003a; Rothman and Manis 2003b). Together, these results open the possibility of a broader range of contributions of “low-threshold” K+ conductances associated with the Kv1 family of channels among tonically firing neurons of the cochlear nucleus.

$Na^+$ channel inactivation and spike threshold adaptation. Another potential candidate mechanism is the spike threshold adaptation that occurs as Na+ channels become inactivated by depolarization. A range of changes to the input-output functions are found in neocortical neurons presented with noise fluctuations that parallel our observations in NA: differentiation that ranged from rapidly convergent to persistently divergent (Arsiero et al. 2007; Higgs et al. 2003; La Camera et al. 2008; Rauch et al. 2003). A simple leaky integrate-and-fire model could explain much of the convergent FI curves but was insufficient to explain the divergent FI curves. Physiology and modeling led Arsiero et al. (2007) to propose that spike threshold adaptation could account for the differences between

| Table 3. Comparison of FI curves across response types, with and without noise |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                                | Integrator                      | Differentiator (Divergent)      | Differentiator (Convergent)     |
|                                | No noise | Noise | No noise | Noise | No Noise | Noise |
| Rheobase, pA                   | 44 ± 53  | 4 ± 40 | 233 ± 134* | −103 ± 106 | 119 ± 57* | 2 ± 36† |
| Midpoint, pA                   | 153 ± 44 | 142 ± 52 | 328 ± 171* | 110 ± 77  | 197 ± 59  | 115 ± 47 |
| Gain, sp/nA                    | 364 ± 244 | 290 ± 200 | 254 ± 118  | 154 ± 69  | 622 ± 264† | 396 ± 172† |
| Maximum firing rate, Hz        | 58.9 ± 25.7 | 62.2 ± 27.2 | 42.1 ± 14.8 | 52.8 ± 15.0 | 914.5 ± 57.8 | 1001 ± 55.4 |
| Dynamic Range, pA              | 227 ± 136 | 280 ± 129 | 210 ± 108  | 425 ± 116 | 157 ± 56  | 226 ± 48† |

Values are means ± SD. *P < 0.05, significantly different from integrator. †P < 0.05, significantly different from divergent.

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convergent and divergent responses. Spike threshold adaptation via Na+ channel inactivation causes noise-driven membrane voltage fluctuations to become relatively more effective at eliciting spikes, resulting in higher firing rates with noise than without at the highest current levels, where inactivation is greatest. Along with $I_{KLT}$, Na+ channel inactivation also contributes to the suppression of action potentials to support phasic firing in MSO neurons (Svirskis et al. 2004).

In NA, the declining action potential amplitude observed in damped neurons indicates possible inactivation of Na+ channels. The addition of noise reduced or eliminated the damping by more effectively eliciting spikes, and damped neurons showed large differentiation. A lesser degree of inactivation in the tonic neurons could have a similar albeit more subtle effect. An investigation of Na+ channel inactivation, spike threshold, and its contribution to noise-driven firing in NA neurons is needed.

Outward Currents That Contribute to Spike Rate Adaptation Affect Rate vs. Time Coding of Noisy Inputs

Neocortical pyramidal neurons have several mechanisms underlying spike rate regulation, including a slow calcium-dependent K+ conductance and a high-threshold voltage-dependent delayed rectifier conductance (Schwindt et al. 1988). Higgs et al. (2006) linked a large-amplitude, slow ($\tau \approx 1$ s) outward current ($I_{AHP}$) with greater gain changes in their firing rate noise sensitivity. Similarly, Prescott and Sejnowski (2008) modeled the roles of two potassium conductances that differed in their voltage-activation regimes. A spike-independent, sub-threshold-activated outward current ($I_M$) provided a sustained outward current that reduced responsiveness to slowly changing signals in favor of responses to rapid fluctuations, improving temporal coding. Conversely, a spike-dependent outward current underlying a medium-duration ($\tau \approx 100$ ms) AHP ($I_{AHP}$) enhanced rate coding at the expense of spike timing reliability driven by the fluctuations in the current drive.

In NA, the slower AHPs that were unique to the tonic III neurons intrinsically regulated the firing, suppressing the depolarizing drive by the current fluctuations between action potential cycles and limiting firing rates. Interestingly, the tonic I and II neurons tended to have fast, deep AHPs. Instead of strongly regulating the firing, however, the AHP was completed before the onset of the next depolarizing fluctuation in the stimulus and therefore had little influence on whether or when the next spike occurred. The fluctuating extrinsic drive was more dominant in these neurons, leading to modified FI curves and greater temporal locking to the stimulus. Thus a critical factor in explaining these results is the time scale of the AHPs relative to the time scale of the voltage fluctuations driven by the noisy currents. In summary, the fluctuation sensitivity in NA tonically firing neurons may rely on several different biophysical mechanisms, leading to their capacity for a combined rate and temporal code, which appears especially suited for the encoding of temporally modulated sound intensity signals. (Table 3)

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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