Published in final edited form as:

Hear Res. 2012 July ; 289(1-2): 13–26. doi:10.1016/j.heares.2012.04.019.

Classification of unit types in the anteroventral cochlear nucleus of laboratory mice

Matthew J. Roos^a and Bradford J. May^b

^aDepartment of Neuroscience The Johns Hopkins University School of Medicine, Baltimore, Maryland ^bDepartment of Otolaryngology-Head and Neck Surgery, The Johns Hopkins University School of Medicine, Baltimore, Maryland

Abstract

This report introduces a system for the objective physiological classification of single-unit activity in the anteroventral cochlear nucleus (AVCN) of anesthetized CBA/129 and CBA/CaJ mice. As in previous studies, the decision criteria are based on the temporal properties of responses to short tone bursts that are visualized in the form of peri-stimulus time histograms (PSTHs). Individual unit types are defined by the statistical distribution of quantifiable metrics that relate to the onset latency, regularity, and adaptation of sound-driven discharge rates. Variations of these properties reflect the unique synaptic organizations and intrinsic membrane properties that dictate the selective tuning of sound coding in the AVCN. When these metrics are applied to the mouse AVCN, responses to best frequency (BF) tones reproduce the major PSTH patterns that have been previously demonstrated in other mammalian species. The consistency of response types in two genetically diverse strains of laboratory mice suggests that the present classification system is appropriate for additional strains with normal peripheral function. The general agreement of present findings to established classifications validates laboratory mice as an adequate model for general principles of mammalian sound coding. Nevertheless, important differences are noted for the reliability of specialized endbulb transmission within the AVCN, suggesting less secure temporal coding in this high-frequency species.

Keywords

peri-stimulus time histograms; onset latency; regularity analysis; prepotential; rate adaptation

1. Introduction

The interface between the auditory periphery and anteroventral cochlear nucleus (AVCN) has been a rich source of information regarding the basic mechanisms of sound coding (Blackburn and Sachs, 1990), auditory development (Feng et al., 2010; Harris et al., 2005; Limb and Ryugo, 2000), and deafness (Lee et al., 2003; Youssoufian et al., 2008). From the perspective of computational neuroscience, the AVCN is an ideal model system because the

© 2012 Elsevier B.V. All rights reserved

Contact information Brad May 521 Traylor Building 720 Rutland Avenue Baltimore, MD 21205 USA bmay@jhu.edu phone: +1 410-614-1878 fax: +1 410-955-1299.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

relatively homogeneous projections of the auditory nerve diverge into parallel processing pathways with unique coding attributes (Young et al., 1988b). The selective sound representations of the AVCN are dictated by variations in synaptic transmission that include the size, number and site of auditory nerve inputs, the intrinsic membrane properties of the target neuron, and a local modulatory circuitry (Trussel, 2002).

Predictive relationships between structural diversity and functional specialization are well defined in the AVCN where neurons with shared cellular morphologies occupy regionalized topographies that can be probed with electrophysiological techniques (Cant and Morest, 1984). Major structure/function relationships have been further confirmed by the histological reconstruction of physiologically characterized neurons (Rhode et al., 1983; Smith and Rhode, 1989). This detailed functional anatomy makes it possible to relate the underlying mechanisms of deafness and other auditory processing disorders to changes in the sound coding attributes of specific cellular populations (Cai, 2007; May et al., 2011).

From the perspective of biophysical mechanisms, the most general AVCN response type is associated with cells that display a multipolar or stellate morphology (Cant and Morest, 1979b). The neurons have elongated dendritic fields that are contacted by numerous bouton synapses from the auditory nerve. When a multipolar cell is depolarized, it emits regularly timed action potentials that conform to the basic Hodgkin-Huxley model of neural membranes (Banks and Sachs, 1991; Lai et al., 1994). Peri-stimulus time histograms (PSTHs) manifest this regularity as a multimodal chopped appearance (Figs. 1C and D). Consequently, the physiological counterpart of the multipolar cell has been designated the chopper unit (Palmer et al., 2003; Pfeiffer, 1966).

Specialized neural responses are produced by cellular morphologies with stunted bush-like dendritic arbors (Cant and Morest, 1979a; Osen, 1969). Because bushy cells receive axosomatic auditory nerve inputs by way of powerful endbulb synapses (Rothman et al., 1993; Ryugo and Fekete, 1982), each auditory nerve input is capable of generating an action potential in the post-synaptic neuron. Additional inhibitory influences constrain firing rates at higher sound levels, preserving temporal precision (Kopp-Scheinpflug et al., 2002; Kuenzel et al., 2011). This secure synaptic coupling transfers the irregular discharge rates of auditory nerve fibers to the bushy cells (Manis and Marx, 1991; Rhode, 2008; Wu and Oertel, 1984), which are known as primarylike units for their physiological resemblance to primary afferents (Figs. 1A and B).

The distinct sound processing roles of chopper and primarylike units are revealed not only by differences in their morphology and temporal response patterns but also by the targets of their axonal projections. Multipolar cells project to processing centers throughout the auditory brainstem, including the cochlear nuclei, periolivary nuclei, lateral lemniscus, and inferior colliculus (Cant and Benson, 2003; Malmierca and Hackett, 2010). The accurate representation of stimulus level by the discharge rates of chopper units suggests that these neurons may be specialized for the coding of spectral cues for sound localization or communication signals (May et al., 1998; Rhode and Smith, 1986). Alternatively, bushy cells provide the major inputs to the medial and lateral superior olive, where temporally precise transmission is essential for the binaural processing of interaural time and level differences (ILDs and ITDs, (Rhode, 2008; Young et al., 1988a).

Despite the increasing importance of laboratory mice as a model for the cellular anatomy and physiology of the cochlear nucleus (Oertel et al., 2008; Webster and Trune, 1982), studies of sound coding in this structure remain rare. Interpretations of the few existing single-unit studies are constrained by the absence of physiological classifications by which results are organized in other species. Functional categorization is essential because genetic

and environmental manipulations may selectively alter the coding properties of a population class. For example, the disruption of the medial olivocochlear system in transgenic mice has been shown to degrade the accuracy of temporal coding among globular bushy cells without altering the coding properties of other AVCN neurons (May et al., 2011).

To enhance the current understanding of sound coding in laboratory mice, the present study describes the PSTH-based classification of single-unit activity in the AVCN of CBA/129 and CBA/CaJ mice. Two genetically diverse strains were tested to verify the generalization of experimental observations among laboratory strains with intact peripheral function. First, a subset of chopper and primarylike units were selected to represent PSTH patterns that have been previously demonstrated in other mammalian species (Bourk, 1976; Palmer et al., 1996; Palmer et al., 2003; Pfeiffer, 1966; Woolf and Ryan, 1985). Then, informative response metrics within the major PSTH types were used to produce a system for the objective classification of single-unit activity (Blackburn and Sachs, 1989). The validity of the resulting decision criteria was verified by the objective classification of the remaining units in the sample. Measures of waveform shapes, synaptic prepotentials, threshold, frequency tuning, spontaneous rate, and latency were also performed to evaluate the natural variation of basic response properties within AVCN response types.

2. Methods

2.1. Subjects

Single-unit responses were recorded in the AVCN of 16 CBA/129 and 12 CBA/CaJ mice (18 male, 10 female). The mice were 1 – 6 months old with a mean age of approximately 3 months. CBA/129 hybrid mice (CBA/CaJ $\times$ 129S6/SvEvTac) were bred in the laboratory and maintained from birth in quiet housing facilities to minimize prior exposure to the ambient noise levels of institutional vivaria (Lauer et al., 2009). CBA/CaJ inbred mice were procured from Jackson Laboratories (stock #000654) at 4 weeks of age and were housed in the quiet facility until tested (typically 1 – 14 days). Because no sex, age, or strain differences were noted in the present analyses, results from all subjects have been combined to produce the largest possible sample of units in multiple PSTH classifications. All animal protocols were approved by the Institutional Animal Care and Use Committee of Johns Hopkins University.

2.2. Surgical procedures

Mice were prepared for surgical recording under urethane anesthesia (1.0 g/kg, IP). Some mice were also given a single dose of xylazine (20 mg/kg IP) 15 minutes prior to urethane administration. When needed, urethane was supplemented at a lower dosage (0.3 g/kg, IP). A regulated heating pad was used to maintain a body temperature of approximately 37° C throughout the experiment.

The subject's head was fixed in standard stereotaxic coordinates with ear bars and a bite bar. The posterior fossa was opened caudal to the lambdoid suture. The lateral cerebellum was partially aspirated to expose the left AVCN. A head-fixation post was bonded to the skull with UV-sensitive dental acrylic. The head post was attached to the stereotaxic frame and the left ear bar was removed to accommodate monaural acoustic stimulation.

2.3. Recording procedures

The recording microelectrode was visually positioned on the dorsal surface of the AVCN, then advanced through the nucleus with a hydraulic micropositioner. The range of the recording track was maximized by orienting the microelectrode at an angle of 25° in the

sagittal plane, so that the electrode passed through the long axis of the nucleus along a dorsal-caudal to ventral-rostral trajectory.

Extracellular recordings were made using glass micropipettes with impedances near 1 M Ω . The electrodes had tip diameters of 1.5 – 2.0 μ m and were filled with 3M NaCl. The electrode signal was bandpass filtered (10 – 5000 Hz) and amplified (3000 – 15000 $\times$). A Schmitt trigger was used to record the timing of action potentials relative to stimulus onsets with 10- μ s resolution. Some recordings were made with 5-M Ω tungsten electrodes (A-M Systems), but micropipettes tended to yield a larger number of well-isolated units in each preparation. Comparisons of PSTH shapes and classification criteria between microelectrode types did not reveal any significant differences. Minimum first spike latency was used to separate primarylike units from auditory nerve fibers (Lieberman, 1993; Spirou et al., 2005). Other response types were easily distinguished from auditory nerve fibers by their unique PSTH shapes.

2.4. Acoustic stimuli

Acoustic stimuli were presented in free-field to minimize surgical manipulations of the outer ear and to avoid issues of closed-field calibration at high frequencies (Pearce et al., 2001). Experiments were conducted inside a double-walled sound-attenuating chamber to control ambient sound levels (Industrial Acoustic Company). The single loudspeaker (Tannoy, Satellite Arena-BL) was positioned approximately 36 cm from the mouse's head at a spatial orientation of 45° azimuth, 0° elevation in the hemifield of the unoccluded left ear. All stimuli were synthesized in MATLAB and generated with multifunction digital processors (Tucker-Davis Technologies, RX6). Sound levels were controlled with programmable attenuators (Tucker-Davis Technologies, PA5) and a power amplifier (Crown D-45). Acoustic calibrations were performed by recording the output of the loudspeaker at the location of the subject's head with a ¼ inch free-field condenser microphone (Bruel & Kjaer, type 4939). Calibrations were completed prior to electrophysiological experiments without a mouse in the testing apparatus. The free-field frequency response of the loudspeaker varied by $\pm$ 10 dB across the range of sampled neurons (6 – 50 kHz).

Neurons with sound-driven activity were isolated by presenting short bursts of tone or noise as the electrode advanced through the AVCN. The BFs and thresholds of well-isolated units were determined by manually sweeping the frequency and level of tone bursts.

Peri-stimulus time histograms (PSTHs) provided the essential response metrics for unit classification (Fig. 1). PSTHs were collected by presenting 300 repetitions of BF tone bursts. The tones were 50 ms in duration (5-ms rise/fall times) and were presented at a rate of 4 bursts/s. The level of the tones was 30 dB relative to the unit's threshold. To mitigate adaptation effects, responses to the first 30 repetitions were not included in the analysis of PSTHs. The remaining spike activity was sorted into 0.1-ms bins relative to stimulus onset.

Rate-level functions (RALVs) provided a precise estimate of the stimulus levels that were unambiguously represented by changes in sound-driven discharge rates. RALVs were collected by presenting a 100-dB range of BF tone levels in ascending steps of 1 dB. The tones were 200 ms in duration (10-ms rise/fall times) and repeated at a rate of 1 tone burst/s. Absolute stimulus levels were adjusted for each unit to sample the complete dynamic range of sound-driven activity. Typically, the lowest level was approximately 10 dB below the BF threshold.

Frequency response maps (FRMs) revealed the combined effects of frequency and level on the unit's excitatory/inhibitory response patterns. FRMs were constructed by presenting a sequence of tone bursts that began at BF and expanded outwardly in frequency steps of 0.04

octaves. A typical sequence included 100 tones spanning 4 octaves. The process was repeated at multiple levels across the dynamic range of on-BF responses, as indicated by RALVs. If the unit was held at the completion of these measures, additional characterizations were performed (e.g., temporal modulation functions, random spectral shapes), somewhat limiting the yield of units from each 2 – 3 h recording session.

2.5. PSTH exemplars

Objective criteria for the categorization of AVCN neurons were derived from the spike time distributions of neurons with shared PSTH shapes. A subset of sampled neurons were selected to represent established chopper and primarylike classifications based on the shape of PSTHs and the regularity of ISIs (Blackburn and Sachs, 1989; Bourk, 1976; Pfeiffer, 1966). For convenience, these neurons are designated PSTH exemplars.

Exemplars sharing the same shape were assessed with quantifiable metrics, such as the latency of onset responses, regularity of ISIs and rate of adaptation. Metrics that demonstrated robust statistical differences between PSTH-defined response types were then used to categorize the remaining units in the sample. The reliability of putative decision criteria was verified by the clustering of objectively categorized units within the parameter space of exemplars.

2.6. Decision criteria

Present computational methods were similar to the previous analysis of PSTH patterns in other species (Blackburn and Sachs, 1989; Bourk, 1976; Young et al., 1988a), with the following exception. Statistical descriptions were based on time bins that contained a fixed number of ISI samples, rather than a fixed duration.

Adaptation patterns were analyzed by calculating the statistical variation of ISIs relative to stimulus onset times. To begin, all spike times were sorted in ascending order regardless of stimulus presentation number. The 27 spikes (representing 10% of the stimulus presentations) with the shortest latencies were selected and their ISIs were determined by measuring the time interval to the next spike in the same stimulus presentation. The mean and standard deviation of the ISIs was calculated. Then, the analysis time window was shifted by one spike to higher values and the process repeated until all spike times were analyzed. A time was assigned to each sampling interval by calculating the mean latency of the 27 spikes. A standardized ISI function was created by linearly interpolating the mean and standard deviation of ISI values at intervals of 0.05 ms.

Discrete samples of 27 spikes (i.e., 10% of the stimulus presentations that contributed to the PSTH) were analyzed because this spike count was small enough to support a detailed temporal analysis of adaptation effects and large enough to minimize the variance between adjacent temporal bins. On rare occasions, an individual ISI value deviated from the mean of the 27 spikes by more than three standard deviations, presumably indicating a false trigger or a missed spike. These outliers were removed, and ISI statistics were recalculated from the remaining values.

2.7. Regularity at stimulus onset

The coefficient of variation (CV) was used to perform the initial separation of generic primarylike and chopper units. This metric was derived from the ratio of the standard deviation to the mean of ISI values (Young et al., 1988a). Chopper units with regularly timed ISIs were expected to generate smaller standard deviations, and therefore lower CV scores than irregular primarylike units.

CV scores cannot be applied arbitrarily to ISI functions because transient and slowly adapting chopper units (Ch-T and Ch-SA) show increased irregularity later in the stimulus. For this reason, generic primarylike and chopper units were identified by their CV scores at the onset of sound-driven activity. The early CV (CV-E) statistic was defined as the median of CV scores within ± 0.1 ms of a unit's median first spike latency (FSL_{median} , as defined below). Quantifiable decision criteria were subsequently established by analyzing the statistical distribution of CV-E scores among primarylike and chopper exemplars.

2.8. Onset precision

The initial onset spikes of generic primarylike units were analyzed in terms of their latency, variability, and temporal separability. The minimum first spike latency (FSL_{min}) reflects the effective synaptic delay of its auditory nerve inputs. Because neurons within the major PSTH types receive different forms of synaptic contact from the auditory periphery, the latency of sound-driven activity is an alternative method for verifying the assumed anatomical basis of physiological classifications. In comparison to chopper units, primarylike units show relatively short latencies because they receive powerful axosomatic inputs from the auditory nerve (Rhode et al., 1983; Ryugo and Sento, 1991). Among generic primarylike (Pri) units, primarylike-with-notch (Pri-N) units are expected to display the shortest FSL_{min} because they receive a greater number of inputs from the auditory nerve (Rhode, 2008).

The FSL_{min} of sound-driven activity is not necessarily indicated by the timing of the first spike after stimulus onset because spontaneous spikes may occur prior to stimulated spikes. To avoid this ambiguity, FSL_{min} was defined as the minimum time interval after stimulus onset that elicited a statistically significant rate increase above the probability of spontaneous activity. This metric was estimated by comparing the number of spikes in a stimulus-on window (stimulated and spontaneous spikes) and a stimulus-off window (spontaneous spikes). The stimulus-on window began at stimulus onset and was adjusted in duration so that the number of stimulus-on spikes exceeded the expected number of spikes in a stimulus-off window of equal duration, under the assumption that the neuron was a Poisson process firing at its spontaneous rate ($p < 0.0001$, (Chase and Young, 2007). Spontaneous activity was sampled during the final 100 ms of the inter-stimulus interval to avoid adaptation effects. When a unit did not exhibit spontaneous activity, a sound-driven response was verified by adjusting the duration of the stimulus-on window until at least five spikes were captured.

Relative to generic primarylike units, the highly convergent inputs of Pri-N units are expected to produce more precisely timed onset responses. The median and standard deviation of first spike latencies (FSL_{median} , FSL_{SD}) were derived from the PSTH by selecting spike times that met the following criteria. The spikes must have preceded FSL_{min} by no more than 0.5 ms or followed FSL_{min} by no more than 10 ms.

The precise FSL_{median} of Pri-N neurons imposes an absolute refractory period that delays the second spike. As a result, the high onset rates of the PSTH are followed by a brief period of inactivity (i.e., notch). The separability of first and second spike latencies was quantified with the Jensen-Shannon Divergence (JSD) statistic, which was computed in MATLAB with the *KLDIV* function. This variant of Kullback-Leibler Divergence indicates the dissimilarity of two probability distributions with finite values that range from 0 to 1 (Antolin et al., 2009). A JSD of 0 indicates identical distributions; a value of 1 indicates two non-overlapping distributions. The prominent notch in the onset responses of Pri-N units is expected to produce JSD scores that approach 1.

The calculation of JSD takes the general form:

$$JSD(F, S) = \frac{1}{2}D(F||M) + \frac{1}{2}D(S||M)$$

$$D(F||M) = \sum_i F(i) \log \frac{F(i)}{M(i)}$$

$$D(S||M) = \sum_i S(i) \log \frac{S(i)}{M(i)}$$

$$M = \frac{1}{2}(F + S)$$

where F and S are the temporal probability distributions for first and second spikes (computed with bins of 0.2 ms), and i is the bin index. M is the midpoint distribution of F and S . D is the Kullback-Leibler Divergence for a discrete random variable.

2.9. Non-linear adaptation

Measures of non-linear (transient) adaptation were designed to quantify rapid changes in sound-driven rates during the initial 10 – 15 ms of sound-driven activity. Ch-S units were expected to maintain high driven rates during this time interval, while Ch-T units were expected to exhibit a sharp rate decrease.

The ISI Adaptation Ratio (ISI_{AR}) was defined as the ratio of the median ISI at the middle of the stimulus (ISI_M) to the median ISI at the onset of the stimulus (ISI_E , Fig. 5A). Because Ch-T units generate longer ISIs after stimulus onset, they were expected to produce high ISI_{AR} scores.

The maximum ISI slope (ISI_{MS}) was calculated by determining the maximum slope for the initial fifteen 1-ms segments of the ISI function (Fig. 5A). Each segment was smoothed with a boxcar averaging filter. Ch-T units were also expected to produce high ISI_{MS} scores.

Linear adaptation—Measures of linear adaptation were designed to capture the progressive rate decrease that is displayed by slowly adapting chopper units (Ch-SA). This objective was achieved by calculating the average ISI slope (ISI_{AS}) from early in the stimulus (ISI_E) to late in the stimulus (ISI_L , Fig. 6A). Because Ch-SA units also exhibit decreased regularity late in the stimulus, a late CV (CV_L) score was derived from the median of CV values that were recorded 20 – 30 ms after the onset of sound-driven activity (Fig. 6B). Relative to Ch-S units, Ch-SA units were expected to show a higher ISI_{AS} and CV_L .

2.10. Waveform shapes and synaptic prepotentials

The spike waveforms of AVCN neurons maintain a systematic relationship with PSTH response types. In domestic cats, most primarylike neurons produce a monophasic action potential that displays an electrical deflection immediately before the generation of an action potential (Bourk, 1976; Pfeiffer, 1966; Young et al., 1988a). Among Pri units, prepotentials

are sufficiently large to be seen on individual traces of spike waveforms (category PP1). Pri-N units exhibit smaller, but consistent prepotentials that are revealed by averaging (category PP2). Based on their strong association with Pri and Pri-N units, prepotentials are assumed to reflect the electrical discharge of the endbulb synapses that connect auditory nerve fibers to spherical and globular bushy cells. Chopper units produce biphasic action potentials without visible prepotentials (category PP3). Representative waveforms from domestic cats are shown in Fig. 8A.

Existing analyses of waveform shapes (Bourk, 1976; Young et al., 1988a) were extended to laboratory mice by recording the digitized action potential waveforms of AVCN neurons. When possible, the analysis was based on spontaneous activity to avoid the correlated electrical interference of nearby neurons. Action potentials were elicited from units without spontaneous activity by presenting near-threshold BF tones. The resulting waveforms were sampled at a rate of 500 MS/s and averaged over 64 consecutive spike acquisitions (Tektronix 1000B digital storage oscilloscope). Prepotentials were identified by a positive deflection in the average waveform that preceded the action potential by less than 0.5 ms.

2.11. BF threshold, excitatory bandwidth, and spontaneous rate

The BF threshold was defined as the minimum sound level that elicited a sound-driven response. Threshold estimates were derived from statistical comparisons of spike rates during the stimulus-on and stimulus-off intervals of RALVs. Stimulus-on (driven) rates were sampled during a 200-ms window that was synchronized with stimulus onset. Stimulus-off (spontaneous) rates were sampled during the final 400 ms of the 800-ms inter-stimulus intervals. As an additional control for adaptation effects, stimulus-off rates were only sampled from the initial 20 stimulus presentations of the RALV (i.e., the lowest presentation levels).

A 50% confidence interval (CI) was calculated under the assumption that the neuron was a Poisson process firing at its spontaneous rate (MATLAB, *poissfit* function, (Cai, 2007). Threshold was defined as the lowest of five consecutive levels where stimulus-on rates exceeded the 50% CI. The probability that five sub-threshold levels would produce a stimulus-on rate exceeding the stimulus-off rate is 0.25^5 ($p = 0.001$).

The bandwidth of frequency tuning was characterized with Q_{10} values that were derived from FRMs. By convention, Q_{10} is the ratio of BF to excitatory bandwidth 10 dB above the BF threshold. Excitatory bandwidth was defined for this calculation as the range of consecutive frequencies where stimulus-on rates exceeded stimulus-off rates, using the same statistical test that was applied during the determination of BF thresholds. Because threshold was determined by the off-line analysis of RALVs, frequency sequences usually were not obtained at exactly 10 dB re threshold. In these instances, responses were linearly interpolated from the closest surrounding levels in the FRM. Similarly, sound-driven rates were interpolated between the 0.04-octave frequency steps to increase the resolution of frequency sampling near the edge of excitatory cutoffs.

3. Results

3.1. Strain differences

Single-unit recordings were made in CBA/129 and CBA/CaJ mice to evaluate the generalization of objective classifications in two strains of laboratory mice with normal peripheral function. For the distribution of scores within each classification metric, Kolmogorov-Smirnov tests were used to compute the statistical significance of strain differences. Because no statistically significant differences were observed, the following results are based on the combined data from both strains.

3.2. Exemplar units

Exemplars from the four major VCN unit types are shown in Fig. 1. For each unit type, the raw spike time raster on the left is summarized by the PSTH on the right. The identification of exemplars was based on subjective (shape) and quantitative criteria (regularity) that were previously established in other species (Blackburn and Sachs, 1989). Primarylike units (Pri) displayed an irregularly timed FSL and short-term rate adaptation during the initial 20-ms of the stimulus presentation. Primarylike-with-notch units (Pri-N) displayed a precisely timed FSL that was followed by an absolute refractory period (notch). Sustained chopper units (Ch-S) showed regularly timed ISIs that gave the PSTH a multi-modal (chopped) appearance. Transient chopper units (Ch-T) exhibited regularly timed ISIs at stimulus onset that rapidly gave way to adaptation effects.

Multiple exemplars within each PSTH classification defined the physiological variability of quantifiable response metrics. The statistical separability of the metrics between PSTH classes created a decision tree for the objective classification of the remaining neurons in the sample. These criteria are summarized in Fig. 2.

3.3. Primarylike versus chopper units (criterion 1)

The gross distinction between primarylike and chopper units is commonly based on the analysis of onset regularity. The distinctive multi-modal visual appearance of the chopper PSTH is produced by the intrinsic membrane properties of multipolar cells which generate regularly timed spike activity (Manis and Marx, 1991). The regularity of sound-driven activity is quantified by the CV statistic (Blackburn and Sachs, 1989; Young et al., 1988a), with chopper units having lower scores than primarylike units.

Figure 3 summarizes differences in the statistical distributions of CV-E scores for exemplar primarylike ($n = 22$) and chopper units ($n = 37$). The exemplar Pri and Pri-N units from Fig. 1 are highlighted in Fig. 3A, and the exemplar Ch-S and Ch-T units in Fig. 3B. The right panel displays CV across the complete stimulus duration, while the left panel highlights the CV-E interval ($\text{FSL}_{\text{median}} \pm 1 \text{ ms}$). Differences in the CV-E scores of the four exemplars are illustrated by their position relative to the gray-filled area in each panel. Both chopper units exhibited lower CV-E scores than the primarylike units. The Ch-T unit produced less regular ISIs later in the stimulus.

Histograms showing the CV-E scores of all primarylike and chopper exemplars are presented in Fig. 3C. The highest scores of the chopper exemplars fell below 0.2, while the lowest scores of primarylike exemplars exceeded 0.25. The remaining units in the sample were categorized as generic primarylike or chopper units if their CV-E scores were within the limits of either distribution. Two units with intermediate scores were not further categorized (NFC).

3.4. Primarylike versus primarylike-with-notch units (criterion 2)

Pri-N units were distinguished from Pri units by the precision of their onset responses. The temporal reproducibility of first spike latencies and ensuing refractory periods produce a robust onset peak and notch that is visible in the PSTH. Figure 4 illustrates these features for the Pri and Pri-N exemplars in Figs. 1A and B. The time scale of the spike time rasters in Fig. 4A have been expanded to show only the first and second spikes of each stimulus presentation. The statistical distributions of the spike times are summarized by the histograms in Fig. 4B.

The mean and standard deviation of first and second spike latencies are presented for 9 Pri and 5 Pri-N exemplars in Fig. 4C. The units are rank ordered by the statistical separation of

first and second spike latencies, as quantified by JSD scores. Units with the most separable spike latencies are shown on the right. First and second spike latencies were highly variable for the population of Pri exemplars, which resulted in substantial overlap between the two distributions. Second spike latencies also were variable for the Pri-N exemplars, but the precision of first spike latencies maintained a clear separation between the distributions.

The mathematical separability of first and second spike latencies was quantified with JSD and FSL_{SD} statistics. When exemplars are plotted along these two dimensions in Fig. 4D, Pri-N units cluster in a region of low FSL_{SD} (precision) and high JSD (separability). Pri units disperse over a greater range of parameter space, but remain spatially segregated from Pri-N units.

Categorization of generic primarylike units was based on how well their FSL_{SD} and JSD scores co-localized with Pri or Pri-N exemplars. Ten units met the criteria for Pri ($n = 4$) or Pri-N classification ($n = 6$). Five units displayed ambiguous scores and were not further categorized (NFC).

3.5. Sustained versus transient choppers (criterion 3)

Chopper units were separated into sustained (Ch-S) and transient (Ch-T) subtypes based on the temporal properties of their ISI functions (Blackburn and Sachs, 1989; Blackburn and Sachs, 1992). Figure 5A contrasts the ISI functions of the Ch-S and Ch-T exemplars in Figs. 1C and D. The Ch-S exemplar produced consistently short ISIs throughout the stimulus presentation because it maintained a high sound-driven rate. The Ch-T exemplar produced ISIs that lengthened abruptly toward the beginning of the stimulus because sound-driven rates rapidly adapted. Median “chopping” frequencies were higher among Ch-T units (726 Hz) than among Ch-S units (325 Hz) prior to adaptation, but both classifications produced a number of units with frequencies exceeding 500 Hz. Discharge rates of this magnitude were uncommon among primarylike units.

The transient adaptation effects of Ch-T exemplars were quantified with the ISI_{AR} and ISI_{MS} statistics. Methods for the calculation of these metrics are related to ISI functions in Fig. 5A. Two-dimensional plots of the metrics are illustrated with 12 Ch-S and 5 Ch-T exemplars in Fig. 5B. All of the Ch-S exemplars produced ISI_{AR} scores that were less than 1.5. Their ISI_{MS} scores were less than 0.5. By contrast, the steep increase in ISI among Ch-T exemplars produced ISI_{AR} scores that were greater than 2.5 and ISI_{MS} scores that were greater than 1. Out of 23 generic chopper units, 20 produced scores that clustered near the Ch-S exemplars. The remaining units were categorized as Ch-T ($n = 2$) or NFC ($n = 1$).

3.6. Sustained versus slowly adapting choppers (criterion 4)

Blackburn and Sachs (Blackburn and Sachs, 1992) identified a third chopper subtype with slowly adapting sound-driven rates (Ch-SA). Ch-SA exemplars display a linear increase in ISI (Fig. 6A) and decreased regularity toward the end of the stimulus (Fig. 6B) that differs from the rapid non-linear adaptation effects of Ch-T units (Fig. 5A). These slowly developing response patterns were quantified with the average slope of the ISI function (ISI_{AS}) and the late CV score (CV-L).

Two-dimensional plots of the ISI_{AS} and CV-L scores of 12 Ch-S exemplars are shown in Fig. 6C. These non-adapting units produced ISI_{AS} scores that were less than 0.04 and CV-L scores that were less than 0.3. Generic chopper units that did not show transient adaptation patterns appear to be distributed along a continuum without a clear categorical boundary. Therefore, the decision criteria for Ch-SA classification was determined by the most extreme scores of the Ch-S exemplars. This approach yielded 5 Ch-S, 13 Ch-SA and 2 NFC assignments.

3.7. Verification of the decision tree

The accuracy of objective classification was verified by examining the PSTH shapes of categorized units. The examples in Figs. 7A and B were assigned to the Pri and Pri-N categories by applying criteria 1 and 2 of the decision tree. The examples in Figs. 7C and D were assigned to the Ch-S and Ch-T categories by criteria 1, 3, and 4. For each case, the categorized units reproduced the major visual and objective criteria of the exemplar units in Fig. 1.

3.8. Waveform shapes and synaptic prepotentials

The action potential waveforms of domestic cats and laboratory mice are compared in Figs. 8A and B, respectively. As illustrated in the figure, striking differences in the waveform shapes of chopper and primarylike units have been noted in cats (Young et al., 1988a). Most Pri neurons produce monophasic action potentials that are preceded by discrete prepotentials (category PP1). Biphasic PP1 responses and monophasic PP2 responses are less commonly observed (Bourk, 1976). Most Pri-N neurons produce monophasic PP2 responses, although larger PP1 prepotentials also are encountered. Chopper units produce biphasic action potentials without prepotentials (category PP3).

In the case of ChS and ChT units, the action potential waveforms of laboratory mice shared the biphasic, PP3 properties that have been previously described in cats. Multiple examples from the two PSTH response types are presented in the right columns of Fig. 8B. Each trace shows the averaged waveform of a different unit. Positive peaks have been aligned in time to enhance the visibility of individual waveforms. Variations in amplitude reflect unit isolation, signal amplification, and electrode position at the time of recording.

One of the most striking physiological differences in the AVCN responses of mice and cats was the action potential shapes of Pri and Pri-N units. Examples of averaged waveforms from mice are presented in the left columns of Fig. 8B. Units in both PSTH classifications produced biphasic action potentials. PP1 prepotentials were never observed. PP2 prepotentials were uncommon. The initial positive deflections that were assumed to indicate PP2 responses tended to be smaller in magnitude, less discrete in time, and typically merged with the initial positive peak of the biphasic action potential. These observations suggest that the synaptic inputs of the auditory nerve to bushy cells are less secure in laboratory mice than in domestic cats.

3.9. Basic response properties of physiologically categorized units

Additional tests characterized the basic response properties of all physiologically categorized units. BF thresholds are plotted in relation to frequency and unit type in Fig. 9A. For comparison, the best threshold curve of auditory nerve fibers is also shown for CBA/CaJ and C57BL/6 mice (Taberner and Liberman, 2005). The best thresholds of AVCN neurons and auditory nerve fibers match closely across a wide range of frequencies, with the most sensitive thresholds falling between 10 – 20 kHz. The major PSTH classifications produced an equivalent range of thresholds at any given frequency. AVCN units appear to be less sensitive than auditory nerve fibers at frequencies below 8 kHz, but this discrepancy is likely due to the sparse sampling of low-BF units in the present study.

Q_{10} measures of frequency tuning are shown in Fig. 9B. Once again, there was good agreement between average physiological responses in the AVCN and auditory nerve (Taberner and Liberman, 2005). As observed in other species, average Q_{10} values increased with BF (Palmer et al., 2003; Rhode and Smith, 1986; Sayles and Winter, 2010). Responses from multiple PSTH classifications were intermingled at any given frequency.

Spontaneous rates are shown in Fig. 9C. Most units from all PSTH classifications exhibited very low spontaneous rates. Higher rates were more likely to be associated with Pri or Pri-N units, which has been previously observed in domestic cats (Blackburn and Sachs, 1989). There was no systematic effect of BF at well-sampled frequencies.

Minimum onset latencies ($FSL_{\min}$) are presented in Fig. 9D. The three classes of primarylike units (Pri, Pri-N, Pri-NFC) produced the shortest latencies at each BF. This effect also has been noted in domestic cats (Blackburn and Sachs, 1989). The rapid activation of primarylike neurons is attributed to the specialized synaptic endbulbs by which they receive auditory nerve inputs (Rhode et al., 1983; Wu and Oertel, 1984).

4. Discussion

The majority of AVCN units were assigned to a specific PSTH response class. As in other species, units with ambiguous or unusual response properties are also encountered in mice. The following discussion summarizes the decision criteria that were applied in these situations, especially where current classification strategies deviated from previous analyses.

4.1. Ambiguities between chopper and primarylike units

The majority of chopper and primarylike exemplars were clearly segregated by differences in the CV-E statistic (Fig. 3). On a few instances (2/74 units), ambiguous CV-E scores were associated with response patterns that could be interpreted as a Pri-N unit with an unusually well-timed second spike or a Ch-T unit with unusually rapid adaptation.

A peak magnitude criterion has been proposed for separating chopper units from primarylike units with regular onset rates (Blackburn and Sachs, 1989; Young et al., 1988a). Chopper units are expected to generate spike counts in the first 2 – 3 peaks of the PSTH on all stimulus repetitions. Therefore, their “spikes per peak” scores are expected to approach 1. Because these ambiguities were rare in mice, the present classification system relied exclusively on CV-E statistics to distinguish generic chopper and primarylike units

4.2. Alternative methods for the classification of primarylike subtypes

Prior classification studies have identified Pri-N units by a subjective inspection of the PSTH (Bourk, 1976; Pfeiffer, 1966). Proposed metrics for the objective classification of this subtype were derived from those descriptions. The precise onset of Pri-N units was translated to the FSL_{SD} statistic, while the refractory notch was captured by the JSD score. The FSL_{SD} statistics of Pri and Pri-N units have been previously measured in domestic cats and are equivalent to the ranges that are reported here for laboratory mice (Young et al., 1988a).

Although chopper units exhibited equivalent onset statistics, exemplar Pri-N units were easily distinguished from Pri units by their low FSL_{SD} and high JSD scores. Exemplar Pri units showed more variable onset responses and less separable first and second spike latencies. Although exemplars demonstrated clear physiological differences, an ambiguity was introduced when these two metrics were extended to a broad sampling of generic primarylike units (Fig. 4D). Several units received Pri-NFC designations because they produced FSL_{SD} statistics that were consistent with the Pri classification but JSD scores that exceeded the highest Pri exemplar.

Attempts to minimize the number of Pri-NFC units failed to identify features that justified their inclusion in either the Pri or Pri-N classification. The tight parameter space of Pri-N exemplars makes it unlikely that this issue would be resolved by adding more Pri-N exemplars to the analysis but it is possible that a larger sample of Pri exemplars would

reduce the range of ambiguous JSD scores. In lieu of these data, present results suggest that FSL_{SD} and JSD scores are a continuous not a categorical property of single-unit responses in the AVCN.

When recording in the AVCN, care must be taken to distinguish between Pri units and their auditory nerve inputs. To avoid this potential artifact, the present recordings were made using metal electrodes or micropipettes with relatively large tips (~ 1.5 to $2.0 \mu\text{m}$) and low impedances ($1 \text{ M}\Omega$). Recordings from small diameter auditory nerve fibers are typically made using micropipettes with fine tips and high impedances ($10 - 30 \text{ M}\Omega$, (Kiang et al., 1965). Scatter plots of first spike latency do not indicate Pri units with shorter latencies than Pri-N units (Fig. 9D), which would suggest erroneously classified auditory nerve fibers.

4.3. Alternative methods for the classification of chopper subtypes

Blackburn and Sachs (1989, 1992) characterized the adaptation patterns of chopper units by a decrease in regularity and increase in ISI that occurred late in the stimulus presentation. The present classification system incorporated a similar approach. Rapidly adapting chopper units were assigned to the Ch-T classification by their high ISI_{AR} and ISI_{MS} scores (Fig. 5C). Both metrics produced a clear separation between Ch-T units and other chopper subtypes. The adaptation patterns of laboratory mice were more categorically distinct than those previously described in domestic cats.

Slowly adapting choppers were assigned to the Ch-SA classification by high ISI_{AS} and CV-L scores (Fig. 6C). Examination of the resulting distributions, however, suggests that the categorization of linear adaptation is not well justified by the quantitative analysis of ISI_{AS} and CV-L scores. Instead, Ch-S and Ch-SA units fall along a continuum of increasing adaptation. Ch-S and Ch-SA designations have been maintained in the present classification system to allow direct comparisons with the descriptions of Blackburn and Sachs (1989, 1992). Nevertheless, the functional importance of rate adaptation may be better understood by emphasizing the shared characteristics of Ch-S and Ch-SA units, and not their enforced categorization.

4.4. Onset units

Onset units emit a precisely timed onset spike following by little or no activity (Blackburn and Sachs, 1989; Pfeiffer, 1966). They are broadly tuned, have a wide dynamic range, and often produce a sustained response when stimulated with broadband noise (Smith and Rhode, 1989). Onset units were rarely observed in our study of the AVCN. The scarcity of this response pattern is most likely due to sampling bias. Onset responses are more common in the posterior VCN (Kane, 1973; Osen, 1969). The present classification system was directed to the anterior subdivision and was designed for units that exhibited sustained responses to BF tones.

Classification ambiguities may arise between the rapidly adapting responses of Ch-T units and the prolonged onset responses of onset chopper (onset-C) units. While these unit types show unique intracellular responses (Paolini and Clark, 1999), extracellular recordings display considerable overlap in physiological properties such as threshold and near-threshold frequency tuning (Jiang et al., 1996). The present study distinguished Ch-T units from onset-C units by their relatively high sustained sound-driven rates (PSTH) and restricted dynamic range (RALV).

Onset (onset-L) units sustain low levels of sound-driven activity and therefore may be confused with primarylike units (Rhode et al., 1983). Blackburn and Sachs (1989) designated ambiguous primarylike/onset units as onset responses if the PSTH displayed precise onset spikes and sustained firing rates below 100 spikes/sec . This criterion was met

by 2 out of 11 Pri-N units in the present sample. All of the units were assigned to the Pri-N classification. Additional criteria based on sustained firing rate, response bandwidth, or dynamic range may be used to discriminate the two unit types, when necessary (Palmer et al., 1996; Winter and Palmer, 1995).

4.5. Unusual units

Chopper (Ch-L) units with low onset rates have been described in domestic cats (Bourk, 1976). Because only a small number of Ch-L units (4/274 chopper units) were observed by Blackburn and Sachs (1989), they were regarded as “unusual units.” Units with these characteristics were more common in mice (7/41 chopper units). In nearly all cases, they met the criteria for classification as Ch-S units.

While most chopper units produce an ISI function that is flat (Ch-S) or adapting (Ch-SA, Ch-T), Blackburn and Sachs (1989) reported a few unusual chopper units with decreasing ISI functions (negative ISI_{AS}). In the present analysis, negative ISI_{AS} scores remained near 0 (Fig. 6C). No clear dichotomy was observed among these units and they were all assigned the Ch-S classification.

Blackburn and Sachs (1989) identified primarylike units with unusually long first spike latencies. Three of these responses are suggested by outliers in Fig. 9D. Given the comparatively small number of primarylike units in the present sample, long latency Pri units may be more common in laboratory mice than in domestic cats. Chopper units with equivalent BFs produced similar latencies, so it is likely that these responses were driven by direct inputs from the auditory nerve.

4.6. Action potential waveforms

A detailed analysis of action potential waveforms is problematic for extracellular recordings because the size, shape, and polarity of the electrical signal is influenced by variations in the physical relationship of the electrode to a propagating current source. Nevertheless, gross waveform features were remarkably consistent for well-isolated AVCN neurons in laboratory mice (Fig. 8B). Chopper and primarylike units produced biphasic action potentials. Chopper units never displayed prepotentials. Primarylike units never displayed clear PP1 responses. The rare instances of potentials that may represent PP2 responses showed poor temporal definition and close proximity to action potentials, which are characteristics that are more consistent with EPSPs (Kuenzel et al., 2011). These results suggest that laboratory mice and domestic cats share similar near-field electrical properties for chopper units but exhibit notable differences in their primarylike responses.

Bourk (1976) reported prepotentials for all Pri units in cats. The majority of units showed large PP1 responses (66%). Pri-N units showed high percentages of both PP1 (34%) and PP2 responses (55%). His descriptions were replicated by Blackburn and Sachs (1989), who examined individual digitized spike waveforms for the presence of PP1 responses (53% Pri, 58% Pri-N). Monophasic action potentials have been linked to primarylike responses by both Bourk (1976) and Young et al. (Young et al., 1988a).

Endbulbs are present on the spherical bushy cells of laboratory mice (Limb and Ryugo, 2000), but appear to be smaller in size and more sparsely branched than the endbulbs of cats (Ryugo and Fekete, 1982). This less robust morphological expression may explain the missing prepotentials and less polarized action potentials of primarylike units in mice. Laboratory rats, a rodent species with more sensitive low-frequency hearing, produce primarylike responses with prepotentials under identical recording conditions (Roos, 2012). It is intriguing to speculate that the evolutionary premium for secure endbulb transmission may be less important for prey species with a strong bias toward high-frequency hearing. In

mice, the functional consequences of this biological variation manifest as reduced temporal fidelity and poor sound localization (Lauer et al., 2011; May et al., 2011).

4.7. Basic response patterns

The basic sound-driven response patterns of physiologically classified AVCN units were similar in laboratory mice to other common animal models. The upper frequency limits of neural BFs and the region of maximum sensitivity were shifted to higher frequencies in mice, but the most sensitive thresholds maintained the general features of mammalian audibility curves (Fig. 9A). Maximum sensitivity followed the best threshold curve of the auditory nerve and was comparable to other species. Primarylike and chopper subtypes did not show strong biases in sensitivity and were broadly distributed in BF.

AVCN neurons with BFs below 6 kHz were not observed in the present study (Fig. 9A). This result differs from the reported frequency range of auditory nerve fibers in laboratory mice (Taberner and Liberman, 2005), but agrees with previous recordings in the AVCN (Luo et al., 2009). The difference may be explained by 3-dimensional reconstructions of frequency representations in the mouse cochlear nucleus (Muniak et al., 2012). The caudal-to-rostral axis of the AVCN displays an orderly tonotopic organization that conforms well with the present sampling of BFs. Neurons with BFs below 6 kHz do not fall along this axis and are not encountered by electrode trajectories that are optimized to sample the main frequency axis of the AVCN.

Frequency responses maps in the AVCN of laboratory mice displayed the same V-shaped excitatory region that has been described in other species (Evans and Nelson, 1973; Shofner and Young, 1985). On average, the bandwidth of frequency tuning followed general mammalian trends (Fig. 9B). Q_{10} values ranged from 2 – 15, increased with BF, and were well predicted by measurements in the auditory nerve (Taberner and Liberman, 2005). Primarylike and chopper units did not show consistent differences in frequency tuning. The variance of Q_{10} values at a particular BF was much larger than previous results from the domestic cat (van Heusden and Smoorenburg, 1983).

The spontaneous rates of AVCN units typically fell below 5 spikes/s in mice (Fig. 9C). Most units displayed some spontaneous activity, and therefore would be classified as type I responses in FRM-based classification systems (Kiang et al., 1965). Some units from all of the major PSTH classifications showed no spontaneous activity, but this so-called type I/III FRM was most common among chopper classifications. Only two units produced spontaneous rates higher than 40 spikes/s. The distribution of spontaneous rates suggests less activity than what has been previously described in domestic cats, where the spontaneous rates of Pri units frequently exceed 100 spikes/s (Blackburn and Sachs, 1989). Anesthesia does not appear to be the source of this species difference. Recordings in mice were conducted under urethane anesthesia, while domestic cats were anesthetized with barbiturates. Urethane is expected to produce less depression of brainstem activity.

The onset latencies of primarylike units were typically shorter than those of chopper units (Fig. 9D), as has been noted in other species. The absolute latencies reported here may appear slightly longer than other published reports because acoustic stimuli were delivered in free-field from a speaker that was located 36 cm from the subject's head. This additional conduction time had no effect on unit classifications that were determined by relative differences in the latency or variability of sound-driven activity.

5. Summary

The essential finding of the present study was that single-unit responses in the AVCN of two common strains of laboratory mice exhibited generic mammalian PSTH response types (Blackburn and Sachs, 1989; Pfeiffer, 1966). Studies in mice have previously reported a preservation of generalized mammalian patterns for auditory nerve physiology (Taberner and Liberman, 2005) and AVCN cellular morphologies (Briner and Willott, 1989; Limb and Ryugo, 2000). Despite these common traits, the absence of prepotentials and the preponderance of neurons with BFs above the upper limits of phase locking suggest that laboratory mice may provide a less representative model of temporal coding in the auditory brainstem.

Acknowledgments

Amanda Lauer conducted the initial assessments of auditory function in CBA/129 mice (auditory brainstem response, distortion product otoacoustic emissions, and acoustic startle responses). This work was supported by NIDCD grants F31 DC010095 and P30 DC005211.

References

- Antolin J, Angulo JC, Lopez-Rosa S. Fisher and Jensen-Shannon divergences: Quantitative comparisons among distributions. Application to position and momentum atomic densities. *J Chem Phys.* 2009; 130:074110. [PubMed: 19239287]
- Banks MI, Sachs MB. Regularity analysis in a compartmental model of chopper units in the anteroventral cochlear nucleus. *J Neurophysiol.* 1991; 65:606–29. [PubMed: 1646868]
- Blackburn CC, Sachs MB. Classification of unit types in the anteroventral cochlear nucleus: PST histograms and regularity analysis. *J Neurophysiol.* 1989; 62:1303–29. [PubMed: 2600627]
- Blackburn CC, Sachs MB. The representations of the steady-state vowel sound /e/ in the discharge patterns of cat anteroventral cochlear nucleus neurons. *J Neurophysiol.* 1990; 63:1191–212. [PubMed: 2358869]
- Blackburn CC, Sachs MB. Effects of OFF-BF tones on responses of chopper units in ventral cochlear nucleus. I. Regularity and temporal adaptation patterns. *J Neurophysiol.* 1992; 68:124–43. [PubMed: 1517819]
- Bourk, TR. Thesis. Massachusetts Institute of Technology; Cambridge, MA: 1976. Electrical responses of neural units in the anteroventral cochlear nucleus of the cat.
- Briner W, Willott JF. Ultrastructural features of neurons in the C57BL/6J mouse anteroventral cochlear nucleus: young mice versus old mice with chronic presbycusis. *Neurobiol Aging.* 1989; 10:295–303. [PubMed: 2682312]
- Cai, S. Thesis. The Johns Hopkins University; Baltimore, MD: 2007. Intensity encoding of ventral cochlear nucleus neurons in normal and deafened cats and correlates of loudness recruitment.
- Cant NB, Morest DK. The bushy cells in the anteroventral cochlear nucleus of the cat. A study with the electron microscope. *Neuroscience.* 1979a; 4:1925–45. [PubMed: 530439]
- Cant NB, Morest DK. Organization of the neurons in the anterior division of the anteroventral cochlear nucleus of the cat. Light-microscopic observations. *Neuroscience.* 1979b; 4:1909–23. [PubMed: 530438]
- Cant, NB.; Morest, DK. The structural basis for stimulus coding in the cochlear nucleus of the cat. In: Berlin, CI., editor. *Hearing Science: Recent Advances.* College-Hill Press; San Diego: 1984. p. 371–421.
- Cant NB, Benson CG. Parallel auditory pathways: projection patterns of the different neuronal populations in the dorsal and ventral cochlear nuclei. *Brain Res Bull.* 2003; 60:457–74. [PubMed: 12787867]
- Chase SM, Young ED. First-spike latency information in single neurons increases when referenced to population onset. *Proc Natl Acad Sci U S A.* 2007; 104:5175–80. [PubMed: 17360369]

- Evans EF, Nelson PG. The responses of single neurones in the cochlear nucleus of the cat as a function of their location and the anaesthetic state. *Experimental brain research. Experimentelle Hirnforschung. Experimentation cerebrale*. 1973; 17:402–27. [PubMed: 4725899]
- Feng J, Bendiske J, Morest DK. Postnatal development of NT3 and TrkC in mouse ventral cochlear nucleus. *J Neurosci Res*. 2010; 88:86–94. [PubMed: 19610111]
- Harris JA, Hardie NA, Bermingham-McDonogh O, Rubel EW. Gene expression differences over a critical period of afferent-dependent neuron survival in the mouse auditory brainstem. *J Comp Neurol*. 2005; 493:460–74. [PubMed: 16261529]
- Jiang D, Palmer AR, Winter IM. Frequency extent of two-tone facilitation in onset units in the ventral cochlear nucleus. *J Neurophysiol*. 1996; 75:380–95. [PubMed: 8822565]
- Kane EC. Octopus cells in the cochlear nucleus of the cat: heterotypic synapses upon homeotypic neurons. *Int J Neurosci*. 1973; 5:251–79. [PubMed: 4132388]
- Kiang, NYS.; Watanabe, T.; Thomas, EC.; Clark, LF. *Discharge Patterns of Single Fibers in the Cat's Auditory Nerve*. MIT Press; Cambridge, MA: 1965.
- Kopp-Scheinpflug C, Dehmel S, Dorrscheidt GJ, Rubsamen R. Interaction of excitation and inhibition in anteroventral cochlear nucleus neurons that receive large endbulb synaptic endings. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2002; 22:11004–18. [PubMed: 12486196]
- Kuenzel T, Borst JG, van der Heijden M. Factors controlling the input-output relationship of spherical bushy cells in the gerbil cochlear nucleus. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2011; 31:4260–73. [PubMed: 21411667]
- Lai YC, Winslow RL, Sachs MB. A model of selective processing of auditory-nerve inputs by stellate cells of the antero-ventral cochlear nucleus. *J Comput Neurosci*. 1994; 1:167–94. [PubMed: 8792230]
- Lauer AM, Slee SJ, May BJ. Acoustic basis of directional acuity in laboratory mice. *Journal of the Association for Research in Otolaryngology : JARO*. 2011; 12:633–45. [PubMed: 21717290]
- Lauer AM, May BJ, Hao ZJ, Watson J. Analysis of environmental sound levels in modern rodent housing rooms. *Lab Anim (NY)*. 2009; 38:154–60. [PubMed: 19384312]
- Lee DJ, Cahill HB, Ryugo DK. Effects of congenital deafness in the cochlear nuclei of Shaker-2 mice: an ultrastructural analysis of synapse morphology in the endbulbs of Held. *J Neurocytol*. 2003; 32:229–43. [PubMed: 14724386]
- Liberman MC. Central projections of auditory nerve fibers of differing spontaneous rate, II: Posteroventral and dorsal cochlear nuclei. *The Journal of comparative neurology*. 1993; 327:17–36. [PubMed: 8432906]
- Limb CJ, Ryugo DK. Development of primary axosomatic endings in the anteroventral cochlear nucleus of mice. *J Assoc Res Otolaryngol*. 2000; 1:103–19. [PubMed: 11545139]
- Luo F, Wang Q, Farid N, Liu X, Yan J. Three-dimensional tonotopic organization of the C57 mouse cochlear nucleus. *Hear Res*. 2009; 257:75–82. [PubMed: 19695320]
- Malmierca, MS.; Hackett, TA. Structural organization of the ascending auditory pathway. In: Rees, A.; Palmer, AR., editors. *The Oxford Handbook of Auditory Science: The Auditory Brain*. Oxford University Press; Oxford: 2010. p. 9-41.
- Manis PB, Marx SO. Outward currents in isolated ventral cochlear nucleus neurons. *J Neurosci*. 1991; 11:2865–80. [PubMed: 1880553]
- May BJ, Prell GS, Sachs MB. Vowel representations in the ventral cochlear nucleus of the cat: effects of level, background noise, and behavioral state. *J Neurophysiol*. 1998; 79:1755–67. [PubMed: 9535945]
- May BJ, Lauer AM, Roos MJ. Impairments of the medial olivocochlear system increase the risk of noise-induced auditory neuropathy in laboratory mice. *Otol Neurotol*. 2011; 32:1568–78. [PubMed: 21956602]
- Muniak MA, Rivas A, Montey KL, May BJ, Francis HW, Ryugo DK. A 3-dimensional model of frequency representation in the cochlear nucleus of the CBA/J mouse. *J Comp Neurol under review*. 2012

- Oertel D, Shatadal S, Cao XJ. In the ventral cochlear nucleus Kv1.1 and subunits of HCN1 are colocalized at surfaces of neurons that have low-voltage-activated and hyperpolarization-activated conductances. *Neuroscience*. 2008; 154:77–86. [PubMed: 18424000]
- Osen KK. Cytoarchitecture of the cochlear nuclei in the cat. *J Comp Neurol*. 1969; 136:453–84. [PubMed: 5801446]
- Palmer AR, Jiang D, Marshall DH. Responses of ventral cochlear nucleus onset and chopper units as a function of signal bandwidth. *J Neurophysiol*. 1996; 75:780–94. [PubMed: 8714652]
- Palmer AR, Wallace MN, Arnott RH, Shackleton TM. Morphology of physiologically characterised ventral cochlear nucleus stellate cells. *Exp Brain Res*. 2003; 153:418–26. [PubMed: 12955380]
- Paolini AG, Clark GM. Intracellular responses of onset chopper neurons in the ventral cochlear nucleus to tones: evidence for dual-component processing. *J Neurophysiol*. 1999; 81:2347–59. [PubMed: 10322071]
- Pearce M, Richter CP, Cheatham MA. A reconsideration of sound calibration in the mouse. *J Neurosci Methods*. 2001; 106:57–67. [PubMed: 11248341]
- Pfeiffer RR. Classification of response patterns of spike discharges for units in the cochlear nucleus: tone-burst stimulation. *Exp Brain Res*. 1966; 1:220–35. [PubMed: 5920550]
- Rhode WS. Response patterns to sound associated with labeled globular/bushy cells in cat. *Neuroscience*. 2008; 154:87–98. [PubMed: 18423882]
- Rhode WS, Smith PH. Encoding timing and intensity in the ventral cochlear nucleus of the cat. *J Neurophysiol*. 1986; 56:261–86. [PubMed: 3760921]
- Rhode WS, Oertel D, Smith PH. Physiological response properties of cells labeled intracellularly with horseradish peroxidase in cat ventral cochlear nucleus. *J Comp Neurol*. 1983; 213:448–63. [PubMed: 6300200]
- Roos, MJ. Thesis. Johns Hopkins University; Baltimore, MD: 2012. Spectral and Temporal Coding in the Rodent Ventral Cochlear Nucleus.
- Rothman JS, Young ED, Manis PB. Convergence of auditory nerve fibers onto bushy cells in the ventral cochlear nucleus: implications of a computational model. *J Neurophysiol*. 1993; 70:2562–83. [PubMed: 8120599]
- Ryugo DK, Fekete DM. Morphology of primary axosomatic endings in the anteroventral cochlear nucleus of the cat: a study of the endbulbs of Held. *J Comp Neurol*. 1982; 210:239–57. [PubMed: 7142440]
- Ryugo DK, Sento S. Synaptic connections of the auditory nerve in cats: relationship between endbulbs of held and spherical bushy cells. *J Comp Neurol*. 1991; 305:35–48. [PubMed: 2033123]
- Sayles M, Winter IM. Equivalent-rectangular bandwidth of single units in the anaesthetized guinea-pig ventral cochlear nucleus. *Hear Res*. 2010; 262:26–33. [PubMed: 20123119]
- Shofner WP, Young ED. Excitatory/inhibitory response types in the cochlear nucleus: relationships to discharge patterns and responses to electrical stimulation of the auditory nerve. *Journal of neurophysiology*. 1985; 54:917–39. [PubMed: 4067627]
- Smith PH, Rhode WS. Structural and functional properties distinguish two types of multipolar cells in the ventral cochlear nucleus. *J Comp Neurol*. 1989; 282:595–616. [PubMed: 2723154]
- Spirou GA, Rager J, Manis PB. Convergence of auditory-nerve fiber projections onto globular bushy cells. *Neuroscience*. 2005; 136:843–63. [PubMed: 16344156]
- Taberner AM, Liberman MC. Response properties of single auditory nerve fibers in the mouse. *J Neurophysiol*. 2005; 93:557–69. [PubMed: 15456804]
- Trussel, LO. Cellular Mechanisms for Information Coding in Auditory Brainstem Nuclei. In: Oertel, D.; Fay, RR.; Popper, AN., editors. *Integrative Functions in the Mammalian Auditory Pathway*. Springer; New York: 2002. p. 72-98.
- van Heusden E, Smoorenburg GF. Responses from AVCN units in the cat before and after inducement of an acute noise trauma. *Hear Res*. 1983; 11:295–326. [PubMed: 6630085]
- Webster DB, Trune DR. Cochlear nuclear complex of mice. *Am J Anat*. 1982; 163:103–30. [PubMed: 7072613]
- Winter IM, Palmer AR. Level dependence of cochlear nucleus onset unit responses and facilitation by second tones or broadband noise. *J Neurophysiol*. 1995; 73:141–59. [PubMed: 7714560]

- Woolf NK, Ryan AF. Ontogeny of neural discharge patterns in the ventral cochlear nucleus of the mongolian gerbil. *Brain Res.* 1985; 349:131–47. [PubMed: 3986582]
- Wu SH, Oertel D. Intracellular injection with horseradish peroxidase of physiologically characterized stellate and bushy cells in slices of mouse anteroventral cochlear nucleus. *J Neurosci.* 1984; 4:1577–88. [PubMed: 6726347]
- Young ED, Robert JM, Shofner WP. Regularity and latency of units in ventral cochlear nucleus: implications for unit classification and generation of response properties. *J Neurophysiol.* 1988a; 60:1–29. [PubMed: 3404211]
- Young, ED.; Shofner, WP.; White, JA.; Robert, J-M.; Voight, HF. Response properties of cochlear nucleus neurons in relationship to physiological mechanisms. In: Edelman, GM.; Gall, WE.; Cowen, WM., editors. *Auditory Function: Neurobiological Bases of Hearing.* Wiley; New York: 1988b. p. 277-312.
- Yousoufian M, Couchman K, Shivdasani MN, Paolini AG, Walmsley B. Maturation of auditory brainstem projections and calyces in the congenitally deaf (dn/dn) mouse. *J Comp Neurol.* 2008; 506:442–51. [PubMed: 18041784]

Highlights

- Single-unit responses were recorded from the anteroventral cochlear nucleus.
- Results from two strains of laboratory mice were compared.
- Responses were classified in terms of peri-stimulus time histograms.
- The analysis replicated previous descriptions in other mammalian species.
- Synaptic coupling was not as secure as in other species.

Fig. 1.

Exemplars displaying the definitive response patterns of four major AVCN response types: (A) Pri, (B) Pri-N, (C) Ch-S, and (D) Ch-T. *Left*: Dot rasters showing spike times relative to stimulus onset for 270 repetitions of a 50-ms BF tone. *Right*: PSTHs summarizing the temporal distributions of the dot rasters.

Fig. 2.

Decision tree for unit classification. Major branch points are defined by the regularity of onset discharge rates (*Criterion 1*), the separability of 1st and 2nd spike latencies (*Criterion 2*), and transient versus slow rates of adaptation (*Criteria 3 and 4*).

Fig. 3.

Criterion 1 of the decision tree. Units were assigned to generic primarylike (Pri) or chopper (Ch) categories based on an analysis of regularity. *A and B*: The CV scores of the four exemplars in Fig. 1 illustrate differences in the regularity of primarylike and chopper units. Classification was based on CV-E scores at stimulus onset (*left inset*). The gray-filled area delineates the ambiguous zone between the two response types. *C*: Histograms comparing the distribution of CV-E scores for Pri and Ch exemplars. Remaining units were categorized as generic chopper or primarylike responses if their CV-E scores coincided with either exemplar distribution. Units with ambiguous scores were not further categorized (NFC). Numerical labels show the location of the four exemplars in *A* and *B*.

Fig. 4.

Criterion 2 of the decision tree. Pri-N subtypes were separated from Pri units by the precision of FSLs. *A*: Temporally expanded dot rasters showing only the first and second spike latencies of the Pri and Pri-N exemplars in Fig. 1. *B*: Histograms of the first and second spike latencies. *C*: The mean and standard deviation of first (filled) and second spike latencies (unfilled) for 9 Pri and 5 Pri-N exemplars. The units have been rank ordered by their JSD scores. *D*: Two-dimensional scatter plot of FSL_{SD} and JSD. Generic Pri units were objectively categorized if their scores fell within regions defined by Pri and Pri-N exemplars. Units with ambiguous scores were not further categorized (Pri-NFC).

Fig. 5.

Criterion 3 of the decision tree. Ch-T were distinguished from generic Ch-S units by non-linear adaptation effects. *A*: Mean ISI functions for the Ch-S and Ch-T exemplars in Fig. 1. The Ch-T unit exhibited rapid adaptation near the beginning of the stimulus. This sharp increase in ISI was quantified by ISI_{AR} and ISI_{MS} statistics. *Shaded regions* indicate time intervals that were used in the calculation of ISI_{AR} . Line segment marks ISI_{MS} . *B*: Two-dimensional scatter plot showing the mutual separation of ISI_{AR} and ISI_{MS} among Ch-S and Ch-T exemplars. Additional generic chopper units were classified as Ch-S or Ch-T if their scores were associated with exemplars. One generic Ch unit resided in an ambiguous region and was not further categorized (Ch-NFC)

Fig. 6.

Criterion 4 of the decision tree. Slowly adapting Ch-SA units were isolated from generic Ch-S units by linear adaptation effects that persisted throughout the stimulus presentation. *A*: Mean ISI functions for the Ch-S exemplar in Fig. 1 and a typical Ch-SA unit. *B*: CV functions for the same units. *Shaded regions* indicate the time intervals that were used to compute ISI_{AS} and CV-L scores. *C*: Two-dimensional scatter plot of ISI_{AS} and CV-L scores for Ch-S exemplars and units that were originally assigned to the Ch-S category because they did not show transient adaptation. Units that were aligned beyond the range of Ch-S exemplars were assigned to the Ch-SA category. Outliers were not further classified (Ch-NFC).

Fig. 7. Objectively categorized units displaying the same response patterns as the Pri, Pri-N, Ch-S, and Ch-T exemplars in Fig. 1. Plotting conventions are described in that figure.

Fig. 8.

Spike waveforms of AVCN units in domestic cats (A) and laboratory mice (B). Individual waveforms of PP1 units show large prepotentials (arrows). PP2 units display a small prepotential that is made visible by the averaging of multiple waveforms. The waveforms of PP3 units do not display a prepotential. PP1 and PP2 responses typically precede monophasic action potentials that were not observed in mice. Waveforms of cats are adapted from Bourk 1976; waveforms of mice show the averages of multiple units with the same PSTH classification. Amplitude scale refers to individual traces from the two units in the leftmost panels of A. Time scale refers to all traces.

Fig. 9.

Basic response properties of physiologically classified AVCN units. *A*: BF thresholds. *B*: Q_{10} values. *C*: Spontaneous rates. *D*: First spike latencies. *Symbols* indicate the physiological classification of individual units. *Numerical labels* identify the four exemplars in Fig. 1. *Dashed lines* describe the best thresholds and average Q_{10} values of auditory nerve fibers (ANF) using data from Taberner and Liberman, 2005.