Progressive Recovery of Cortical and Midbrain Sound Feature Encoding Following Profound Cochlear Neuropathy.

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Progressive recovery of cortical and midbrain sound feature encoding following profound cochlear neuropathy.

A dissertation presented
by
Anna Chambers
to
The Division of Medical Sciences
in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy
in the subject of
Neurobiology

Harvard University
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Progressive recovery of cortical and midbrain sound feature encoding following profound cochlear neuropathy.

To enable the identification and localization of sounds in our environment, auditory brain centers must form representations that accurately encode distinct acoustic properties, but also integrate those properties to support a unified percept of an auditory object. These parallel operations of decomposition and integration are carried out by hierarchically organized processing regions which progressively reformat peripheral electrical impulses into signals that may be integrated into higher order brain circuits. To investigate the nature of these transformations and their vulnerability to hearing loss, I recorded extracellular responses in the auditory midbrain and cortex of awake mice. The first aim of this project was to study the multiparametric tuning characteristics of single neurons using an online stimulus optimization algorithm. Closed-loop stimulus tailoring rapidly revealed diverse multiparametric tuning, and further revealed the conservation of response sparseness between the two areas. I then tracked the recovery of central feature encoding in mice with profound cochlear neuropathy. I recorded from midbrain and cortex at two timepoints after nerve degeneration, observing a progressive recovery of responsiveness in both areas, which occurred earlier and was more robust in the cortex. Concurrently, several aspects of the once-precise temporal response properties in midbrain were persistently degraded, and classification of speech tokens in the cortex did not recover to control levels of accuracy. I hypothesize that compensatory central plasticity may support the recovery of feature encoding in the auditory pathway to a large extent, although various aspects of temporal encoding remain impaired. This may underlie the observation that some human patients with auditory neuropathy have
profound deficits in speech comprehension despite having normal hearing thresholds. Finally, I tested the effect of AUT3, a novel positive modulator of the Kv3.1 potassium channel, on the encoding and classification of pulse trains and speech tokens in the midbrain. I observed that adjusting the excitability of central auditory neurons with this compound can partially restore the precision and reliability of spiking responses after hearing loss.
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General Introduction

Central auditory processing confers onto an individual the capabilities of recognizing the identity of a sound source, localizing the sound source, attributing meaning to the sound, and storing it in memory. The auditory system shares basic functional requirements with other sensory systems, such as the transformation of sensory events into electrochemical signals to be propagated to higher brain centers, and the formation of labelled lines which create conserved topographic maps. However, the auditory system faces unique, modality-specific challenges. Among those challenges, I identify three particular aspects of auditory processing which form the conceptual backbone for the studies presented in this thesis.

First, the auditory system faces the challenge of computing many crucial features of sound stimuli centrally rather than inferring those features from the pattern of stimulation at the level of the sensory epithelium. To overcome this challenge, the auditory system is organized into a processing hierarchy comprised of discrete nuclei capable of performing computations on incoming inputs, as well as transforming electrical signals originating from the periphery into progressively abstracted representations of the original stimulus waveform (Wang, 2009). The transformations that occur between the inferior colliculus (IC) of the midbrain, which represents the first center of major convergence of auditory inputs along the pathway, and the auditory cortex (ACtx), are especially difficult to study because there is not a single type of stimulus which is known to drive auditory cortical neurons particularly well. Further, the manner in which auditory cortical neurons signal tuning preference – with regard to fluctuations in the rate and timing of cortical neuron action potentials – is a disputed topic within the field, subject to different interpretations depending on the experimental preparation, recording technique, and manner of acoustic stimulation (Hromadka et al., 2008, Wang 2005). The first chapter of this thesis attempts to resolve this dispute by utilizing an
unbiased, automated stimulus optimization technique to progressively tailor stimuli to neural preference in the IC and ACtx according to real-time fluctuations in firing rate. I show that the technique rapidly and reliably elicits multiparametric receptive field estimates from single neurons of awake mice in both brain areas, and further reveals fundamental transformations in those representations which may not be revealed by open-loop receptive field characterization.

Second, the auditory system faces the challenge of creating sound feature representations that are robust to the degradation of peripheral inputs. Hearing loss is the most common sensory deficit in humans (Geleoc and Holt, 2014), and has a complex etiology which includes genetic or metabolic disease, acoustic trauma, and aging. A specific type of hearing loss which has received increasing attention in recent years is due to the degeneration of auditory nerve fibers, accompanied by the sparing of cochlear hair cells (Starr et al., 2000). Peripheral neuropathy is often characterized by normal hearing thresholds, however close examination of perceptual capabilities in the clinic reveals a dichotomy in terms of the discrimination of temporal cues versus other types of cues such as sound level (Zeng 2005). In these patients, the representation of temporal fluctuations in sound signals appears to be corrupted, such that performance in some tasks, such as speech comprehension, is severely impaired, while other tasks, such as level discrimination, are spared. As described in Chapter 2, I chemically induced peripheral neuropathy in adult mice and recorded extracellular midbrain and cortical responses from awake animals at discrete time points relative to the induction of neuropathy. I show that compensatory plasticity mechanisms may account for the presence of robust feature encoding in higher brain regions for features encoded by firing rate.

The auditory cortex is a region of the processing hierarchy that transforms precisely timed inputs from lower regions into rate-based abstractions of temporally modulated stimuli. It is unknown to what degree the fidelity of these abstractions depends upon the precision of temporal...
encoding in the brainstem and midbrain, as sluggish ACtx neurons do not directly inherit the time-locked structure of afferent inputs. Results I describe in Chapter 2 suggest that the persistent temporal degradation of signals in the IC may be related to deficits in ACtx encoding of speech stimuli.

Finally, a significant challenge to human auditory processing and perception lies in the current paucity of therapeutic options for suprathreshold hearing impairment. Although a large portion of hearing loss patients benefit substantially from amplification devices such as hearing aids, or cochlear implants which deliver direct electrical stimulation to the auditory nerve, a growing number of studies are devoted to the segment of the patient population unaided by these technologies. For them, an understanding of the central deficits in sound processing, particularly related to the maladaptive changes in cell excitability after peripheral insult, are necessary in order to develop potentially life-changing therapeutic options. In Chapter 3 of this thesis, I describe experiments which test a novel modulator of a potassium channel that is both widely expressed in the auditory system and necessary for proper auditory function, the delayed rectifier potassium channel Kv3.1. Kv3.1 is modulated in its expression level and pattern by acoustic experience, in particular after hearing loss. I show that systemic administration of this compound in mice with profound peripheral neuropathy may provide a temporal encoding benefit in order to functionally supplement the brain’s intrinsic compensatory plasticity observed in Chapter 2.

Taken together, these studies provide a multifaceted perspective on fundamental auditory encoding principles, how they are affected by a common sensory impairment, and how we may intervene to correct maladies in central encoding by adjusting cell excitability, all in the context of an intact, functioning network in vivo.
Chapter 1: Online stimulus optimization rapidly reveals multidimensional selectivity in auditory cortical neurons

*Contributing authors:* Anna R. Chambers, Kenneth E. Hancock, Kamal Sen, Daniel B. Polley

*Author contributions:* ARC and DBP designed optimization algorithm and experiments, KEH implemented stimulus optimization in MATLAB, ARC carried out experiments and analyzed data, KS provided useful analysis tools.
Abstract

Neurons in sensory brain regions shape our perception of the surrounding environment through two parallel operations: decomposition and integration. For example, auditory neurons decompose sounds by separately encoding their frequency, temporal modulation, intensity, and spatial location. Neurons also integrate across these various features to support a unified perceptual gestalt of an auditory object. At higher levels of a sensory pathway, neurons may select for a restricted region of feature space defined by the intersection of multiple, independent stimulus dimensions. To further characterize how auditory cortical neurons decompose and integrate multiple facets of an isolated sound, we developed an automated procedure that manipulated five fundamental acoustic properties in real time based on single-unit feedback in awake mice. Within several minutes, the online approach converged on regions of the multidimensional stimulus manifold that reliably drove neurons at significantly higher rates than predefined stimuli. Optimized stimuli were cross-validated against pure tone receptive fields and spectrotemporal receptive field estimates in the inferior colliculus and primary auditory cortex. We observed, from midbrain to cortex, increases in both level invariance and frequency selectivity, which may underlie equivalent sparseness of responses in the two areas. We found that onset and steady-state spike rates increased proportionately as the stimulus was tailored to the multidimensional receptive field. By separately evaluating the amount of leverage each sound feature exerted on the overall firing rate, these findings reveal interdependencies between stimulus features as well as hierarchical shifts in selectivity and invariance that may go unnoticed with traditional approaches.

Introduction
Sensory neurophysiologists face a dilemma when choosing a stimulus feature space to explore in an experiment. Due to what is commonly referred to as the *curse of dimensionality* (Bellman, 1961), a space that captures the full range of complexities of high-dimensional, naturally occurring sensory events is intractably large, and an exhaustive search of this space is not feasible. Instead, most experimenters choose to vary one or two stimulus dimensions while holding others at fixed, predetermined values. Whereas neurons at each successive stage of the visual cortical hierarchy have been shown to operate on stimulus features of increasing complexity and abstraction, auditory cortical neurons are driven by both simple and complex sound features that vary across all possible derivatives of spectral, temporal, intensive, and dichotic variations in the sound pressure waveform (Wollberg and Newman, 1972; Wang et al., 1995; deCharms et al., 1998; Bizley et al., 2009; Carruthers et al., 2013). Thus, stimulus dimensionality reduction is particularly problematic for studies of sensory coding at higher levels of the central auditory pathway where neurons are driven by a restricted and unpredictable set of sound features within a vast parameter space (Hromadka et al., 2008; Schneider and Woolley, 2011).

Multiparametric stimulus search paradigms that are effective and efficient, yet as unbiased and inclusive as possible, are of great interest to the field (Edin et al., 2004; Benda et al., 2007; Lewi et al., 2011; DiMattina and Zhang, 2013). To this end, a variety of approaches for neural characterization have been described that provide alternatives to probing receptive field organization with discrete, arbitrary stimuli. These procedures aim to fully characterize the stimulus-response relationship via online system identification (Wu et al., 2006; Lewi et al., 2009; DiMattina and Zhang, 2011; Lewi et al., 2011), or are guided by specific aspects of the neural input-output function, such as mutual information (Machens, 2002; Sharpee et al., 2004; Lewi et al., 2011), and spike count (Nelken et al., 1994; Bolinger and Gollisch, 2012).
Inspired by an approach to study complex shape encoding in the visual cortex (Yamane et al., 2008), we implemented a rapid, reliable, and computationally inexpensive stimulus evolution technique to tailor stimulus features to the neural receptive field according to real time variations in firing rate. As applied here, the closed-loop optimization technique aimed to characterize the relationship between firing rate and stimulus optimality in the primary auditory cortex (A1) and central nucleus of the inferior colliculus (ICc) of awake animals. There has been much debate regarding the nature of cortical representations of optimized stimuli, with both sustained, high firing rate responses and low rate, highly precise responses reported in the literature (DeWeese et al., 2003; Wang et al., 2005; Hromadka et al., 2008). We sought to provide a specific test of whether stimulus preference is represented by the disproportionate growth of spiking during the steady-state portion of the neural response. In addition, we used this approach to illuminate whether and how stimulus invariance and selectivity changed across distinct nodes of the sensory processing hierarchy.

Materials and Methods

Surgical procedures. All procedures were approved by the Animal Care and Use Committee at Massachusetts Eye and Ear Infirmary and followed the guidelines established by the National Institutes of Health for the care and use of laboratory animals. Male CBA/CaJ mice aged 8-10 weeks were brought to a surgical plane of anesthesia with ketamine/xylazine (induction with 120 mg/kg ketamine and 12 mg/kg xylazine, with 60-80 mg/kg supplements as necessary). The core body temperature of the animal was maintained at 36.5°C with a homeothermic blanket system. Using a scalpel, a small craniotomy was centered over right primary auditory cortex or right central nucleus of the inferior colliculus, leaving the dura mater intact. The brain surface was covered with sterile ointment. Chronic implants consisted of multichannel silicon probes (177 µm² contact area, 100 µm
contact separation; NeuroNexus Technologies) arranged in a 4X4 configuration mounted onto a bidirectional microdrive (A1) or 16X1 stationary probe configuration (all ICc, some A1). The A1 implant was positioned by first mapping the cortex to delineate the low-high-low caudal-rostral best frequency (BF) gradient that uniquely identifies the orientation of A1 and the anterior auditory field (Hackett et al., 2011; Guo et al., 2012). The ICc was identified based on a low-to-high best frequency gradient along the dorsal-ventral axis of a single shank multichannel probe. A1 and ICc implants were performed in separate groups of mice. Bone wax (3M) was packed around the margins of the craniotomy to protect the probes and brain surface, and the microdrive (for A1 implants) and headstage connector were affixed to the skull surface using acrylic bonding material (C&B MetaBond, Parkell). Ground wires were implanted outside of the auditory cortex and the animal was given post-surgical subcutaneous injections of buprenorphine (0.05 mg/kg) and saline (0.5 cc) to reduce pain and dehydration, respectively. A clear plastic cylinder was affixed to the perimeter of the A1 craniotomy to protect the probes and brain surface.

**Recording procedures.** At least 48 hours after the implant surgery, the animal was placed in a small, acoustically transparent chamber built to be just large enough for its body (3.5 cm X 7 cm). The chamber was tapered at one end to encourage the mouse to face a consistent direction, and was too narrow to allow the mouse to turn its head by more than a few degrees in either direction. The mouse was continuously video monitored during recording to ensure that it was still and its eyes open, and recording was paused if the mouse reared or moved its head position from the target position. Cortical probes were moved slightly (roughly 25 – 50 µm increments) to increase single unit yield between recording sessions. Single units were characterized with the adaptive online procedure if they exhibited a response above spontaneous rate to any of 633 randomly selected
sound stimuli. All neurons tested with the evolutionary procedure were included in the dataset, regardless of efficacy. Raw signals were digitized at 32-bit, 24.4 kHz (RZ5 BioAmp Processor; Tucker-Davis Technologies) and stored in binary format. Single units were isolated online and then further de-noised offline, as necessary, using a PCA-based sorting method in SpikePac software (Tucker-Davis Technologies).

Acoustic stimuli. Stimuli were generated with a 24-bit digital-to-analog converter (National Instruments model PXI-4461) and presented via four free-field electrostatic speakers (Tucker-Davis Technologies) positioned around the mouse restraint chamber. Stimuli were calibrated before recording sessions using a wide-band ultrasonic acoustic sensor (Knowles Acoustics, model SPM0204UD5). Other than the FRAs and STRFs, stimuli were 400 ms in duration with a 600 ms interstimulus interval and 4 ms raised cosine onset/offset ramps.

Simulated receptive fields. For each simulated neuron, five tuning functions were chosen with replacement from a randomly varied set of five tuning 'types':

1. Sigmoid:

\[ F(x) = \frac{1}{1 + e^{-kx}} \]

Where \( k \) was drawn randomly from the interval from -1 to 1.

2. Gaussian:

\[ F(x|\mu, \sigma) = \frac{1}{\sigma \sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \]

Where \( \mu \) and \( \sigma \) were drawn randomly from the interval from 1 to 20 and 1 to 5, respectively.

3. Difference of Gaussians

\[ F(x|\mu, \sigma_1, \sigma_2) = \frac{1}{\sigma_1 \sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma_1^2}} - \frac{1}{\sigma_2 \sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma_2^2}} \]
Where $\sigma_1$ and $\sigma_2$ were drawn randomly from the interval from 1 to 5.

4. **Sum of Gaussians**

$$F(x \mid \mu_1, \mu_2, \sigma_1, \sigma_2) = \frac{1}{\sigma_1\sqrt{2\pi}} e^{-\frac{(x-\mu_1)^2}{2\sigma_1^2}} + \frac{1}{\sigma_2\sqrt{2\pi}} e^{-\frac{(x-\mu_2)^2}{2\sigma_2^2}}$$

Where $\mu_1$ and $\mu_2$ were drawn randomly from the interval from 1 to 20, $\sigma_1$ and $\sigma_2$ were drawn randomly from the interval from 1 to 5.

5. **Flat tuning** (no change in response)

20 samples were taken along each feature dimension. A maximal firing rate, chosen at random from a range typical of A1 single unit firing rates (from roughly 20 to 100 Hz) was selected for each modeled neuron. Tuning functions evaluated at each sample represented the neuron’s fractional response of maximal firing rate (the peak of each tuning function was 1), and the fractional responses at each feature dimension were multiplied together to reveal the firing rate evaluated at that intersection of features (i.e. the predicted firing rate to each stimulus). Spontaneous responses with random noise were also added before calculating the final response magnitude of the model neuron to each stimulus.

*Simulating conventional methods of stimulus search.* For one- and two-dimensional stimulus optimization, three or four stimulus features were held constant at a random, arbitrary value while the remaining dimensions were varied along their entire sampling range (20 samples). Simulated neuron responses were calculated at each sample, taking the values of the other (constant) stimulus dimensions into account. The maximal response from this procedure was then normalized to the response of the neuron to its globally optimal stimulus. To simulate an iterative optimization method we first
ordered the stimulus dimensions arbitrarily for each neuron. Then, starting with the first stimulus dimension, we varied one dimension at a time along its entire sampling range and chose the ‘optimal’ value for that dimension according to the model neuron response, while other, non-optimized dimensions were held at an arbitrary constant (Wang et al., 1995). This response was calculated as described above: each tuning function evaluated at a specific intersection of feature dimensions revealed a fractional response of maximum for each model neuron. The fractional responses were multiplied together to create the final fractional response of maximum, and this number was multiplied by the model neuron’s maximal firing rate to reveal the response to each stimulus. The tested dimension was held at its optimal value and we then iteratively optimized the remaining dimensions until peaks along every dimension were defined. Although this procedure is multidimensional in nature, the stimulus dimensions are varied one at a time rather than simultaneously, and it is non-exhaustive in that most stimulus feature combinations are not tested.

*Evolutionary stimulus search procedure.* A stimulus set varying along five acoustic parameters (center frequency (4 kHz – 64 kHz in 0.1 octave increments), level (10 dB – 60 dB in 10 dB increments), spectral bandwidth (pure tone – 1.25 oct band in 0.25 octave increments), sinusoidal amplitude modulation frequency (unmodulated [0 Hz] – 70 Hz in 10 Hz increments), and speaker location (all permutations of left, right, top, center) yielded a pool of 177,120 potential stimuli. Thus, each individual stimulus was a sinusoidally amplitude modulated sound (unless amplitude modulation of zero happened to be selected) whose carrier was either a pure tone or a band-limited noise of a particular spectral bandwidth. Each run of the adaptive search procedure was initialized with 50 stimuli from this pool, selected at random. Firing rate responses (the average of two repetitions) were calculated during the stimulus window (0-400 ms) and all stimuli played in the experiment thus far were rank-ordered at the end of each generation. At the conclusion of one generation, all
stimulus-evoked spike counts were rank-ordered and the top ten most effective stimuli overall were used as ‘breeders’ for the next generation. The ‘offspring’ for each breeder stimulus was created by randomly shifting one or more acoustic parameters to its nearest-neighbor value (for example, if the level of a breeder stimulus was 40 dB, its offspring could have a level of 30 dB, 40 dB, or 50 dB, as the sampling density for level was 10 dB). After the first generation, 80% of stimuli were chosen randomly from all possible offspring identified by the evolutionary algorithm, while 20% of stimuli were chosen randomly from the entire acoustic feature space to avoid focusing on local maxima and mitigate potential decreases in firing rate response magnitude due to adaptation. The most effective stimulus from the first generation (the ‘yardstick’ stimulus) was repeated in every subsequent generation to estimate the overall response stability across generations. Most A1 single units were subjected to two separate runs of the procedure with independently seeded starting conditions to estimate the reliability of the evolutionary procedure.

Movement artifact rejection. Two criteria were used to safeguard against contamination of neural responses by infrequent movement artifacts. First, a global ceiling on firing rate was set (200 Hz for cortex, 400 Hz for midbrain). Any stimuli corresponding to neural responses exceeding this ceiling were excluded from the dataset. Second, responses to stimuli across two repetitions were compared. If the neural responses to the two repetitions differed by more than 25 Hz, at least one presentation was considered an artifact and the stimulus was excluded from the dataset. In addition, artifacts were removed at the level of primary data acquisition (TDT SpikePac) by employing common mode rejection, wherein activity consistent across all channels is considered non-neural in origin, and subtracted from the neural trace which is used to inform the evolutionary algorithm.
Frequency Response Areas (FRAs). In a subset of single units (n = 12), FRAs were measured to cross-reference the ‘best’ features selected by the adaptive search procedure. FRAs were measured with pseudorandomly presented tone pips (50 ms duration, 4 ms raised cosine onset/offset ramps, 0.5-1 s intertrial interval) of variable frequency (4 – 64 kHz in 0.1 octave increments) and level (0 – 60 dB SPL in 5 dB increments). A total of 533 unique frequency-level combinations were presented twice for a given single unit. FRA analysis was performed as outlined in (Guo et al., 2012)). Briefly, a poststimulus time histogram (PSTH) was calculated with 1 ms bin size, and windowed to identify sound-driven spikes. Response onset was the point at which firing rate exceeded the spontaneous rate by at least 4 SD. The offset was set at the point where firing rate decreased to less than 5 SD above the spontaneous rate. The BF was associated with the highest spike count summed across all sound levels.

Spectrotemporal Receptive Fields (STRFs). In a subset of single units, STRFs were measured for cross-referencing purposes. A 60s long dynamic random chord stimulus was used which consisted of tone pulses (20 ms duration, 5 ms raised cosine onset/offset ramps) randomly and independently chosen from 20 ms bins in time, 1/12 octave bins covering 4 – 64 kHz in frequency and presented from 20-75 dB in 5 dB bins. For each single unit, the stimulus was repeated 20 to 40 times. Automatic Smoothness Determination was used to improve estimates (Sahani and Linden, 2003; Sahani et al., 2013), in which Bayesian techniques are used to obtain optimal spectral/temporal smoothing and scaling parameters for the neural data. In order to estimate a functional transformation of stimulus input which would yield the recorded neural response, a discrete-time Wiener filter is usually utilized with reverse correlation (Aertsen and Johannesma, 1981). However, regression combined with
Bayesian smoothing and scaling parameters avoids the overfitting to noise typical of the Wiener filter. We did not employ a static nonlinearity to determine spike rate from the STRF.

Radial visualization method. The RADVIZ method is a dimensionality reduction technique meant for plotting high-dimensional data onto radial axes (Ankerst et al., 1996). Each stimulus is represented as a point on a radial plane, which is connected to five equally spaced anchor points on the perimeter of the plane by five springs. Spring constants $k$ are determined by the five acoustic parameters of the stimulus. The position of the point is determined by the equilibrium position of the connected springs. The radial plane was superimposed onto a hue/saturation color map so that the position of the point also represented a color.

Sparseness metric. Sparseness (Willmore et al., 2011) of a response distribution was defined as follows:

$$ S = 1 - \frac{E[r]^2}{E[r^2]} $$

where $E[.]$ is the expected value. Using this definition, sparseness equals zero for a dense code and 1 for a sparse code. An intuitive definition for lifetime sparseness would be the peakedness of a response distribution of each neuron, where lifetime sparseness would be high for a neuron that was silent for most stimuli but occasionally exhibited high response rates. For each neuron, a lifetime sparseness metric, $S_t$, was obtained by calculating the sparseness of its responses to all randomly presented stimuli. These lifetime sparseness estimates were then averaged across all $n$ units in the A1 or IC population. For the simulations, sparseness was calculated according to the responses of simulated neurons to 300 randomly chosen stimuli from the five dimensional feature space.
In contrast to the work of Willmore et al. (2011), the stimuli used to elicit responses that went into the lifetime sparseness calculation described here were randomly chosen, and were not tailored to the receptive field of the neuron. Therefore, sparseness values may be skewed upward due to the lack of a prior probability of eliciting a spike, and may not be suitable for direct comparison.

\[
\bar{S}_L = \frac{1}{n} \sum_{i=1}^{n} s_{Li} = \frac{1}{n} \sum_{i=1}^{n} \left[ 1 - \frac{E[r_{ij}]}{E[r_{ij}^2]} \right]
\]

Results

Low-dimensional stimulus optimization results in lower probabilities of maximizing model neuron responses, even when responses are dense.

In order to test the effectiveness of various conventional and adaptive stimulus search methods, we first simulated the responses of generic neurons with tuning across five arbitrary stimulus dimensions (Fig. 1.1). Receptive field properties of the simulated neurons were determined by randomly setting the selectivity and shape of the tuning function (See Materials and Methods) with replacement from the following list: sigmoidal, Gaussian, difference of gaussians, sum of gaussians, and flat (no tuning). Fig. 1.1A shows the five-dimensional tuning for two model neurons that exemplify dense or sparse responses (see Materials and Methods). The tuning functions for each parameter were then combined in a multiplicative manner to create the five-dimensional receptive field of each model neuron.

Non-exhaustive stimulus search was simulated with a conventional strategy in which one or two stimulus dimensions were varied while all others were held at arbitrary constant values (Fig. 1.1B).
1.1B, left and middle). This strategy is common in neurophysiological characterizations in which the representation of a small number of stimulus features is the focus of the study. However, in model neurons with complex, multidimensional feature selectivity and sparse responses—meant to simulate receptive fields in higher order cortical areas—these stimulus search paradigms often fail to capture the full range of responsiveness of the neuron. Even in cases of low sparseness, where neurons are likely to respond robustly to more stimuli, the one- and two- dimensional search techniques are insufficient to maximize model neuron firing rates.

Another method common to neurophysiological experiments is to iteratively optimize multiple stimulus features in a fixed order. Successive low-dimensional optimizations may work well for neurons with certain types of receptive fields, particularly if feature tuning is combined in a linear fashion to determine the neuron’s output. Indeed, this method has been shown to be successful at eliciting sustained responses from auditory cortical neurons (Wang et al., 2005). However, because sound features are not varied simultaneously, nonlinear interactions between stimulus features may be missed. In our simulation, this method was successful in eliciting maximal responses from model neurons with low to moderate sparseness values (Fig. 1.1B), but was not reliably effective for the most sparse responses. Depending on the particular application, neurophysiology experiments often set stimulus values that are not explicitly the focus of the experiment at constant - though not arbitrary - values. The simulations shown here represent a generic data set outside of any particular sensory modality. Nevertheless, they demonstrate that even in cases of low sparseness, where neurons are likely to respond robustly to more stimuli, the one- and two- dimensional search techniques are insufficient to maximize model neuron firing rates.
FIGURE 1.1. Varying more parameters in the search paradigm increases the likelihood of obtaining maximal responses from simulated neurons.

(A) Five tuning functions, chosen with replacement from a list of five possibilities (Gaussian, Sigmoid, Sum of Gaussians, Difference of Gaussians, No Tuning), were assigned to each model neuron to represent the change in response across variations of five arbitrary features. Shown are the five tuning functions for two example model neurons, with differing sparseness. Each ‘feature’ (1-5) represents an arbitrary stimulus dimension, to which the simulated neuron will exhibit the plotted tuning. The type of tuning was chosen from the 5 possible tuning types outlined in the Materials and Methods section. (B) Three stimulus search strategies commonly used in sensory neurophysiology experiments were tested for their efficacy at eliciting maximal responses from model neurons. In the first strategy (1.1B, left), one feature (chosen at random from five total features in the stimulus space) was varied while the other four features were held at an arbitrary constant (randomly chosen for each feature). The same procedure was performed for varying two features (1.1B, middle). Finally, all features were varied iteratively, in a random order (1.1B, right).
Figure 1.1 (continued)

A

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B

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A nearest-neighbor stimulus search strategy successfully optimizes stimuli along five dimensions for simulated neurons.

Given that conventional, low dimensional or iterative search methods could not reveal sparse responses in model neurons, we sought to implement a closed loop, adaptive search technique that would simultaneously vary at least five stimulus dimensions while avoiding null response regions of stimulus space and effectively capturing maximal responses. Beginning with a set of 50 randomly seeded stimuli, model neurons were tested with an evolutionary search procedure meant to resemble processes of natural selection and social behavior (Bleeck et al., 2003, Kennedy and Eberhart, 1995). In each subsequent generation, 10 stimuli were chosen at random and 40 stimuli were chosen based on model neuron feedback, using one of two potential strategies. In the first strategy ('trait swapping,' Fig. 1.2A, left), ‘offspring’ stimuli were created from a combination of traits from two breeder 'parents'. Breeding pairs were selected at random from the top ten most effective stimuli. The ‘genetic penetrance’ was set to 100% for each stimulus dimension, meaning each trait in the offspring was copied from one of the two parent stimuli at random. In the second strategy ('nearest neighbor,' Fig. 1.2A, right), a five dimensional cloud of nearest neighbors to each breeder stimulus was defined, and each offspring stimulus dimension was set to one of the neighboring values for the corresponding stimulus.

After each generation of 50 stimuli, the stimuli were re-ordered to update the ten most effective overall breeders, which were then used to seed the next generation. The simulations ran until the mean response magnitude of the breeder stimuli reached at least 80% of the model neuron's maximal response magnitude. In most runs of the simulation, this was accomplished in 10 generations or fewer. However, the trait swapping approach produced more runs that failed to reach the criterion level of effectiveness within 10 generations (Fig. 1.2B, black lines), indicating that the
algorithm may have perseverated in a local maximum of the simulated neuron’s receptive field. Overall, the nearest neighbor algorithm was able to identify effective regions of the 5-dimensional response manifold more reliably and rapidly as determined from 1000 simulated neurons equally distributed along the sparseness axis ($p < 0.01$, two-sample Kolmogorov-Smirnov test; Fig. 1.2C and D).
FIGURE 1.2. Two closed-loop, evolutionary search strategies identify maximum response regions with differing degrees of efficiency.

A closed loop evolutionary stimulus search was simulated (A) using two different evolutionary algorithms. In both cases, an initial generation of 50 stimuli was randomly chosen from the five-dimensional stimulus space. In subsequent generations, 10 stimuli were chosen at random and 40 stimuli were chosen based on one of the two evolutionary strategies. In the first strategy (‘trait swapping’), pairs of ‘breeder’ stimuli were chosen from the top 10 stimuli of the previous generation, ordered by response magnitude. For each stimulus feature, the child stimulus inherited its trait from one of the two parents, chosen at random (black outline). In the second strategy (‘nearest neighbor’), a five-dimensional cloud of nearest neighbors was defined around each breeder stimulus, and offspring traits were chosen at random from this cloud. (B and C) Mean response magnitude of the breeder stimuli at each successive generation, for 80 runs each of the two search strategies. The algorithm ran until a mean breeder response magnitude of 0.8 or higher was reached. Black lines indicate runs where the criterion was not met after 10 generations. Color scale indicates sparseness. (D) Cumulative probability distribution of the number of generations necessary to reach the criterion for both search strategies, for 1000 runs of each.
Figure 1.2 (continued)
Evolutionary stimulus search rapidly shifts the distribution of firing rate responses of A1 neurons toward maximal observed values.

Having tested our stimulus evolutionary procedure in silico, we then performed extracellular single-unit recordings from the primary auditory cortex of awake mice to confirm its utility in vivo. We populated the sensory feature space with acoustic parameters to which cortical neurons have been shown to display selectivity: carrier frequency, spectral bandwidth, intensity, amplitude modulation rate, and location (Merzenich et al., 1975; Phillips and Irvine, 1981; Schreiner and Mendelson, 1990; Middlebrooks et al., 1994; Read et al., 2001; Bizley et al., 2009; Yin et al., 2011b; David and Shamma, 2013); Fig. 1.3A. Upon permuting across a typical range and sampling resolution, this created a set of roughly 175,000 potential stimuli. When stimuli were selected at random, A1 single unit responses (Fig. 1.3B, top, normalized to peak firing rate observed for the unit) were heavily skewed toward minimal or no response; high firing rates were extremely rare (Fig. 1.3B, bottom and Fig. 1.3C).

Beginning with a randomly seeded set of 50 stimuli, neurons were tested with the nearest-neighbor algorithm of the evolutionary search procedure (Fig. 1.3D). Each stimulus was 400 ms long with 600 ms between trials, such that six generations of the evolutionary search procedure would take 10 minutes. From our simulation experiments, six generations often elicited maximal responses from even the most sparse model neurons (Fig. 1.2C), and ten minutes was well within the timeframe of single unit experiments in vivo. For each subsequent generation of 50 stimuli, 39 offspring evolved from the top ten fittest breeders. Ten stimuli were chosen at random to avoid perseverating in local peaks within the preferred stimulus manifold, and increase the likelihood of arriving at a globally optimal stimulus. A single ‘yardstick’ stimulus was repeated in each subsequent generation to gauge the level of response adaptation. Firing rate responses were averaged across two
repetitions of each stimulus. Fig. 1.3E shows an example of an evolving stimulus subset from one breeder and the progressive growth of sound-evoked spike rate across subsequent generations of its offspring.

Firing rate adaptation has been identified as a potential hindrance to online stimulus optimization (DiMattina and Zhang, 2013). The tendency for neural responses to lose sensitivity for similar stimuli repeated over time could interfere with the adaptive procedure, especially in later generations when the algorithm might have converged on a narrow region of the stimulus manifold. In our experiments, however, the mean firing rate to each generation of breeder stimuli increased (red line, Fig. 1.3F) despite the concurrent growth of response adaptation (blue line, Fig. 1.3F), reflected in the tendency of neuronal firing rate to decrease over repeated presentations of the ‘yardstick’ stimulus. This trend was observed in the group data, where responses to the same stimulus adapted over generations (Repeated measures ANOVA, F(5,50) = 26.77, p < 0.001; Fig. 1.3G, blue line) and there was a significant increase in responses to breeder stimuli over generations (F(5,50) = 173.1, p < 0.0001; Fig. 1.3G, red line), while responses to randomly selected stimuli did not change over generations (F(5,50) = 0.73, p = 0.6; Fig. 1.3G, black line).
(A) The relevant stimulus space was constructed using five basic acoustic features, shown in the thumbnails from their minimum (left) to maximum (right) values in the experiment. A cartoon shows the location of the four speakers relative to the animal's head. For location (right bottom), presence of a blue line indicates an active speaker. (B) Example SU recording trace from A1. (Bottom) Distribution of normalized firing rates of A1 single units (n = 50) to all randomly chosen stimuli from a 5-dimensional feature space. (Top) (C) Example responses of one A1 unit to 50 randomly chosen stimuli. Each thumbnail represents one stimulus: spectrogram represents frequency content (y-axis), temporal modulation (x-axis) and level (brightness). Blue lines represent location of speakers playing the stimulus: left, top, right and center speakers (clockwise from left side of thumbnail). Grayscale background indicates the unit firing rate to the corresponding stimulus. Thumbnails are rank-ordered by firing rate response. (D) Cartoon of closed-loop stimulus search procedure, adapted from Benda et al (2007). (E) Example of a breeder stimulus, initially selected at random (top thumbnail, same schematic as in (C), that evolved to elicit higher firing rates in subsequent iterations (bottom 3 rows) by adjusting features to nearest neighbor values. (F) Distribution, per generation (from top to bottom, generations 1-6) of normalized firing rate responses of an A1 single unit to randomly chosen (grey bars) versus evolving (black bars) stimuli. Elapsed time is indicated to the right of each histogram. Top histogram is the first generation, comprised entirely of randomly chosen stimuli. Dashed lines indicate mean firing rate responses to random (black), breeder (red), and yardstick (blue) stimuli, per generation. (G) Mean normalized fitness (firing rate) ± SEM per generation for randomly chosen stimuli, breeder stimuli (top 10 stimuli), and yardstick stimuli (top stimulus from first generation, repeated in each subsequent generation). Despite significant adaptation of firing rate to effective
stimuli (blue line), fitness of the breeder stimuli increases across generations (red line) and remains significantly higher than randomly chosen stimuli (black line).
Figure 1.3 (continued)
The evolutionary procedure consistently converged onto an effective stimulus set across separate runs initialized with distinct random stimuli.

In a subset of neurons (n = 31), the evolutionary procedure was run twice in order to verify convergence onto a reliably effective stimulus subset. A radial visualization technique (see Materials and Methods) assigned each stimulus a color based on its five parameter values (Fig. 1.4A). Stimuli from two runs of the procedure for each of three example A1 single units are ranked according to firing rate (Fig. 1.4B-D). Each bar represents a single stimulus whose height and color represent its firing rate response and stimulus attributes, respectively.

These examples convey three important points about the evolutionary procedure and its effectiveness. First, color converges strongly toward the right side of each bar plot, indicating that within each run, maximum firing rates tended to be elicited by very similar stimuli. Second, color convergence between two consecutive runs that were initially seeded with independent, random stimuli converged onto highly similar effective stimuli, indicating that the evolutionary procedure was reliable at identifying highly effective regions of the receptive field for each neuron. Third, performing separate runs with highly similar convergence confirmed that instead of focusing on local maxima, the procedure truly converged on regions of receptive field space that reliably drove A1 neurons at maximal firing rates.

We quantified the degree of convergence by independently correlating the averaged parameters of the top ten stimuli from each of two runs (Fig. 1.4E). Significant correlations are noted for the comparison of each stimulus property (r > 0.43 and p < 0.05 for all; as a categorical variable, stimulus location was not amenable to parametric correlation).
FIGURE 1.4. Adaptive stimulus search reliably converges on consistent areas of feature space across runs initialized with distinct pedigrees.

(A) Individual stimuli were plotted onto a circular hue/saturation map using the radial visualization technique (see Materials and Methods) according to the values of the five stimulus parameters, shown as equally spaced anchor points on the color map. The position of each stimulus determines its color. (Right circle) The most ‘fit’ stimulus (in terms of firing rate) from each of 78 runs of A1 single units are plotted onto the circular map. (B-D) Example sets of stimuli from two separate runs of three individual A1 single units. Each stimulus is represented by a vertical bar, and bars are rank-ordered on the x-axis by firing rate response, which is also represented by the y-axis height (most effective stimuli appear to the right of each plot, and are tallest). Color represents stimulus features, therefore more similar colors are more closely spaced on the hue/saturation map with radial visualization. Plots show (1) convergence to a set of effective stimulus features, and (2) stable convergence to the same features across two separate runs initialized with distinct randomly chosen stimuli. (E) Correlation of best features determined from two separate runs of the adaptive search procedure from the same A1 single unit. The most effective value across all parameters (shown in separate plots) are determined by taking the mean value of the top ten stimuli from each iteration of the adaptive search procedure.
Figure 1.4 (continued)

A

B

C

D

E

Preferred center freq (kHz)  | Preferred level (dB SPL)  | Preferred sAM freq (Hz)  | Preferred spec. BW (oct)
--- | --- | --- | ---
run 2  | run 2  | run 2  | run 2
4  | 8  | 10  | 1.25  
6  | 16  | 35  | 1  
8  | 32  | 60  | 0.5  
10  | 64  | 60  | 0.5  

$r = 0.8843$  
$p < 0.01$

$r = 0.7315$  
$p < 0.01$

$r = 0.4372$  
$p < 0.05$

$r = 0.6637$  
$p < 0.01$
The stimulus search procedure shows agreement with other well-tested methods, but allows for simultaneous testing of variation along many feature dimensions.

Stimulus preference defined by the evolutionary procedure (Fig. 1.5A) was cross-validated against pure tone receptive field estimates (Fig. 1.5B) and the spectrotemporal receptive field (STRF) obtained with a dynamic random chord stimulus (deCharms et al., 1998; Linden et al., 2003) (n = 20, Fig. 1.5C). We observed a substantial agreement in preferred frequency between the FRA, STRF, and evolutionary search (Fig. 1.5A-C), across the sample (FRA/ES: r = 0.98, p < 0.01; STRF/ES: r = 0.96, p < 0.01; Fig. 1.5D). Importantly, though, multidimensional search consistently elicited higher peak firing rates than a pure tone at the best frequency for every neuron tested in A1 and the ICc, an auditory midbrain structure (Fig. 1.5E).

A subset of neurons (n = 20) was subjected to a subsequent test in which a single stimulus parameter was varied while the other four were held at their optimal value, as determined from the evolutionary search procedure (Fig. 1.5F). This procedure revealed the leverage of each individual sound dimension on the firing rate of the neuron. As the example in Fig. 1.5F illustrates, A1 neurons may display band-pass, low- or high-pass selectivity across multiple feature dimensions, demonstrating the utility of unbiased search strategies. Our experiments were conducted using a discrete stimulus space, therefore a neuron with a monotonically increasing tuning function, such as that seen to center frequency in Fig. 1.5F, did not undergo further optimization in order to reveal a globally optimal center frequency. Rather, the preferred frequency would be defined as the highest point in the discrete tuning function. A glyph plot (Fig. 1.5F, bottom right) was constructed from this unit where each spoke represents the normalized difference in firing rate between the peak and the trough of the tuning function across each parameter. Glyph plots of all A1 single units subjected to this procedure are shown in Fig. 1.5G. The heterogeneity of spoke lengths relative to other...
spokes in each glyph as well as other glyphs in the population further illustrate the complex interplay of combinatorial sensitivity and tolerance to multidimensional stimuli.
FIGURE 1.5. Adaptive stimulus search performs well against conventional methods, but allows for unbiased search across more stimulus dimensions.

(A) Scatter plot of firing rate versus center frequency (kHz) of all stimuli from one run of the evolutionary procedure in an example neuron. Stimuli were varying across four other dimensions not plotted here. Red line represents a 4th order polynomial fit of the mean firing rate across frequency. Red arrow indicates the preferred frequency. (B) A frequency-response area measurement from the same neuron as in A. Red arrow indicates the best frequency of the FRA (C) An STRF measurement from the same neuron as in A and B. Red arrow indicates best frequency, which closely corresponds to values obtained from the FRA measurement and evolutionary search. (D) Summary of concordance between the best frequency obtained with the evolutionary procedure versus the FRA (red) and the STRF (blue) (n = 12). (E) Peak firing rates from A1 (blue) and ICc (red) were calculated as the peak 50-ms response bin across all stimuli presented. Open circles represent units that did not display a significant response above spontaneous rate to any pure tones, however were responsive to one or more stimuli from the evolutionary search procedure. (F) Example one-dimensional tuning functions from a single A1 neuron where each dimension of the preferred stimulus (obtained from the evolutionary search procedure) was varied at a time while others were held constant. Filled and unfilled arrows indicate the actual peak and the optimized value obtained for that parameter in evolutionary search, respectively. Glyph plot represents the difference in firing rate between the maximum and minimum values of each one-dimensional tuning function, meant to estimate the leverage of each stimulus dimension on the maximal response of the neuron. (G) Glyph plots constructed from all A1 neurons subjected to one-dimensional variations of the preferred stimulus. Lines indicate same parameters as schematized in Fig 1.5F (n = 20).
Figure 1.5 (continued)
A1 neurons display a sparse code across some acoustic dimensions, with a dense code across others.

As shown in Fig. 1.6A, only variations across center frequency, and to a lesser extent location, could modulate the optimized stimulus response of A1 units from maximal to minimal firing rates. Other stimulus features modulated the firing rate by about 50% on average (significant differences across parameters indicated with horizontal lines; paired t-test, with Bonferroni correction, significance level is $p < 0.005$), indicating that spike rate remained relatively invariant across large variations in multiple parameters. This was somewhat surprising given that neural codes have been theorized to maximize efficiency such that fewer metabolically expensive action potentials would ideally be utilized to represent stimuli (a sparse code) (Barlow, 1961; Olshausen and Field, 1996). As an extension of these ideas, in a system where stimulus representation is progressively less linear as one ascends the processing hierarchy, at each stage the most efficient stimulus representation would be increasingly sparse. This hypothesis has been made in the visual (Willmore et al., 2011) and auditory (Wang, 2007; Atencio et al., 2012) systems.

Experimental evidence indicated, however, that in the visual system sparseness may instead be balanced between different stages of the sensory processing hierarchy. This preservation of sparseness can be attributed to balanced increases of tolerance and selectivity across identity-preserving and identity-determining variations, respectively (Rust and DiCarlo, 2012). Consistent with the observation that intensity invariance, though not commonly seen in subcortical areas, is present in A1 single units (Sadagopan and Wang, 2008), we observed that the fractional change in peak firing rate ($(\text{peak-trough})/\text{peak}$) across level variation was lower in A1 than for ICc neurons, indicating a higher tolerance of A1 maximal responses to variations of intensity (Fig. 1.6B). Further, we calculated the number of standard deviations between the peak of the tuning functions and the
mean (z-score) as a proxy of selectivity and found that center frequency was associated with higher z-scores in A1 than ICc (paired t-test, p < 0.01) —consistent with sharper frequency tuning observed in primate A1 (Bartlett et al., 2011) (Fig. 1.6C). As in the visual system, the shifting balance of selectivity and tolerance for stimulus features in two hierarchically related brain areas was associated with no net change in lifetime sparseness (see Experimental Methods) between ICc (n = 12) and A1 (n = 50) units (Fig. 1.6D,E).

Although overall responses were not significantly more sparse in A1 than ICc, a prominent hypothesis for sound coding at higher levels of the central auditory pathways holds that the sustained manner of stimulus-evoked responses, rather than the overall rate is indicative of stimulus preference (Wang et al., 2005; Wang, 2007). Namely, responses in A1 single units in awake animals are theorized to systematically shift from phasic (onset) to tonic (sustained) firing (Fig. 1.6F) as the stimulus is progressively fit to the most effective regions of the multidimensional receptive field. We therefore organized A1 neural responses into an onset portion (0-50 ms) and a sustained portion (51-400 ms) to gauge potentially independent effects of stimulus optimality on firing rate and sparseness during the two epochs. We hypothesized that, if the aforementioned model were correct, the steady-state portion of the response would grow disproportionately as stimuli more closely approximated the preferred region of the multidimensional receptive field.
FIGURE 1.6. The evolutionary stimulus search procedure allows the observation that stimulus preference is not disproportionately indicated by a tonic response in awake A1, and A1 units are not sparser than IC units.

(A) Mean (± SEM) normalized peak minus trough firing rates across A1 units (n = 20) for 1-D tuning functions in which one parameter (on x-axis) was varied while other parameters remained at optimized values. Black lines indicate significant differences between indicated groups (p < 0.005, paired t-test, with Bonferroni correction for multiple comparisons). (B) Same as in A, but for IC units (n = 8). (C) Z-score of center frequency and intensity tuning functions of IC and A1 neurons. (D) Cumulative probability distribution of normalized firing rates in A1 and IC to randomly selected, five dimensional stimuli (A1 n = 50, IC n = 12). (E) Lifetime sparseness (median ± IQR) of A1 and IC neurons to 100 randomly selected, five-dimensional stimuli. (F) Example raster plots of phasic and tonic responses from cortical single units. (G) Distribution of firing rate responses (black line) from one example A1 neuron (76 stimuli, obtained by starting at the preferred stimulus from evolutionary search and varying one parameter at a time along its relevant range). Colored boxes indicate quartiles according to proportion of maximal response. (H) For A1 neurons, mean normalized PSTHs to stimuli falling within each quartile of the response distribution (indicated by color corresponding with Fig 4C, n = 20). PSTHs are normalized to the peak 20 ms bin for each neuron. (I) Onset (0-50 ms) versus sustained (51-400 ms) firing rate (mean, normalized) to stimuli in each quartile of the response distribution in A1. Mean ± SEM along each axis indicated in black outlined circles. (J) Lifetime sparseness (median ± IQR) of A1 units in response to 100 randomly chosen five-dimensional stimuli using only the onset (left) or sustained (right) portions of the response.
Figure 1.6 (continued)
Contrary to the model’s prediction, we found that onset and sustained firing rates tended to increase together across the population of recorded neurons (Fig. 1.6G-I), as shown in the population PSTHs in response to progressively optimized stimuli (Fig. 1.6H; colors indicate which quartile of the stimulus-response function for each neuron contributed to the PSTH, one example of this function for an A1 neuron is shown in Fig. 1.6G). We found no evidence indicating disproportionate increases in the steady-state epoch of the response (paired t-test, p>0.05 for all quartiles). As expected, sparseness values were not significantly different when the firing rate was calculated only from the onset or sustained epoch (Fig. 1.6J). Maximum overall firing rates for all A1 single units are shown in the histogram in Fig. 1.7A. The same results were observed in the ICc (paired t-test, p > 0.05 for all quartiles; Fig. 1.7B-C). For direct comparison with previous findings in marmoset A1, we plotted the onset response index, or the ratio of the onset to sustained response, for the preferred and non-preferred stimuli of A1 and IC neurons (Fig. 1.7D). Our results indicate that the transition from the non-preferred to preferred receptive field was not accompanied by a shift from onset to sustained firing.
FIGURE 1.7. A shift from phasic to tonic firing is not seen in single units recorded from awake mouse A1 and ICc.

(A) Maximum firing rates (sp/s) for all A1 neurons. (B) For IC neurons, mean normalized PSTHs to stimuli falling within each quartile of the response distribution (indicated by color corresponding with Fig 4G). PSTHs are normalized to the peak 20 ms bin for that unit. (C) Onset versus sustained firing rate (mean, normalized) to stimuli in each quartile of the response distribution in IC. Mean ± SEM along each axis indicated in black outlined circles. (D) Onset Response Index (ratio of onset to sustained responses; ORI) plotted for A1 and IC neurons, where the optimal stimulus was found via the evolutionary procedure and each stimulus parameter was varied individually along its entire sampling range. “Preferred” stimuli were those that fell within the top quartile of response magnitude, “non-preferred” were those that fell within the bottom quartile but still exhibited responses above spontaneous rate.
Figure 1.7 (continued)
Discussion

Early stages of auditory processing feature a variety of biophysical and synaptic specializations that allow neurons to precisely synchronize action potential timing to spectral, temporal, and dichotic features within the acoustic source signal. As afferent activity is propagated through the central auditory neuroaxis, the functional organization changes to support more integrative and contextual processing. At the level of the auditory cortex, the high-fidelity representations from earlier stages have been almost entirely reformatted to rate-based abstractions of the original signal (Wang, 2007). This shift can be attributed, in part, to the emergence of higher-order neurons that behave more like nonlinear feature detectors than generalized spectral and temporal analyzers (Young et al., 2005; Wang, 2007; Nelson and Young, 2010; Schneider and Woolley, 2011; Atencio et al., 2012; Schinkel-Bielefeld et al., 2012; Yaron et al., 2012). Much can be learned about sensory encoding from the exhaustive characterization of neural tuning to isolated acoustic attributes. However, the approach has its limitations in terms of constructing a comprehensive and unifying theory of receptive field structure in higher-order auditory areas. The approach described here, in keeping with an emerging body of literature on sensory stimulus optimization, facilitates the adoption of unbiased stimulus selection procedures by relying on easily implemented and theoretically intuitive design principles.

Besides a genetic algorithm (Bleeck et al., 2003; Yamane et al., 2008; Hung et al., 2012), other methods have been described for firing rate maximization, such as local hill-climbing or gradient search (O'Connor et al., 2005; Foldiak, 2011; Koelling and Nykamp, 2012), as well as simplex search (Nelken et al., 1994). A potential benefit of the genetic algorithm is that rather than updating the position of a single point, or simplex of points, in stimulus space, the algorithm used here allows the
exploration of diverse regions of feature space by optimizing a group of breeder stimuli as well as incorporating random ‘mutations’ into each generation. These features make the search strategy less likely to perseverate in local firing rate maxima. In addition, some stimulus parameters may not be well defined along a gradient, and may be better suited to an evolutionary search or other similar strategies, such as particle swarm optimization (Kennedy and Eberhart, 1995).

The evolutionary procedure allows the experimenter to obtain information about stimulus selectivity across at least five separate acoustic feature dimensions, whereas traditional methods typically vary only across a frequency/intensity or spectrotemporal stimulus space, and force other potentially relevant parameters to an arbitrary constant. In addition to variations of amplitude-modulated pure tones and band-limited noise in order to maximize firing rate, the evolutionary approach is flexible enough to accommodate a discrete or continuous feature space in which complex stimuli such as vocalizations could be used to optimize not only firing rate, but any number of characteristics of the neural response. Further, the approach does not assume a linear stimulus-response function. These qualities highlight potential advantages of a genetic optimization algorithm over traditional techniques such as the STRF (Aertsen and Johannesma, 1981; Klein et al., 2000), typically defined as the best linear fit between a spectrogram of the stimulus and the evoked spike rate.

Response maximization was explored in this study in order to describe the relationship between stimulus preference and firing rate in A1 single units. However, with the evolutionary optimization approach, the stimulus manifold is sampled sparsely and unevenly as compared to STRF estimates derived from continuous, dynamic, and uncorrelated stimuli such as ripple noise (Escabi and Schreiner, 2002). In addition, the online characterization used here did not generate a testable model of the receptive field organization, as has been described in a recent conference.
abstract (Feng et al., 2012) or other methods that maximize the mutual information between the stimulus and the response (Machens, 2002; Machens et al., 2005; Nelken et al., 2005) to characterize a maximally informative stimulus distribution (Sharpee et al., 2004). Along similar lines, other studies have used principles of information theory to adaptively optimize the expected information gain from the parameters of a receptive field model and the stimulus (Paninski, 2005; Paninski et al., 2007).

Although the model neurons on which the closed-loop procedure was initially tested exhibited a clearly defined maximum response, not all sensory neurons display this property. For example, neurons could be modeled with increasing responsiveness along a stimulus dimension such that the optimal stimulus can only be described by subjecting the response to a power constraint (Berkes and Wiskott, 2006). Indeed, some A1 neurons in our study exhibited monotonically increasing tuning functions across at least one stimulus parameter (Fig. 1.5F), and their preferred stimulus features was not adequately assessed using the discrete stimulus space chosen for the study. Yet others may display plateaus or saddle shapes in their tuning functions which render multiple stimuli along particular feature dimensions as eliciting a peak response (DiMattina and Zhang, 2013). Therefore, in practice it is not necessarily useful to speak of only one ‘globally optimal’ stimulus. Rather, a major benefit of online optimization is that it allows the experimenter to define a large and diverse stimulus parameter space without making assumptions as to which parameters may exert the most leverage over the neural response.

The procedure described here was able to rapidly (10 minutes for our paradigm though the exact time will vary depending on the stimulus space and brain region studied) optimize five-dimensional stimuli for cortical and midbrain neurons, which is well within the timeframe of a typical single unit recording in an unanesthetized animal. However, it is likely that some
experimenters may wish to increase the dimensionality of their stimulus space or perform a more exhaustive search of the space, necessitating longer recording times. Therefore, the procedure could be a starting point for a more exhaustive, open-loop receptive field characterization, or alternatively could be expanded to more fully delineate the topology of a higher dimensional feature space, rather than just its peak.

Through the use of adaptive, multidimensional stimulus search, we have shown that different acoustic properties have distinct amounts of leverage over the firing rate response to an optimized stimulus. Further, the leverage of some properties, such as intensity, can be related to the stage of the processing hierarchy from which neural responses were obtained (Fig. 1.6). Side-by-side comparisons of tuning to multiple features allow the experimenter to see relationships, such as the interplay between tolerance and selectivity, which may underlie broader neural coding principles in a sensory system, such as the preservation of sparseness along a processing hierarchy. In the auditory system, the fact that both phasic and tonic responses have been observed to varying degrees in A1 has kept the field in disagreement regarding the relationship between neural preference and firing mode (DeWeese et al., 2003; Wang et al., 2005; Hromadka et al., 2008; Qin et al., 2009). Further discrepancies have been noted in the experimental techniques utilized across A1 studies – sparse representations have been observed using cell-attached recordings, while higher firing rates are typically elicited with extracellular recordings. Hromádka and Zador, using cell-attached recordings, have reported a diverse array of responses across A1 cells, including sustained responses. It is possible that a subset of A1 neurons does, in fact, shift its firing mode from phasic to tonic with stimulus optimization, but for practical purposes the entire acoustic space remains experimentally intractable, making ‘optimization’ a relative and incomplete term. However the efficient and reliable approach described here provides reasonable evidence indicating that neural preference in mouse A1 is not necessarily marked by a shift from phasic to tonic firing (Fig. 1.6).
Sensory neuron firing rates are often used as a read-out for experimental manipulations such as behavioral training, overexposure or deprivation. With conventional methods, changes in feature selectivity to the manipulated parameters are studied in relative isolation, however multidimensional search allows for feature selectivity to be observed within the context of a complex, and potentially more naturalistic, stimulus. Further, the ability to study neural responses outside of the typical constraints of stimulus selection creates opportunities to test principles of neural encoding which may be consistent across sensory systems dealing with varying degrees of stimulus complexity. Heterogeneity in cortical responses has, for the most part, functioned as a hindrance to the understanding of sound representations. By working towards automated methods for auditory experiments, the complex and unpredictable nature of neural responses in cortex can instead be a valuable asset for delineating the neural mechanisms that support auditory perception.
Chapter 2: Compensatory gain control partially recovers sound feature encoding in midbrain and cortex of mice with profound cochlear neuropathy

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Author contributions: ARC and DBP designed experiments, ASE and YY provided reagents and technical assistance, MCL performed cochlear histopathology and analysis, JPW provided speech stimuli and classifier model code, ARC performed all other experiments and analysis.
Peripheral deafferentation occurs routinely in the auditory system as a consequence of acoustic trauma, ototoxic insult, or aging. Central neurons corresponding to deafferented regions of the cochlea exhibit changes in sound-evoked tuning, wherein Hebbian mechanisms guide a shift in central responsiveness toward intact regions of the periphery. In addition, central neurons maintain activity levels based on non-Hebbian homeostatic mechanisms involving synaptic or intrinsic changes to compensate for reduced sensory drive. The degree to which these central compensatory processes are involved in recovery following hearing loss, as well as their consequences for perception, are a topic of great interest in the field both from a clinical and basic research perspective. We recorded from the inferior colliculus (IC) and primary auditory cortex (A1), in awake mice after at least 90% of Type-I auditory nerve fibers were eliminated unilaterally via administration of ouabain, a Na\textsuperscript{+}/K\textsuperscript{+} ATPase pump inhibitor. Thresholds for brainstem evoked potentials were substantially elevated. However, behavioral detection thresholds were near normal, indicating that neural activity at higher stations of the auditory pathway could support perception. We recorded central representations of tones and broadband pulse trains in order to address a three-fold hypothesis: First, we predicted that the rapid expansion of representations of the normal auditory nerve would occur in addition to a slower potentiation of the remaining inputs from the profoundly degenerated nerve. Second, we predicted that the latter potentiation would occur to a greater extent in A1 than IC. Third, we predicted that this plasticity would support the recovery of a spike rate code for sound features. We found that unit thresholds were lower than those predicted from brainstem metrics, indicating a central compensatory plasticity. A1 evoked firing rates with contralateral stimulation were preserved at near normal levels. Finally, using vector strength and
classifier models to test the integrity of temporal encoding in IC and A1, the neurophysiological and behavioral categorization of rapidly modulated sounds was impaired while rate encoding mechanisms recovered to a greater extent over time. These studies form a conceptual framework for understanding the contributions of competitive and non-competitive plasticity to recovery from hearing loss, as well as the limits of perceptual ability that can be preserved via the brain’s intrinsic recovery mechanisms.

**Introduction**

The central nervous system displays an incredible capacity for network-level and cell-intrinsic changes to compensate for peripheral damage. In the auditory system, some degree of sensory disruption via disease, noise trauma, aging, or ototoxic drugs is extremely common in the human population. It is estimated that for individuals 12 years of age or older in the United States, nearly 1 in 5 has some degree of bilateral or unilateral hearing loss (Lin et al., 2011). Widely utilized avenues for hearing loss treatment currently consist of amplification of incoming acoustic stimuli with hearing aids, or electrical stimulation of auditory nerve fibers with cochlear implants (Eshraghi et al., 2012). These devices have proven enormously successful for a large portion of hearing loss patients, but therapies for individuals who exhibit normal audibility in quiet, but difficulty in certain tasks such as speech comprehension, are currently inadequate (Giraudet and Avan, 2012; Geleoc and Holt, 2014), largely due to a lack of understanding of the way in which specific peripheral insults affect the central encoding of sound features.

Attention in the research community has recently focused on suprathreshold hearing impairment in patients with some degree of peripheral nerve fiber loss. Auditory nerve fiber
degeneration can occur as a normal consequence of aging, as well as in cases of genetic disorder, metabolic disease, ototoxic drugs, and noise exposure (Starr et al., 2000; Michalewski et al., 2005; Bharadwaj et al., 2014). Interestingly, behavioral characterization of neuropathy patients have shown that despite exhibiting normal audibility in quiet, patients have a dramatically reduced capacity for hearing in challenging conditions. When tested on perceptual tasks in a laboratory setting, certain aspects of sound feature discrimination, such as sound level discrimination and sound localization with interaural level cues, are intact at normal or near-normal levels. However, perceptual tasks involving the discrimination of temporal cues, such as gap detection, slow and fast temporal modulation discrimination, and sound localization with interaural timing difference cues, are severely impaired even with the aid of amplification (Kraus et al., 2000; Rance et al., 2004; Zeng et al., 2005). Further, neuropathy patients have particular difficulties with speech comprehension.

The first aim of the study described here is to shed light on mechanisms which allow for intact, normal threshold audibility in patients with such profound auditory nerve fiber degeneration as to entirely degrade the auditory brainstem response (ABR) signal. To do so, Type 1 spiral ganglion neuron degeneration was induced unilaterally in mice with ouabain, a Na\(^+\)/K\(^+\)-ATPase pump inhibitor which, when applied at the round window at a concentration of 1mM, spares cochlear hair cells, leaving cochlear amplification mechanisms intact (Schmiedt et al., 2002; Lang et al., 2005; Yuan et al., 2014). Recordings from the midbrain and cortex of these mice one week and one month after ouabain treatment revealed progressive central compensatory plasticity that restored low threshold, frequency-tuned responses in both brain areas at one month post-treatment. This recovery was more prominent in the cortex, where many aspects of sound feature encoding appeared normal at the one month time point. These results are in agreement with an emerging body of literature studying the phenomenon of central gain enhancement after peripheral insult in
the auditory system (Sun et al., 2008; Sun et al., 2012; Chen et al., 2013; Niu et al., 2013; Chen et al., 2014).

The second aim of the study is to investigate the underlying causes of specific impairments in temporal processing in humans with severe auditory nerve fiber degeneration. One model of temporal degradation of neural responses after nerve fiber loss states that since recruitment and convergence of stochastic nerve fiber responses to temporally modulated stimuli enhance temporal synchronization in the cochlear nucleus, a smaller number of available fibers would render brainstem synchronization significantly degraded (Zeng et al., 2005). The results of the first aim built a hypothesis wherein central compensatory plasticity, though capable of regulating and enhancing firing rate responses to sound stimuli after peripheral insult, did not in turn compensate for temporally degraded brainstem responses. Experiments in the second aim tested whether midbrain multiunit responses, which in normal hearing animals exhibit well-synchronized, reliable responses to broadband pulse trains up to approximately 300 Hz would show any impairment even as thresholds progressively recovered to normal levels. Further, speech classification tested at the level of the auditory cortex aimed to investigate whether poorly timed midbrain responses would coexist with any deficits in cortical encoding of complex, temporally modulated stimuli.

Results from the second aim showed that although some recovery of midbrain synchronization to rapidly modulated stimuli was observed, overall levels of synchronization as well as spike timing variability and adaptation to repeated pulses were persistently degraded in both the one week and one month time points after ouabain treatment, despite a concurrent recovery (and enhancement in cortex) over time of sound level encoding and tone frequency encoding. Further, a persistent deficit in cortical classification of human speech tokens was observed, consistent with studies from other groups showing deficits in speech token encoding by cortical responses after
noise exposure. Taken together, these results suggest that although central compensatory plasticity displays a remarkable capacity to restore normal or even enhanced representation of sound features over time, the precision of timing of central auditory responses after nerve fiber loss still poses a significant problem for higher auditory processing of temporally complex stimuli, potentially underlying the perceptual deficits seen in humans with this condition.

**Materials and Methods**

*Animals and Ouabain Application:*

All procedures were approved by the Animal Care and Use Committee at Massachusetts Eye and Ear Infirmary and followed the guidelines established by the National Institutes of Health for the care and use of laboratory animals. All experiments were performed on male CBA/CaJ mice, 8-10 weeks old. For near complete unilateral cochlear denervation, a 1 mM solution of ouabain octahydrate (Sigma) and sterile water was applied to the round window niche, as described previously (Lang et al., 2005; Yuan et al., 2014). Animals were anesthetized with ketamine (120 mg/kg, IP) and xylazine (12 mg/kg, IP), with half the initial ketamine dose given as a booster when required. A post-auricular incision was made and connective tissue as well as underlying muscle and the facial nerve were separated to reveal the bulla via blunt dissection and held in place with small animal surgical retractors (Fine Science Tools). A small opening was made in a thin section of the bulla with the tip of a 28.5-gauge needle. The ouabain (1-2 ul, 1mM in sterile water) was applied using the same 28.5-gauge needle with the sharp end cut off, attached to a 0.5-cc syringe. The ouabain was applied at 15 minute intervals, 6 times (clearing the solution at each interval with
absorbent paper points before reapplying) for a total of 90 minutes of exposure time. The muscle and connective tissue were replaced over the bulla opening and a preliminary DPOAE and ABR measurement (see subsequent Methods) was made to confirm functionality of outer hair cells and expected ABR threshold shifts as a result of the ouabain application. The incision was sutured and the animal was given Buprenex as an analgesic before being transferred to a warm cage. All ouabain treated animals underwent additional DPOAE and ABR testing one week after the procedure, with the untreated ear serving as the control.

*Cochlear Function Tests:*

Auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) were recorded as previously described (Chambers et al., 2012; Yuan et al., 2014). Mice were anesthetized with ketamine and xylazine, and placed on a homeothermic heating blanket during testing. ABR stimuli were 5-ms tone pips (pure tone frequencies tested: 8, 16 and 32 kHz) with a 0.5 ms rise-fall time delivered at 30/s. Sound level was incremented in 5-dB steps, from 20 dB to 80 dB sound pressure level (SPL). Threshold for ABR was defined as the lowest stimulus level at which a repeatable morphology could be identified in the response waveform. DPOAEs were recorded for primary tones with a frequency ratio of 1.2 and with the level of the f2 primary 10 dB less than f1 level, incremented together in 5-dB steps. The 2f1-f2 DPOAE amplitude and surrounding noise floor were extracted.

*Tissue Processing and Immunostaining:*
Tissue processing was carried out as described previously (Yuan et al., 2014). Briefly, cochleae were dissected and perfused through the round window and oval window with 4% paraformaldehyde in phosphate-buffered saline, then post-fixed in the same solution. Cochleae were dissected into half-turns for whole-mount processing. Immunostaining began with a blocking buffer (PBS with 5% normal goat or donkey serum and 0.2-1% Triton X-100) for 1 to 3 hr at room temperature and followed by incubation with a combination of the primary antibodies listed (see Figure Legends).

Image Acquisition and Morphometric Analysis:

Lengths of cochlear whole mounts were measured and converted to cochlear frequency (Muller et al., 2005). Confocal z-stacks from each ear were obtained in the inner hair cell (IHC) and outer hair cell (OHC) area using a high-resolution glycerin-immersion objective (63x) and x3.18 digital zoom and a 0.25 um z-spacing on a Leica SP5 confocal microscope. For each stack, the z-planes imaged included all synaptic elements in the x-y field of view. The field of view for each stack encompassed ~10 IHCs, or ~11 OHCs from each row. Image stacks were ported to image-processing software (Amira, Visage Imaging), where synaptic ribbons, glutamate receptor patches, and hair cells were counted using the “connected components” feature of the Amira software. Juxtaposition of ribbons and receptor patches was assessed by high-magnification reimaging of all the synaptic elements in each z-stack as an array of thumbnail projections, each centered on the x, y, z coordinate of an element identified in the Amira analysis (Liberman et al., 2011). Counts of spiral ganglion neurons (SGNs) were made in confocal images of mid-modiolar sections (14-um thickness) through Rosenthal’s canal, immunostained with anti-Tuj antibodies. The cochlear frequency correlate of each half-turn visible in these mid-modiolar sections was determined as described previously (Stankovic
et al., 2004). In each case, the total number of SGNs was counted in three mid-modiolar sections through Rosenthal’s canal.

**Monaural stimulation and earplug controls:**

Experiments were conducted using only monaural stimulation – mice were briefly anesthetized with isoflurane (5% induction, 2% maintenance) before each experiment so that a dense foam earplug (3M), cut to fit the ear canal of a mouse (~2mm diameter) could be secured inside the external ear canal of the ear that was not being stimulated during the experiment. In order to minimize acoustic crosstalk through the animal’s earplug and across the head, a global ceiling on stimulus level (70 dB for tones, 80 dB for band-limited noise) was set, and animals were tested at the end of the experiment with earplugs in both ears, to ensure that any response elicited from monaural stimulation was eliminated with plugging the previously stimulated ear. This global ceiling was determined from recordings from the IC with and without earplugs, to determine the threshold shift of unit responses as measure of acoustic crosstalk (Figure 2.1). Adult (8-10 wks) male CBA mice (n = 2) were anesthetized with ketamine and xylazine and a craniotomy was performed over the right IC (Fig 2.1A). The right (ipsilateral) ear was subjected to tympanic membrane rupture and middle ear bone removal (indicated by cyan X) in order to diminish the contribution of this ear to the sound-evoked responsiveness in the IC. In the control conditions (Fig 2.1B-C, black lines), a speaker was placed at the left ear in order to record the normal thresholds of unit activity to stimulation of the contralateral ear. In the crosstalk recording configuration (Fig 2.1B-C, red lines), an earplug was placed in the left ear while the speaker was placed at the right ear, so that threshold shifts for unit recordings would reflect attenuation conferred by the mouse’s head as well as the earplug. Acoustic stimuli were broadband chirps (Fig 2.1B) and pure tones from 4-64 kHz (Fig 2.1C). Chirp stimuli
were well attenuated. For pure tones, the majority of units showed no measureable sound-evoked response within the SPL range presented in the presence of the earplug and head attenuation. However, some responses remained at thresholds lower than 70 dB, indicating that the earplug minimized but did not completely avoid acoustic crosstalk through the head and earplug. Therefore, each recording experiment described in the further sections of this chapter involved a control experiment with a double earplug condition. Sites which still showed responsiveness with double earplugs could not be attributed unequivocally to one ear and were therefore not included in analysis.

In order to confirm the contribution of type I SGN neurons to the progressive recovery of sound responsiveness in central brain regions, mice were subjected to a complete SGN destruction protocol where in bilateral ouabain was applied, and histological analysis indicated that 100%, rather than 90-99%, of SGNs had been degenerated (n = 2). These animals showed 100% crossing behavior in the auditory avoidance task to a 70 dB broadband chirp train, however their crossing percentage dropped to zero after ouabain treatment. Further, unit recordings from the IC taken 3 months after ouabain treatment yielded no discernable sound-evoked activity in response to pure tones (Fig 2.1D, red lines; black lines are spike counts from a normal animal, for reference). This experiment indicated that the recovery of sound-driven responsiveness seen in other animals with remaining SGN fibers could be attributed to activity propagated through those fibers, rather than type II fibers.
FIGURE 2.1 Anesthetized recordings from the IC confirm utility of earplugs for isolating sound stimulation to one ear, complete destruction of type I fibers causes loss of sound-driven activity, even after 3 months of recovery.

Anesthetized recordings from the IC, wherein the attenuation across the head and earplug were measured (schematized in A), indicate that chirp stimuli are effectively attenuated up to approximately 80 dB (B), and pure tones are mostly attenuated up to 70 dB (C), but not completely, therefore subsequent experiments required a double earplug control for tone stimulation. Complete destruction of type I fibers resulted in a loss of all sound-driven activity, as measured via unit recordings 3 months after ouabain treatment (D).
Figure 2.1 (continued)
Behavioral experiments

The acoustic startle response (ASR) is a reflexive behavior mediated by a brainstem circuit, wherein the presentation of a sudden, loud acoustic stimulus results in a measurable and reliable startle. Mice that had undergone ouabain treatment were fitted with a earplug in either their ouabain-treated or control ear prior to behavioral testing. While still sedated under isoflurane, the subject was placed in a small cage (approx. 3” by 4”) resting on top of a piezoelectric sensor plate. This setup was housed in the dark within a sound attenuated chamber lined with acoustic foam, while an infrared camera captured the mouse to monitor movement throughout testing. The animal was allowed to recover from anesthesia for 10 to 15 minutes, and visual monitoring confirmed that the animal was alert. An additional 5 to 10 minutes was taken for the animal to explore its cage and calm sufficiently to minimize movement. After recording commenced, a loud (70-110 dB SPL), rapid (50 ms, 0.1 ramp) noise burst was presented at varying time intervals (15-20 sec) from a speaker (Tucker-Davis Technologies) placed directly above the cage, at a distance of approximately 8”. The voltage trace measured from the sensor plate gave a metric of the forcefulness of the animal’s startle response.

To test conscious perception, an active avoidance task was developed. Mice that had undergone ouabain treatment were allowed approximately three weeks to recover before starting the behavior training paradigm. The mice were trained to cross from one side of a rectangular behavioral chamber to another upon the presentation of a 8 kHz pure tone in order to avoid a mild footshock. The rectangular chamber was approximately 5” by 11”, with a floor constructed from thin (1/4” diameter) metal bars connected to the shock device (Coulbourn Instruments). The walls of the chamber were constructed from nylon tubing (approx. 5/8” diameter, 10” height), which was arranged vertically in order to minimize the mouse’s ability to climb and avoid the shock. A free-
field electrostatic speaker (Tucker-Davis Technologies) was placed directly above the behavioral chamber, as well as a webcam for automation of the behavior protocol. Automation was carried out via custom software (LabView) that split the image of the rectangular chamber into two zones and detected the presence of the mouse (detected as a deviation from a ‘background’ image) in one of the zones. Once the animal stayed in one zone for 5 s (holding period), the 8 kHz tone was played. The tone played for variable lengths of time depending on the stage of training (5-10 s). If the software detected that the animal had crossed to the other side of the chamber during tone presentation, the trial ended and a variable intertrial interval (25-35 s) commenced. If the animal did not cross during tone presentation, the footshock was delivered at the offset of the tone. The shock was delivered for 15 s or until the animal crossed to the other side of the chamber, and then the intertrial interval began. Mice typically crossed within 1 or 2 seconds after shock onset. If a mouse did not cross during the duration of the 15 s shock period and instead froze, training ceased for the day in order to avoid excessive stress and learned helplessness.

In the first stages of training (2-3 days), animals were initially allowed to explore the behavioral chamber for 5-10 minutes, and were then shaped on the crossing paradigm only, without the tone presentation. This allowed the animal to learn that crossing to the other side of the chamber ended the footshock. Once the animal escaped the footshock within 15 s with 100% reliability, the tone was presented prior to the shock. At early stages of training, the tone was presented at 90 dB for 7-10 s, and as animals reached 75-100% performance, the length of the tone presentation was shortened to 5 s, and the level of the tone was varied randomly from 10-90 dB. Finally, the animal performed the task with an earplug in either the ouabain-treated or control ear, and results were compared.
Chronic electrode implantation.

Male CBA/CaJ mice, 8-10 weeks old and if ouabain-treated, at least one week after ouabain procedure, were brought to a surgical plane of anesthesia with ketamine/xylazine (induction with 120 mg/kg ketamine and 12 mg/kg xylazine, with 60-80 mg/kg supplements as necessary). The core body temperature of the animal was maintained at 36.5°C with a homeothermic blanket system. Using a scalpel, a small craniotomy was centered over right primary auditory cortex and/or right central nucleus of the inferior colliculus, leaving the dura mater intact. The brain surface was covered with sterile ointment. Chronic implants consisted of multichannel silicon probes (177 µm² contact area, 100 µm contact separation; NeuroNexus Technologies) arranged in a 4X4 configuration mounted onto a bidirectional microdrive (A1) or 16X1 stationary probe configuration (all ICc, some A1). The A1 implant was positioned by first mapping the cortex to delineate the low-high-low caudal-rostral best frequency (BF) gradient that uniquely identifies the orientation of A1 and the anterior auditory field (Hackett et al., 2011; Guo et al., 2012). The ICc was identified based on a low-to-high best frequency gradient along the dorsal-ventral axis of a single shank multichannel probe. Most animals received both implants so that recordings could be made in the awake state in both brain areas simultaneously. Bone wax (3M) was packed around the margins of the craniotomy to protect the probes and brain surface, and the microdrive (for A1 implants) and headstage connector were affixed to the skull surface using acrylic bonding material (C&B MetaBond, Parkell). Ground wires were implanted outside of the auditory cortex and the animal was given post-surgical subcutaneous injections of buprenorphine (0.05 mg/kg) and saline (0.5 cc) to reduce pain and dehydration, respectively. A clear plastic cylinder was affixed to the perimeter of the A1 craniotomy to protect the probes and brain surface. Animals were allowed to recover for at least 48 hours after the surgery before recordings were performed.
Acoustic stimuli:

Stimuli were generated with a 24-bit digital-to-analog converter (National Instruments model PXI-4461). For DPOAE and ABR tests, as well as during electrode implant surgery, stimuli were presented via acoustic assemblies consisting of two miniature dynamic earphones (CUI CDMG15008–03A) and an electret condenser microphone (Knowles FG-23339-PO7) coupled to a probe tube. Stimuli were calibrated at the tympanic membrane in each mouse before recording. In awake recordings, stimuli were presented via a free-field electrostatic speaker (Tucker-Davis Technologies). Free-field stimuli were calibrated before recording with a wide-band ultrasonic acoustic sensor (Knowles Acoustics, model SPM0204UD5).

Data processing:

Raw signals were digitized at 32-bit, 24.4 kHz (RZ5 BioAmp Processor; Tucker-Davis Technologies) and stored in binary format. Subsequent analyses were performed in MATLAB 2011a (MathWorks). The signals were notch filtered at 60 Hz and then bandpass filtered at 300–5000 Hz with a fifth-order acausal Butterworth filter. MU spikes were detected as threshold-crossing events using an adaptive threshold (3.5 SDs from the mean of a 10 s running average for standard MU recordings).

PSTH Classifier Model

In order to classify stimuli based on neural responses, a Euclidean distance-based PSTH classifier model (Foffani and Moxon, 2004) was utilized. In the training stage of classification, a template is
defined by calculating the number of spikes in each bin of a 10-ms binned PSTH for either and individual site or an ensemble of sites. This template is equivalent to the concatenation of all PSTHs from the population of neurons. One template is created for each stimulus. For cross-validation purposes, n-1 (n = total number of trials) trials are used to create the template, and the single remaining trial PSTH is used for the testing phase. The single trial PSTH is classified as being elicited by a particular stimulus if the Euclidean distance between the trial and the template for that stimulus is minimal compared to all other templates. This distance is calculated by summing the square differences between each spike count from the template and the trial.

*Frequency response areas (FRAs):*

Frequency response areas (FRAs) were measured with pseudorandomly presented tone pips (50 ms duration, 4 ms raised cosine onset/offset ramps, 0.5–1 s intertrial interval) of variable frequency (4–64 kHz in 0.15 octave increments) and level (0–70 dB SPL in 5 dB increments). Each tone pip was repeated twice and responses were averaged.

*Chirp trains and vector strength calculation:*

Frequency-modulated chirps were 1 ms in duration, and spanned 4-64 kHz. The FM rate was calculated to compensate for the mouse basilar membrane group delay (Spankovic et al., 2008) in order to generate a powerful, synchronous activation across the cochlear frequency axis. Vector strength of responses to chirp trains was calculated as described by Yin et al (2011a). Briefly, the standard formula for vector strength is as follows:
VS = \sqrt{\frac{\sum_{i=1}^{n} \cos \theta + \sum_{i=1}^{n} \sin \theta}{n}}

Where VS is the vector strength, n is the number of spikes over all trials, and θ is the phase of each spike in radians. Phase-projected vector strength is calculated as follows:

VS_{pp} = VS \cos(\phi_t - \phi_c)

Where VS_{pp} is the phase-projected vector strength per trial, VS_t is VS per trial, and \phi_t and \phi_c are the trial-by-trial and mean phase angle in radians. Cycle-by-cycle vector strength is calculated similarly to VS_{pp}, except that it is calculated on a cycle-by-cycle basis. A VS_{pp} value is generated per cycle, and VS_{pp} values over all cycles are averaged together to generate the VS_{cc}.

*Model fits of rate-level functions:*

Rate-level functions were calculated from responses to broadband noise. Only stimulus driven sites were included in the analysis. The monotonicity index (MI) was calculated as described in Watkins and Barbour (2011):

\[ MI = \frac{rate \ at \ highest \ level - spont \ rate}{max \ rate - spont \ rate} \]

So that a purely monotonic tuning function would have an MI of 1. Responses were smoothed with a 3-point moving average and fit with a two-tailed, six parameter Gaussian function as described in Watkins and Barbour. If the response was nonmonotonic, the upper and lower ranges of the function were fit separately. Only one fit was required for a monotonic response.
\[ r(l) = a \exp \left[ -\frac{(l - \mu)^2}{2\sigma^2_{low}} \right] + c_{low} \quad \text{for } l \leq \mu \]

\[ r(l) = a \exp \left[ -\frac{(l - \mu)^2}{2\sigma^2_{high}} \right] + c_{high} \quad \text{for } l > \mu, \]

Where \( r \) is the firing rate at sound level \( l \), \( a \) is the amplitude, \( \mu \) is the best level, \( \sigma^2 \) is the variance, and \( c \) is the offset. The model parameters \( a, \mu \) and \( \sigma^2 \) were optimized. The best level was the peak of the function (if there was a plateau in the function, the levels included in the plateau were averaged), and the threshold was calculated as the point in the model fit at which the normalized firing rate exceeded 20\% of max, with 1 dB resolution. An r-square cutoff was designated (0.7) to limit the analysis only to rate-level functions adequately fit by the model.

**Results**

**Ouabain selectively degenerates type I SGN fibers while leaving hair cells intact**

In order to induce peripheral neuropathy in mice, ouabain was applied at the round window under ketamine-xylazine anesthesia. Upon applying 1mM ouabain for six 15-minute intervals, as shown in previous studies in gerbil and mouse (Schmiedt et al., 2002; Yuan et al., 2014), ouabain selectively destroyed at least 90\% of SGNs while sparing inner and outer hair cells. The extent of ouabain-induced neuropathy was assessed both functionally and histologically, 30 days after ouabain
treatment (Fig. 2.2). In all cases, ouabain was applied unilaterally so that a within-animal control was available.

The aim of the current study was to produce near-complete SGN loss across the cochlear frequency axis, while sparing enough functional inner hair cell (IHC)-SGN synapses to support sound perception and sound-driven activity along the auditory pathway. In order to obtain precise counts of functional IHC-SGN synapses after ouabain treatment, cochlear whole mounts were immunostained with the following markers as described previously (Liberman et al., 2011; Yuan et al., 2014): (1) CtBP2, a defining feature of the hair cell ribbon synapse (Khimich et al., 2005), (2) GluA2, a glutamate receptor expressed post-synaptically (Matsubara et al., 1996; Frank et al., 2010; Liberman et al., 2011), and (3) high molecular weight neurofilament, a marker for afferent and efferent nerve fibers in the organ of Corti (Fig. 2.2A). Analysis of confocal z-stacks reveals closely juxtaposed IHC synaptic ribbons (red) and postsynaptic glutamate receptors (green), which were identified as functional synapses. In untreated ears, each IHC contained up to 10-20 synapses depending on its location along the cochlear frequency axis (Fig. 2.2B, black line). In ouabain-treated ears, synapse counts 30 days after treatment revealed less than one synapse per IHC (Fig. 2.2B, green line).

Investigations into cochlear function were performed via auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) measurements. The ABR was measured via scalp electrodes in ketamine-xylazine anesthetized mice using tonal stimuli at 8, 16 and 32 kHz. In normal ears, the measured evoked potential is comprised of peaks at characteristic latencies which correspond to strong, synchronous activity at various stages of peripheral and brainstem processing (Fig. 2.2D, inset, black line). The first peak of the ABR corresponds to activity generated from the eighth nerve. Thresholds for ABR wave 1 were elevated in ouabain-treated ears by approximately 40
dB on average compared to control ears in the same mice (Fig. 2.2C, beige lines). ABR wave 1 growth functions (Fig. 2.2D) showed a dramatic reduction in amplitude in ouabain-treated ears (green lines, bold is the average across animals) relative to control ears (black lines).

In the same ears, DPOAEs were measured to assess hair cell function without the requirement of IHC-SGN synaptic transmission. In healthy ears, DPOAEs are generated by the cochlea as a byproduct of outer hair cell electromotile activity, in response to two tones of a specific frequency ratio (Kemp, 1978). A robust emission at the frequency 2f2-f1 may be measured via a microphone placed in the external auditory canal (Kujawa et al., 1995). In ouabain-treated ears, DPOAE thresholds were not significantly altered relative to control ears (between groups ANOVA, F(2) = 1.36, p = 0.27; Fig. 2.2C, purple lines), indicating that outer hair cell function was normal. As noted in a previous study (Yuan et al., 2014), the presence of a small peak in the ABR at a latency normally associated with the rising phase of wave 1, referred to as wave 1a and thought to originate from inner hair cell receptor potentials at high SPLs, was an added indication of normal hair cell functionality (Fig. 2.2D, inset).
Figure 2.2. Ouabain treatment causes profound, selective Type I spiral ganglion neuron degeneration, but auditory avoidance behavior is intact.

(A) Cochlear histopathology from a control (top) and ouabain-treated (bottom) adult mouse. Juxtaposition of immunostained synaptic ribbons (CtBP2; red) and glutamate receptors (green) indicate a functional synapse. Inner hair cells (IHCs) are outlined in white. (B) Synaptic counts per IHC across the cochlear frequency axis. Black line is averaged control, green line is averaged ouabain-treated count 30+ days after ouabain treatment. (C) Outer hair cell function is normal, as measured via distortion product otoacoustic emission (DPOAE) thresholds (squares = individual cases, purple lines = mean across time and frequency). (C–D) Auditory brainstem response (ABR) wave 1 thresholds (C; circles = individual cases, beige lines = mean) and amplitudes (D) reflect the massive loss of nerve fiber response. (E) The acoustic startle reflex is absent in ouabain-treated animals. (F) Animals were trained on an auditory avoidance task, and learned to cross from one side of a rectangular behavioral chamber to another during the 500 ms presentation of an 8 kHz tone in order to avoid a mild footshock. (G) Animals crossed with significantly higher probability at sound levels above 30 dB (behavior below 30 dB largely reflected spontaneous crossing behavior) whether their ouabain-treated ear (black bars) or their control ear (green bars) was plugged, indicating that sound perception could be mediated through the ouabain-treated ear. Errorbars are mean +/- SEM.
Figure 2.2 (continued)
Ouabain treatment results in a loss of brainstem-mediated behavior, but preservation of cortically-mediated behavior

In order to investigate the effects of profound SGN loss on auditory-mediated behaviors, ouabain-treated animals were tested in two behavioral paradigms, 30 days after unilateral ouabain treatment. In all experiments, animals were briefly anesthetized with isoflurane so that a dense foam earplug could be placed in one ear. Auditory behavior could thus be attributed to monaural stimulation of either the control or ouabain-treated ear.

The acoustic startle response (ASR) was measured in awake mice by placing each subject in a small, acoustically transparent chamber resting on a piezoelectric sensor plate and recording responses to presentations of rapid white noise bursts at high SPLs. Compared to stimulation of the untreated ear, ouabain treatment induced a near-complete loss of the ASR at the levels tested (Fig. 2.2E). In humans with profound neuropathy, the absence of acoustic reflexes can be accompanied by intact sound perception at normal thresholds (Zeng et al., 2005), therefore we designed a behavioral paradigm to test sound detection in mice with an operant task. Prior to ouabain treatment, mice were trained on an auditory avoidance task (AAT), wherein the presentation of a sound stimulus (8 kHz, 500 ms in duration) at various sound levels signaled the mice to cross from one side of a rectangular behavioral chamber to the other in order to avoid a mild foot shock (Fig. 2.2F). Mice readily learned this task, and were re-tested 30 days after ouabain treatment. Crossing probability at levels > 30 dB SPL when stimulating both the control and ouabain-treated ears (Fig. 2.2G) indicated that ouabain treatment spared enough SGNs to support sound perception and thus, sound-driven activity at higher stages of auditory processing.
A progressive recovery of sound responsiveness occurs in midbrain and cortex, but not in the auditory nerve, over the course of one month.

The presence of sound detection behavior in ouabain-treated animals one month after treatment motivated the investigation of sound-driven activity at higher stages of auditory processing. Mice were implanted with chronic multichannel recording electrodes in the central nucleus of the inferior colliculus (IC) and primary auditory cortex (ACtx). Three groups of animals were tested: control, in which both ears were untreated (n = 3) or subjected to sham treatment (n = 2; sterile water application at the round window) in the ear contralateral to recording, a group in which the ear contralateral to recording had been treated with ouabain seven days prior (n = 5) and a third group in which the ear contralateral to recording had been treated with ouabain thirty days prior (n = 5) (Fig. 2.3A-C). Responses from IC and ACtx were recorded simultaneously in awake, passively listening animals which were mildly restrained in a small, acoustically transparent sound chamber roughly the length and width of their body, requiring animals to face a consistent direction. Stimuli were presented via free-field speakers located on either side of the animal’s head. Sound stimulation was monaural – in each recording, either the contralateral or ipsilateral ear was plugged with a dense foam earplug and sound stimuli were played from the opposite speaker.

Fig. 2.3D shows example rate-level functions for ABR wave 1 and IC or ACtx multiunit recording sites. Stimuli were tones, and responses were averaged across frequency (8, 16, 32 kHz for ABR, 4-64 kHz for IC/ACtx tonal responses). From left to right, examples are taken either from a control, 7 day post-ouabain, or 30 day post-ouabain animal. Although ouabain treatment shows a dramatic degeneration of SGNs within minutes of application, remaining nerve fibers continue to degenerate over the course of several months following treatment (Yuan et al., 2014). This
progressive loss of SGNs is reflected in the ABR wave 1 amplitude, which shows a high-threshold response that is dramatically attenuated relative to control at 7 days, and a flat response at 30 days (Fig. 2.3D, bottom row). By contrast, higher brain regions showed an opposite trend across timepoints. In the midbrain (Fig. 2.3D, middle row), some responses were recorded at the 7 day timepoint, though their thresholds were high. At 30 days, responses were significantly increased relative to the 7 day group, although still attenuated relative to control. The exemplified trends for ABR and IC responses were robust across the population (ABR: between-groups ANOVA F(2) = 52.04, p < 0.001; IC: F(2) = 171.54, p < 0.001; Fig. 2.3E, left and middle panels).

An even more dramatic recovery over time was seen in the cortex (Fig. 2.3D, top row). At 7 days, some gain functions looked very similar to control responses (Fig. 2.3D, top row, middle panel), however when averaging all sites, the amplitude of cortical responses was attenuated relative to control (between-groups ANOVA, F(2) = 19.18, p < 0.001; Fig. 2.3E, right). However, in the 30 day recording group, responses were not only elevated relative to the 7 day group, but trended toward enhancement relative to control (Fig. 2.3D, top row, 2.3E, right). This suprathreshold enhancement of central sound responses has been observed in other hearing loss models, such as IHC loss via systemic carboplatin administration in chinchilla (Qiu et al., 2000), although the degree of peripheral lesion was not as dramatic as the ouabain treatment described here.
Figure 2.3. Responses to tones recorded in higher brain areas recover over the course of one month, while ABR responses diminish with nerve fiber loss. The most dramatic gain of response amplitude is observed in the auditory cortex.

(A,B) 16 channel silicon multielectrodes were chronically implanted in the central nucleus of the inferior colliculus (IC) and the primary auditory cortex (ACtx) of adult animals with or without unilateral ouabain treatment (contra to recording sites). (C) Schematic of experimental and control groups. Control animals underwent sham (sterile water applied at the round window) or no treatment, while unilaterally ouabain treated animals received multielectrode implants at either 7 days or 30 days post treatment. (D) Example pure tone-elicited growth functions from ABR wave 1b, IC multiunit, and ACtx multiunit signals. Insets show overlaid PSTHs from 20 dB and 70 dB stimulus levels. (E) Mean ABR wave 1b, IC, and ACtx pure tone growth functions, averaged across tone frequency and including all sites, whether responsive or not. Error bars are mean +/- SEM.
Figure 2.3 (continued)
Robust encoding of sound level by firing rate is observed in IC and ACtx 30 days after ouabain treatment

In order to assess the effect of ouabain treatment and the subsequent progressive compensatory plasticity observed in the IC and ACtx on feature encoding for responsive sites only, multiunit sites were analyzed for significant activity above baseline. A 10-ms binned peri-stimulus time histogram (PSTH) was constructed from a site’s response to the stimulus, and a site was included in feature encoding analysis if at least one of the 10-ms bins during the stimulus presentation period had a spike count greater than three standard deviations above the mean spontaneous spike count.

Patients with auditory neuropathy have been shown to retain sound detection ability at normal thresholds despite a lack of measurable ABR, and perform well on sound level discrimination tasks (Zeng et al., 2005). In our mouse model, tone detection behavior mediated by hearing through the ouabain-treated ear scaled with sound level in a manner comparable to that observed through the control ear (Fig. 2.2G), indicating that the encoding of sound level in higher brain regions could be intact. In order to test the hypothesis that compensatory gain control after ouabain treatment restored firing rate responses to a degree that supports the accurate encoding of sound level, rate-level functions from sound responsive sites in the IC and ACtx were studied at both timepoints relative to ouabain treatment.

Rate-level functions for each sound responsive site were created from responses to band-limited noise (4-64 kHz, 100 ms in duration) at various sound levels (0-80 dB SPL, 5 dB steps). Responses to each sound level were averaged across 20 repetitions of the stimulus. A wide variety of function shapes were observed, especially in the cortex, including purely monotonically increasing...
functions, monotonically increasing functions which saturated at high sound levels, and nonmonotonic functions which peaked at sound levels lower than 80 dB. In order to capture the diversity of tuning types, each rate-level function was fit with a six-parameter split Gaussian function (Watkins and Barbour, 2011). For nonmonotonic functions, upper and lower dynamic ranges were fit separately (Fig. 2.4A-B). Three metrics calculated from model fits of rate-level functions are shown in Fig. 2.4. The threshold is defined as the point on the model fit, to the nearest dB, where the response reaches 20% of maximum. Best level is the peak of the function, and the monotonicity index (MI) is the response at best level divided by the response at the highest level tested (80 dB SPL). Values of MI near 1 indicate a purely monotonic response, while values near zero indicate a peaked, nonmonotonic function.

At 7 days after ouabain treatment, thresholds were significantly elevated in sound-responsive sites of the IC and ACtx (IC: between groups ANOVA, corrected for multiple comparisons, F(2) = 58.61, p < 0.001; ACtx: F(2) = 25.81, p < 0.001; Fig. 2.4C-E; circles represent individual sites, adjacent black lines represent population mean +/- standard deviation). At 30 days, thresholds in both brain regions were still significantly elevated relative to control, however a significant lowering relative to the 7 day timepoint was observed (IC: between groups ANOVA, corrected for multiple comparisons, p < 0.001 for IC and ACtx; Fig. 2.4C, F). Analysis of best level revealed that for IC units, which preferred high sound levels in all groups, ouabain did not have a significant effect (between groups ANOVA, F(2) = 2.6, p = 0.08; Fig. 2.4D). However, cortical multiunits in control animals exhibited a broader range of best levels, which were significantly elevated at 7 days after ouabain treatment (between groups ANOVA, corrected for multiple comparisons, F(2) = 4.68, p < 0.05) but were not significantly different from control after 30 days of recovery (between groups ANOVA, p = 0.89). Monotonicity indices in ACtx were significantly increased at 7 days after ouabain treatment (between groups ANOVA, F(2) = 4.47, p < 0.05; Fig. 2.4E), reflecting a
significant threshold shift and lack of responsiveness at low sound levels. However, by 30 days, a wider range of indices was observed such that as a population, indices were not significantly in cortex different compared to control (between groups ANOVA, corrected for multiple comparisons, p = 0.97). In IC, MIs were skewed toward values near 1 at all timepoints, and there was not a significant effect of ouabain treatment (between groups ANOVA, F(2) = 2.55, p = 0.08; Fig. 2.4H).
Figure 2.4. Between 7 and 30 days, rate-level functions indicate recovery of low threshold, non-monotonic responses in both ACtx and IC

(A,B) Example monotonic (A) and nonmonotonic (B) rate-level functions, measured with broadband noise from 0 to 80 dB SPL. Functions were fit with a six-parameter split Gaussian model (beige indicates rising fit, green indicates falling fit). Letters and key indicate how threshold (20% of max response of the model fit), best level (peak of the model fit) and monotonicity index (MI) were calculated. (D-F) Threshold, best level, and MI for all sound-responsive A1 units while stimulating the ouabain-treated (contra) ear. (G-I) Same metrics for IC sound-responsive units. Open circles are individual sites, errorbar indicates mean +/- SD
Figure 2.4 (continued)

A  B  C

Normalized response
Normalized response

I  II  III
II  I

Stimulus level (dB SPL)
Stimulus level (dB SPL)

I = Threshold
II = Best level
III/II = Monotonicity index

D  E  F

ACTx

Threshold (dB SPL)

Control  7 days  30 days

G  H  I

Inf. colliculus

Threshold (dB SPL)

Control  7 days  30 days

Monotonicity index

Control  7 days  30 days

Control  7 days  30 days
Encoding of pure tone frequency is preserved in ouabain treated mice

To investigate the encoding of pure tone frequency, frequency-response areas (FRAs; stimuli were pure tones from 0-70 dB SPL, 4-64 kHz in 0.15 octave increments) from all sound responsive multiunit sites were analyzed. Spike-count tuning functions centered at best frequency (BF) and taken from three levels surrounding the best level are plotted in Fig. 2.5. Tuning quality was assessed with a d-Prime metric described previously (Guo et al., 2012).

In control animals, IC multiunit FRAs displayed low threshold responses with high tuning quality (Fig. 2.5A, top). Control ACtx FRAs were similarly low threshold (Fig. 2.5B, top). Upon recovering from ouabain treatment, IC FRAs exhibited significantly lower peak spike count responses and tuning quality at the 7 day timepoint (between groups ANOVA, corrected for multiple comparisons, F(2) = 19.99, p < 0.001; Fig. 2.5C, D, left) which recovered, though not to the level of control, at the 30 day timepoint (between groups ANOVA, corrected for multiple comparisons, p < 0.01). The enhancement of spike count in response to pure tones was predominantly manifested as an increase of peak responses, rather than a broadening of tuning (Fig. 2.5C). By contrast, ACtx spike count tuning functions in sound-responsive sites were similar to control at the 7 day timepoint, and significantly enhanced relative to control at 30 days (between groups ANOVA, corrected for multiple comparisons, F(2) = 5.05, p(Ctrl vs 7 days) = 0.6, p(Ctrl vs 30 days) = 0.03). Tuning quality stayed stable in ACtx across the three experimental groups such that ouabain treatment had no significant effect on d-Prime values (between groups ANOVA, F(2) = 3.3, p = 0.05; Fig. 2.5D, right). These results suggest that although in normal conditions, ACtx pure tone responses are significantly lower in spike count, and noisier in terms of tuning quality, than IC
responses, they are significantly more robust to the dramatic loss of cochlear output induced by ouabain treatment.
Figure 2.5. Tone frequency tuning functions show a progressive enhancement over time to stimulation of the ouabain-treated ear, which is most dramatic in the cortex.

(A,B) Example frequency response areas (FRAs) from IC (A) and ACtx (B) multiunit sites in control (top) and 30-day after ouabain (bottom) conditions. Colormap indicates firing rate, y-axis indicates level (0-70 dB SPL, 5 dB steps), x-axis indicates frequency (4-64 kHz, 0.15 octave steps) (C) Frequency tuning functions, taken from the average of the three best levels and centered at BF, for IC (left) and ACtx (right) in control, 7 day, and 30 day conditions. Data are averaged across sites and animals. (D) dPrime for FRAs were calculated by sampling 30 tone-frequency combinations from tone-driven (shown in example in A) and tone-unrelated portions of the FRA (repeated 1000x), then dividing the difference in the mean firing rate divided by the average SD.
Figure 2.5 (continued)
Investigating temporal encoding after ouabain treatment

In psychophysical studies of human patients with auditory neuropathy, specific impairments in the perception of temporal cues such as gaps in noise, slow and fast temporal modulations, and interaural time differences have been observed, despite the absence of confounding factors such as audibility and cochlear compression, and retention of normal performance on other tasks such as level discrimination (Zeng et al., 2005). Further, AN patients have reported profound difficulties in speech comprehension, for which hearing aids offer little to no advantage (Kraus et al., 2000). Recent physiological and psychophysical experiments in humans have implicated a decline in the encoding precision of subcortical responses to suprathreshold sound stimuli which correlates with degraded performance in behavioral paradigms requiring discrimination of spectrotemporal details of complex sound stimuli (Bharadwaj et al., 2014). Notably, these subjects did not show significant hearing threshold shifts.

The convergence of multiple stochastic auditory nerve fiber responses is thought to enhance temporal precision in cells of the cochlear nucleus (Oertel et al., 2000). Thus, a reduction in the number of SGNs with ouabain treatment could have a detrimental effect on temporal encoding in the brainstem, propagating to higher processing centers such as the midbrain. Although cortical responses to rapid amplitude modulations tend to be poorly synchronized spike rate-based abstractions of synchronized inputs from lower areas, there is some evidence that a loss of brainstem and midbrain temporal precision could contribute to a deficit in hierarchical abstraction across serially connected auditory cortical regions (Juarez-Salinas et al., 2010). However, these experiments were carried out in aged primates, where the loss of temporal precision due to SGN degeneration as a function of age likely interacts with central changes in sound processing due to aging per se.
In order to first investigate the integrity of midbrain temporal encoding in ouabain-treated animals, a rapid pulse train stimulus was presented to the three experimental groups of animals at 20 dB above the response threshold for a single pulse. Subsequent analyses included: (1) the coefficient of variation of first spike latency, (2) the adaptation index (AI) to a 10 Hz pulse train, and (3) cycle-by-cycle vector strength (VScC) of the response. Each individual pulse was a frequency-modulated chirp (Fig. 2.6A), spanning 4 to 64 kHz in frequency and latency-adjusted to match the basilar membrane group delay in mouse, thus eliciting a strong, synchronous activation across the cochlear frequency axis (Spankovich et al., 2008). Trains of chirp stimuli (500 ms stimulus duration, 700 ms interstimulus interval) were presented at rates of 1-320 Hz, at 1/5th octave increments. In control animals, chirp trains at suprathreshold levels elicited robust, highly synchronized responses in IC multiunits (Fig. 2.6A).

Several aspects of temporal precision and reliability are degraded in the IC after ouabain treatment

In order to investigate the temporal precision of IC multiunit responses after ouabain treatment, the coefficient of variation, defined as the ratio of the standard deviation to the mean, of first spike latency to a single chirp stimulus at best level was analyzed (Fig. 2.6B). This metric was significantly elevated in the 7 day physiology group, and persistently elevated at 30 days, despite concurrent recovery of lower response thresholds and compensatory gain on the magnitude of responses (between groups ANOVA, F(2) = 22.14, p < 0.01). There was no significant difference noted between this metric at 30 days versus 7 days (between groups ANOVA, corrected for multiple comparisons, p = 0.29).
Figure 2.6. Precise and reliable response timing in the IC recovers over the course of 30 days, though not completely

(A) Example IC raster from one sound-responsive channel to a 15 Hz broadband pulse train (1 ms chirp, expanded below). Blue inset shows the response timing across trials to the first chirp. (B) Coefficient of variation (ratio of standard deviation to the mean) of the first spike latency to a single chirp at best level. (C) Cycle-by-cycle vector strength in sound-responsive units in the three experimental groups. (D) Adaptation index, defined as the average firing rate to the second through last pulse in a train, divided by the firing rate to the first pulse. Open circles indicate individual sites, errorbars indicate mean +/- SD.
Figure 2.6 (continued)
The increased variability of latencies observed after ouabain treatment suggested that responses to trains of chirps could be more poorly synchronized. To test this hypothesis, a variant of the classic vector strength metric was used in order to reveal both the temporal precision of IC responses to rapid pulse trains, and the ability of IC multiunits to sustain responsiveness to each individual pulse in the train for the duration of the 500 ms-long stimulus. The phase-projected vector strength (VSpp) was first calculated (Yin et al., 2011a) which differs from the standard vector strength (VS) in that it penalizes single-trial VS values if they are not in phase with the global response. This metric is designed to counteract spuriously high VS values due to low firing rates. In order to investigate the ability of IC multiunits to follow a rapid chirp train, cycle-by-cycle vector strength (VScc) was determined by calculating the VSpp on a cycle-by-cycle basis rather than on the cycle histogram. Thus, the single VScc metric encompassed both the ability of IC responses to precisely time-lock to a chirp train, and its reliability in keeping up with the train over multiple cycles of the stimulus.

IC multiunit responses in normal (untreated or sham-treated) animals display significant VScc values up to chirp train frequencies approaching 300 Hz and beyond, however one week after ouabain, VScc values were substantially lower on average for sound-driven sites, and cutoff rates of synchronization were significantly lower as well (between groups ANOVA, $F(2) = 63.24$, $p < 0.001$). In animals that had had one month of recovery after ouabain, VScc values were substantially higher than the 7 day group, however synchronization was still significantly degraded relative to control (between groups ANOVA, corrected for multiple comparisons, $p < 0.01$).

An intact ability to detect sounds, but impairment in spectrotemporal analysis, could be mediated by the increased adaptation of central responses to temporally modulated stimuli, compared to control. In this scenario, adaptation refers to the decrease in firing rate to each chirp in
a rapid train, relative to the firing rate response to the first chirp. In order to investigate this in ouabain-treated animals, an adaptation index (AI) metric was calculated for each responsive site in the three experimental groups. The firing rate to each chirp in a 10 Hz train was calculated by counting multiunit spikes within a 15 ms window surrounding the initial chirp, with a 5 ms response latency relative to the stimulus built in to account for synaptic latencies en route to the midbrain. The firing rate in response to all chirps after the first chirp were averaged and divided by the firing rate to the first chirp.

In control animals, IC multiunits displayed facility in maintaining a steady firing rate response to all chirps in the 10 Hz train, therefore AI values hovered around 1 (Fig. 2.6D). However, after ouabain treatment, AI values decreased significantly (between groups ANOVA, corrected for multiple comparisons, F(2) = 10.16, p < 0.01). AI values recovered to levels indistinguishable from control after 30 days of recovery, however (between groups ANOVA, corrected for multiple comparisons, p = 0.07).

These results indicate a persistent deficits in temporal encoding in the IC following ouabain treatment. They are consistent with a model that proposes that a failure to recruit stochastically responsive SGN responses creates temporally desynchronized responses in early brainstem processing centers. In addition, there is also evidence that after hearing loss, intrinsic biophysical properties of neurons at multiple stages of the auditory processing hierarchy undergo extensive changes. Global changes in excitability of individual cells could further compound the ability of IC neurons to integrate inputs from lower brainstem areas (Auerbach et al., 2014). These issues may be relevant for therapeutic options seeking to adjust central excitability in the absence of technologies to regenerate SGNs, and are discussed in more detail in the following chapter.
PSTH-based classification of speech tokens is persistently degraded in the cortex 30 days after ouabain treatment

Amplitude modulations are present in most species-specific vocalizations, including human speech. The importance of temporal modulations for speech comprehension is illustrated in work showing that the removal of spectral information from human speech recordings does not significantly affect the correct comprehension ability of young adult listeners with cochlear implants (Shannon et al., 1995). It has been suggested that amplitude modulations may underlie listeners’ ability to discriminate phonemic components of sound (McGettigan et al., 2014). The applicability of speech as a discriminative stimulus in animal models of hearing loss is illustrated in studies reporting intact detection but impaired discrimination of speech tokens in noise-exposed rats (Centanni et al., 2014; Reed et al., 2014), as well as impaired cortical encoding of stop consonants in noise-exposed cats (Tomita et al., 2004; Aizawa and Eggermont, 2006). Given that the encoding of rapidly modulated stimuli in the midbrain was persistently degraded in the IC at 30 days after ouabain treatment, human speech tokens were presented during recordings in the three experimental groups, to test the hypothesis that ouabain treatment would result in the impairment of speech encoding by the midbrain and/or cortex at 30 days.

Human speech tokens were consonant-vowel-consonant combinations, spoken by a female voice and digitally pitch-shifted to span the relevant hearing range in mouse (Fig. 2.7A-B). Most tokens differed only by the identity of the initial consonant (there were 6 possible consonants and 2 vowels that varied across the stimulus set). Voice onset time (VOT) is the distinguishing feature of the set of plosive consonants utilized, and denotes the length of time between initiation of the stop consonant and vibration of the vocal folds. Voiced stop consonants (/b/, /g/, and /d/) are
characterized by short VOTs while unvoiced stop consonants (/p/, /k/, and /t/) contain longer VOTs – as the VOT is increased, categorical perception in humans abruptly switches from voiced to unvoiced between 20 and 40 ms (Steinschneider et al., 1999).

Speech tokens were presented to mice during recording sessions in the three experimental groups, at 20 dB above threshold. Neural responses from IC and ACtx were then utilized to train a PSTH-based classifier model (Foffani and Moxon, 2004). The model chose an ensemble of 20 sites at random from the population of sound-responsive sites and created a template of PSTHs to n-1 trials of all speech stimuli. Single trial ensemble PSTHs were then classified based on their Euclidean distance from the templates. This process was repeated 20 times in each brain area and experimental group. Confusion matrices were then constructed to depict the probability of correct classification (indicated in the diagonal, Fig. 2.7C; ACtx data 2nd row, IC data 3rd row) and correct classification probability was averaged over all speech tokens (Fig. 2.7E, G).

IC responses displayed close to ceiling performance in control (Fig. 2.7F, left) and 30 day (Fig. 2.7F, right) groups, significantly improving over classification accuracy of sound-responsive sites in the 7 day group (Fig. 2.7F, middle). Classification performance between sites from control and 30 day groups statistically indistinguishable (between groups ANOVA, F(1) = 0.23, p = 0.64). In ACtx, sound responsive ensembles performed well on speech classification in the control condition (Fig. 2.7E, left). Performance was significantly degraded 7 days after ouabain treatment (Fig. 2.7E, middle) and had recovered substantially in the 30 day group (Fig. 2.7E, right), but notably, was still significantly degraded relative to control performance (between groups ANOVA, F(1) = 38.98, p < 0.001).
Figure 2.7. PSTH-based classification of consonant-vowel-consonant speech tokens is indistinguishable from control at 30 days post-ouabain in the IC, recovers incompletely in ACtx

(A,B) Spectrograms of an example speech token ("teed"), recorded from a female human speaker (A) and pitch-shifted to the mouse hearing range (B). Formants are highlighted in yellow. (C) Diagram of confusion matrix for speech tokens, with values on the diagonal indicating correct classification. (D,F) Confusion matrices generated from an ensemble of 20 sound-responsive sites, in control, 7 day, and 30 day conditions for ACtx (D) and IC (F). Colormap indicates probability of veridical classification (E,G) Ensembles were drawn 20 times from the population to produce mean correct classification. Errorbars indicate mean +/- SEM.
Figure 2.7 (continued)
Ensemble classification of chirp rate and sound level recovers significantly from 7 to 30 days, with specific classification deficits reflecting perceptual deficits in neuropathy patients published previously.

The PSTH ensemble classifier model was then extended to neural responses to broadband noise of varying sound level, and chirp trains of varying frequency, presented at 20 dB above threshold (Fig. 2.8). Overall, when classification accuracy was averaged across stimuli, IC responses showed an initial classification deficit at 7 days after ouabain (between groups ANOVA, corrected for multiple comparisons, F(2) = 29.52, p < 0.001; Fig. 2.8B, D, bar plots). By contrast, ACtx responses did not show a significant decline in classification accuracy after ouabain, in either the 7 or 30 day group (between groups ANOVA, F(2) = 2.22, p = 0.12; Fig. 2.8A, C, bar plots). However, upon closer examination of the classification accuracy of specific stimuli, notable shifts in ensemble encoding emerge.

For the classification of sound level, in both brain areas accuracy shifted from lower sound levels to high sound levels. This shift could be explained simply by the persistence of a threshold shift in some sites, however human psychophysical data suggests that even at suprathreshold levels, human neuropathy patients show some deficits in discriminating lower sound levels (Zeng et al., 2005). In the case of chirp train classification, performance is similar in the IC when comparing the control and 30 day groups, indicating the recovery of some synchronization of the response, which will have a dramatic effect on classification performance. The relationship between classification and synchronization is further illustrated by the increase in classification errors at higher chirp rates, where vector strength cutoffs indicate sharp decreases in synchronization behavior of multiunit responses. In ACtx, where responses are generally poorly synchronized and instead convey
information about chirp train frequency through firing rate, accurate performance shifts from high chirp rates in the control condition to low chirp rates in the 30 day group. Psychophysical evidence implicates hearing loss in a specific impairment of modulation frequency discrimination at high rates where the transformation from precisely time-locked responses to firing rate responses may be disrupted by neuropathy either because temporal encoding deficits inherited from the brainstem, or de novo processing deficits generated by inhibitory dysregulation in response to a loss of cochlear output (Gold and Bajo, 2014).
Figure 2.8. Mean ensemble classification of sound level and pulse rate at 30 days post ouabain treatment is not significantly different from control classification

(A,B) Confusion matrices and mean correct classification (right) from ensemble classification of sound level (broadband noise from 0-80 dB SPL) in A1 (A) and IC (B). Errorbars indicate mean +/- SEM. (C,D) Confusion matrices and mean correct classification (left) from A1 (C) and IC (D) ensembles for pulse rate using chirp stimuli from 1 to 320 Hz (1/5th octave steps). Ensembles consisted of 20 sites, drawn 20 times at random from the sound-driven population.
Figure 2.8 (continued)
Comparison of ipsilateral and contralateral tonal responses indicates that compensatory plasticity following ouabain treatment is a competitive process

A large body of literature in sensory plasticity has described the dynamic representation of spared inputs in response to peripheral lesion (Calford, 2002; Irvine, 2007; Kilgard, 2012). Unilaterally applied ouabain spared the inputs ipsilateral to recording in each brain area. Therefore two potentially distinct plasticity mechanisms could be at play: the enhancement of the representation of the spared contralateral inputs, and the enhancement of the representation of the normal ipsilateral inputs. The ipsilateral ear served as a within-animal control for cochlear function, histology, and behavior studies but also provides a significant driving input to both IC and ACtx, where ipsilateral representations are enhanced after loss of input from the contralateral ear. This initial enhancement can be viewed as an unmasking of previously ‘silent’ ipsilateral influence on excitatory responses in both brain regions, and has been observed in previous studies of unilateral hearing loss. However, the subsequent recovery (and enhancement in ACtx) of responsiveness to spared inputs from the contralateral (ouabain-treated) ear in the current study motivated the testing of a hypothetical model of compensatory plasticity. In one instantiation of the model, a nonselective gain control mechanism – mediated through either network-level alterations or homeostatic, cell-intrinsic changes in excitability, or both – a global enhancement of excitatory inputs increases the gain on both contralateral and ipsilateral afferent inputs to IC and ACtx. In another instantiation of the model, resources devoted to the enhancement of spared contralateral inputs competes with, and prevents, the enhancement of intact ipsilateral inputs.

In order to test this model, sites which has been stimulated monaurally in the contralateral and ipsilateral ears during the same experimental recording session (without moving probes mounted on a microdrive) were tested to reveal the balance of responsiveness to either ear.
Normalized frequency tuning functions centered at best frequency (BF) were taken from stimulation of each ear. Ipsilateral tuning functions were assigned negative values (from 0 to -1) while contralateral tuning functions were assigned positive values (from 0 to 1). Each site’s tuning functions were normalized to the higher peak firing rate from either of the two ears. The normalized functions were then added together, such that resultant negative values indicated an ipsilateral preference at the specified frequency separation from BF, and positive values indicated contralateral preference.

Fig. 2.9A depicts contra/ipsi preference in an example control animal. Strong contralateral dominance is shown in the IC (Fig. 2.9A, right) while a slightly weaker contra dominance, and some broad ipsilateral frequency responses, are observed in the cortex (Fig. 2.9A, left). At 30 days after ouabain treatment, the distribution of recovery was bimodal. In half of the animals included in this analysis (n = 4), a strong ipsilateral preference was shown (Fig. 2.9B, left and right taken from same animals) in both brain areas. In these animals, little or no responsiveness to the contralateral ear was observed, indicating that this group did not experience compensatory plasticity after ouabain treatment which was sufficient to restore contralateral excitatory drive. In the other half of the group, robust representation of the contralateral (ouabain-treated) inputs were observed at 30 days. Interestingly, these animals not only exhibited strong contralateral dominance, but had little or no responsiveness to the ipsilateral, intact ear.

The either/or nature of central responsiveness after recovery from ouabain treatment was illustrated in the inverse relationship between the number of well-tuned ipsilateral versus contralateral sites in both brain areas (Fig. 2.9C). Further, graded increases in firing rate were directly related to the preference index, defined as the value of the summated frequency tuning functions at BF (negative for ipsilateral dominance, positive for contralateral dominance; Fig. 2.9D). These
relationships point to the competitive nature of compensatory plasticity after ouabain treatment. The degree of contralateral dominance was related to the precise number of synapses present in the histological analysis, indicating that there is a point of synaptic loss beyond which compensatory plasticity cannot recover sound representations, but before which there is a nonlinear gain in the capability of recovery.
Figure 2.9. After 30 days, sites in ACtx and IC show a strong preference for either the intact or ouabain-treated ear.

(A-C) Normalized frequency tuning functions, centered at BF (y-axis indicates frequency separation from BF, 0.15 octave steps) from ipsi (negative values) and contra (positive values) stimulation were subtracted from each other. B shows overlaid functions from two animals who showed little or no responsiveness to the ouabain-treated ear. C shows overlaid functions from animals with robust recovery, displaying a strong contra preference. (D) Proportion of well-tuned ipsilateral sites was negatively correlated with that of contra sites. (E) Preference index (mean response at BF, ipsi is negative) was positively correlated with the contra FR divided by ipsi FR.
Figure 2.9 (continued)
Application of ouabain, a sodium/potassium ATPase pump inhibitor, at the round window, selectively degenerated Type 1 SGNs while leaving cochlear hair cells intact. Consistent with the clinically defined characteristics of profound auditory neuropathy, ouabain-treated mice had no discernable ABR wave 1 in the treated ear, but retained DPOAEs at normal thresholds. Behaviorally, animals displayed no acoustic startle reflexes when using the ouabain treated ear, however they displayed sound detection behavior at normal thresholds when tested with an operant task. These characteristics were also consistent with behavioral results from the patient population.

Simultaneous recordings from the IC and ACtx of mice treated with ouabain in the ear contralateral to electrode implantation at one week and one month after treatment revealed a progressive recovery of sound feature representations which was more pronounced, and possibly earlier, in the ACtx. This recovery was reflected in the significantly lowered thresholds seen at the 30 day timepoint, as well as the increase in sound evoked firing rates. The evoked rate at high sound levels in the ACtx was also elevated relative to control, mirroring results from other studies of hearing loss-inducing central gain enhancement. However, other aspects of sound encoding, such as the precision and reliability of spike timing in the IC and the classification of human speech tokens in ACtx, were persistently degraded even as sound detection behaviors and low sound evoked activity thresholds in both brain areas were observed.

Universality of central gain enhancement across hearing loss models
There is extensive evidence \textit{in vivo} for compensatory central gain enhancement following peripheral insult in a variety of hearing loss models (Auerbach et al., 2014). A meta-analysis of the literature offers an opportunity to separate peripheral effects from central plasticity by limiting lesions and cell damage to functionally distinct cochlear constituents and searching for commonalities among the midbrain, thalamic and cortical profiles of recovery.

Noise trauma is the most common type of peripheral insult used to study hearing loss, and exerts detrimental effects on both inner and outer hair cells (although outer hair cell damage is more prominent), as well as causing significant SGN loss (Dallos and Harris, 1978; Liberman and Dodds, 1984a, b; Liberman and Kiang, 1984). Some models of central gain enhancement argue that outer hair cell damage is critical for the phenomenon to be observed (Jastreboff, 1990; Stypulkowski, 1990), however studies utilizing carboplatin in chinchillas, which selectively damages IHCs, observed significant gain enhancement despite preservation of OHCs, indicating that OHC loss is not necessary for enhancement to occur (Chen et al., 2013; Chen et al., 2014). The present results with the ouabain model add validity to this hypothesis, as DPOAEs were not significantly affected by ouabain treatment (Fig. 2.2). This evidence indicates that gain enhancement is indeed a long term adaptive plasticity mechanism which occurs centrally.

**Temporal dynamics of compensatory plasticity in the midbrain and cortex**

In this study, IC and ACtx responses were recorded at two time points relative to nerve degeneration: one week and one month. At the 7 day timepoint, overall percentages of sound-driven sites relative to all sites recorded are lower than control, however the sites that were sound-responsive in the cortex show less impairment relative to control in terms of level and frequency
tuning, as well as pulse train classification, than their IC counterparts. This suggests that either ACtx is more robust to the immediate effects of ouabain treatment than IC, or that both brain areas suffer from substantially lowered sound-evoked firing rates and sound feature encoding capacity immediately after ouabain treatment, which recovers to a greater extent, more rapidly in the cortex.

Although the answer to this question is not available as yet specifically in the ouabain model, several studies tracking the recovery of central responsiveness after peripheral insult in the auditory system suggest that the latter scenario is true (Popelar et al., 1987; Syka et al., 1994; Salvi et al., 2000; Syka and Rybalko, 2000; Sun et al., 2008; Norena et al., 2010; Sun et al., 2012). A study comparing round-window compound action potential (CAP) recordings with evoked potential recordings from the IC and ACtx revealed an enhancement of rate-level functions in ACtx as soon as one hour after exposure (Syka et al., 1994). This enhancement relative to control was not seen in the IC or CAP recordings. Over the course of two weeks, ACtx rate-level functions progressively recovered lower thresholds and slopes, although responses at high levels were still significantly enhanced relative to control. Conversely, rate level functions for the IC and CAP did not recover to control amplitudes over the same time course. Studies using more intense or prolonged exposure have observed an initial suppression of rate-level functions in ACtx one hour after exposure which recovers and is enhanced on a longer timescale of days or weeks (Syka and Rybalko, 2000; Norena et al., 2010).

Differences in compensatory changes after ouabain treatment between the IC and ACtx

The differences observed in this study between the IC and ACtx in terms of the temporal dynamics and magnitude of compensatory recovery could be due to intrinsic mechanisms which allow ACtx to exhibit a greater degree of plasticity and gain control of firing rate responses than the
IC. However, the difference could also be due to an inherited response in ACtx which reflects the diversity of projections along the auditory pathway between the two brain regions. For example, approximately one-third of IC neurons projecting to the medial geniculate body of the thalamus (MGB) are GABAergic inhibitory projections (Winer et al., 1996; Peruzzi et al., 1997). Differences in compensatory gain control in distinct cell types could underlie either induction or depression of gain control in ACtx. Further, descending projections from ACtx can modulate firing rate responses in the IC in a complex manner on both short and long time scales (Suga et al., 2000; Zhang and Suga, 2000), implicating a top-down role of the ACtx in potentially driving or modulating IC plasticity. Studies characterizing compensatory changes in sound responsiveness across various cell types and changes in functional connectivity between the IC, MGB, and ACtx in response to peripheral nerve fiber loss are necessary for understanding the origins and circuit-level mechanisms of central gain enhancement, however several models for these mechanisms have been constructed based on current lines of evidence (Auerbach et al., 2014; Gold and Bajo, 2014).

Alterations in inhibitory and excitatory synaptic responses after peripheral insult

Many studies have shown alterations to inhibitory synapses after hearing loss, both biochemically and functionally at various stages of the auditory pathway. Long-lasting decreases in functional glycinergic markers in the cochlear nucleus suggest changes in inhibitory function at the very first stages of central processing (Hildebrandt et al., 2011; Middleton et al., 2011). Further, blocking GABAergic inhibition in dorsal cochlear nucleus slices from noise-exposed mice resulted in greater enhancement relative to control, implicating a decrease in inhibition for gain enhancement following hearing loss in this brain structure. In the IC and ACtx, a decrease in GABAergic
inhibition has also been found after noise exposure (Kotak and Sanes, 1995; Vale and Sanes, 2000; Wang et al., 2006; Yang et al., 2011). In ACtx, GABA enhancement via local application of vigabatrin was shown to suppress the enhancement of firing rates in ACtx observed after salicylate-induced IHC loss (Lu et al., 2011). Functional subtypes implicated in gain control in ACtx include parvalbumin positive (PV+) interneurons, which have been shown to provide dynamic gain control and mediate level tuning in normal-hearing animals (Atallah et al., 2012; Moore and Wehr, 2013), and vasoactive intestinal polypeptide (VIP) expressing interneurons, which may be implicated in compensatory gain control via their specific inhibition of PV+ interneurons (Pi et al., 2013).

Thickening in post-synaptic densities in the ventral cochlear nucleus, as well as increases in AMPA receptor surface labeling in ventral and dorsal cochlear nucleus after peripheral deafferentation and unilateral cochlear ablation, respectively, suggest an enhancement in excitatory strength in response to the loss of cochlear output (Gulley et al., 1977; Rubio, 2006). Increased EPSCs concomitant with decreased IPSCs in IC and ACtx after cochlear ablation suggest a bidirectional modulation of responses which may also underlie compensatory changes in the ouabain model.

**Alterations in intrinsic excitability of neurons after peripheral insult**

In order to maintain the stability of neural networks, Hebbian forms of plasticity may be counteracted by non-Hebbian homeostatic plasticity, a regulatory mechanism which allows neurons to modulate their intrinsic excitability in response to associative changes in synaptic strength (Turrigiano et al., 1994; Turrigiano and Nelson, 2000). Homeostatic gain control may be an important factor in the compensatory changes following sensory deprivation, such that central
auditory neurons could increase their excitability in response to a decrease in afferent input, thus balancing the firing rate outputs of networks of neurons (Turrigiano and Nelson, 2004). Homeostatic plasticity has been proposed as a mechanism for the noise-trauma induced hyperactivity in the central auditory pathway (Schaette and Kempter, 2006; Norena, 2011; Yang et al., 2011; Auerbach et al., 2014; Gold and Bajo, 2014). Several studies have shown responses in the IC and ACtx of gerbils after cochlear ablation which resemble synaptic scaling, a key mechanism of homeostatic plasticity wherein neurons may globally renormalize synaptic strength in response to long-term increases or decreases in activity levels (Vale and Sanes, 2002; Kotak et al., 2005; Kotak et al., 2008). Sensory deprivation studies in the visual and auditory system have suggested a critical period dependence for homeostatic mechanisms, but cases of profound peripheral insult, such as retinal ablation, have provided evidence for homeostatic mechanisms in adult animals (Keck et al., 2013).

**Implications of changes in central encoding for behavior and perception**

Although unilaterally ouabain-treated mice exhibited normal sound detection behavior assayed with an operant task, it is unknown whether the ACtx deficit in speech classification would manifest itself perceptually as a deficit in a discriminative behavior. However, as the animal model described here met the standard clinical criteria for auditory neuropathy, including intact sound detection, the evidence from IC and ACtx recordings suggest that this model may prove quite useful in discerning the neurophysiological basis for the specific temporal perceptual deficits in humans.

In adult rats subjected to noise trauma, both behavioral and neurophysiological characterizations of responses to human speech tokens have been studied (Reed et al., 2014) which
suggest that the neuronal encoding deficits observed in central auditory regions after peripheral insult occur alongside significant perceptual deficits in discrimination, but not detection, of speech stimuli. Further behavioral characterization of ouabain-treated mice will reveal whether the perceptual deficits seen in noise-exposed rats are also seen when peripheral insult is restricted to auditory nerve fibers rather than hair cells.

It is also notable that ouabain-treated mice had no prior exposure to the human speech tokens, and unlike mouse vocalizations, the stimuli had no hedonic meaning to the animals. These experiments have revealed both recovered aspects and persistently degraded aspects of acoustic feature encoding separated from the cognitive effects of attention or species-specific adaptations to particular types of vocalizations. In addition to the potential impairments in the neural representation of the acoustic properties of stimuli, human neuropathy patients are subject to the additional influence of attention and experience on detection and discrimination. Recent attention has been devoted to the characterization of suprathreshold perceptual attributes of human neuropathy patients required to make use of selective attention (Bharadwaj et al., 2014). These experiments have suggested that when identifying sound sources from a competing stream, neuropathy patients showed, first, a wide variability in terms of accuracy, and second, a tendency to make mistakes primarily in selecting the correct stream via attentional mechanisms, rather than simply mishearing the sound stimuli themselves. This evidence opens many interesting avenues for research into the ability of recovered sound representations after hearing loss to support the complex and challenging processing required for selective auditory attention. Further, central representations of sound stimuli may be modulated by behavioral training. Thus, targeted behavioral paradigms, either introduced during the initial recovery from peripheral auditory trauma or long afterward, may be useful in directing or enhancing compensatory plasticity in higher brain regions such as ACtx (Kilgard, 2012; Whitton et al., 2014).
Chapter 3: AUT3, a Kv3.1 positive modulator, improves IC temporal encoding in mice with profound cochlear neuropathy

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Author contributions: ARC, CL, and DBP designed experiments, ASE and CL provided reagents and technical assistance, MCL performed cochlear histopathology and analysis, JPW provided speech stimuli and classifier model code, ARC performed all other experiments and analysis.
Abstract

The amount and type of voltage-gated potassium (Kv) channels expressed in the cell membrane of a neuron are major determinants of its intrinsic electrical excitability. Kv3.1 is a high-threshold delayed rectifier channel that is widely expressed in fast spiking neurons throughout the central auditory pathway. Kv3.1 rapidly repolarizes the membrane potential during spiking, effectively shortening the refractory period and thus enabling neurons to sustain high firing rates in the face of high frequency synaptic inputs. Deprivation or enrichment of auditory experience has been shown to powerfully modulate the level of Kv3.1 expression in the auditory brainstem, thereby revealing a potential therapeutic target for rescuing temporal processing irregularities that accompany peripheral nerve degeneration. We tested the hypothesis that systemic injections of AUT3, a positive modulator of Kv3.1, would rapidly restore aspects of auditory temporal encoding that are known to be substantially degraded following extensive loss of Type I spiral ganglion neurons in adult mice. Here, we characterized the acute effects of AUT3 administration on extracellular multiunit responses recorded simultaneously at two stations of the central auditory pathway via implanted silicon probes: the inferior colliculus (IC) and primary auditory cortex (A1). All experiments were performed in awake mice and provided a comparison of two dosages of AUT3 (30 mg/kg and 60 mg/kg) against a drug vehicle condition. Consistent with the localization of Kv3.1 to principal excitatory neurons of the central nucleus of the IC, we found that increasing concentrations of AUT3 increased stimulus-evoked responsiveness of IC sites while enhancing synchronization and trial-by-trial reliability of spike timing. By contrast, Kv3.1 is expressed in fast-spiking inhibitory interneurons in the auditory cortex, where we observed that AUT3 suppressed responses evoked by the same stimuli. Changes in IC spike rate and timing induced by AUT3 administration translated to an overall improvement in the accuracy of temporal modulation rate coding, as determined by a PSTH-based classifier model. These findings support the potential
therapeutic applications of Kv modulation for auditory CNS disorders such as auditory neuropathy spectrum disorder and tinnitus, which may be rooted in hyperexcitable and temporally dysregulated spiking at key stages of central auditory processing.

**Introduction**

The mammalian auditory system is capable of such precise temporal encoding as to support the discrimination of rapid amplitude modulations and subtle timing differences between sounds originating from distinct spatial locations (Frisina, 2001; Lestienne, 2001). At sound frequencies below 2 kHz, mammalian auditory neurons are capable of precisely time-locking their responses to the fine structure of a stimulus (Joris and Yin, 1995; Joris, 1996). In order to achieve such precision, auditory neurons must be capable of firing action potentials at very high rates. This ability is largely conferred to a neuron by the properties of the complement of ion channels it expresses.

Neuronal excitability is greatly influenced by potassium currents (Peusner et al., 1998). Potassium channels affect the membrane potential, shape of the action potential, and the firing rate of a neuron (Bean, 2007). Because auditory function is so closely linked to the precision and high rates of neuronal firing, the mammalian auditory system has been extensively studied in the context of potassium channel regulation and dysfunction (Joris, 1996; Oertel, 1999; Trussell, 1999; Kaczmarek, 2006; Schneggenburger and Forsythe, 2006). The intrinsic excitability of auditory neurons has been shown to be dynamic, rapidly adjusting relative to a changing acoustic environment (Song et al., 2005). Further, changes in potassium channel expression along the central auditory pathway have been observed after hearing loss (von Hehn et al., 2004), motivating an understanding of their role in the processing of already degraded peripheral outputs.
Kv3.1 channels enable rapid firing in auditory neurons

Current for the repolarization of an action potential is provided by the family of delayed rectifier channels (Hodgkin and Huxley, 1952), of which the voltage sensitive Kv3.1 potassium channel is a member (Swanson et al., 1990). Unlike other members of the delayed rectifier family, however, Kv3.1 channels activate and deactivate rapidly with changes in voltage (Kanemasa et al., 1995). Their activation takes place at membrane potentials more positive than -10 mV and deactivation generally occurs before the end of an action potential. Due to this rapid deactivation, neurons with Kv3.1 currents can fire at high rates because of their narrow action potentials and the lack of a relative refractory period, present in other cells with slowly deactivating outward potassium currents (Kanemasa et al., 1995). The function of Kv3.1 currents in rapid action potential firing has been studied extensively in the medial nucleus of the trapezoid body (MNTB). In vitro slice recordings have shown that in response to current pulses of less than roughly 400 Hz, neurons fire a precisely timed action potential to each stimulus (Wang et al., 1998). In mice lacking the Kv3.1 gene, the ability to sustain firing after the first pulse of a 200 Hz stimulus in the MNTB is lost (Macica et al., 2003), illustrating the necessity of the current in this auditory structure for conveying information about high-frequency stimuli.

Modulating Kv3.1 channels after peripheral auditory insult

Kv3.1 transcription is regulated by the Ca\(^{2+}\)-cAMP-responsive element binding protein (CREB) transcription factor. CREB is activated and phosphorylated in response to the external
stimulation of a cell. Intracochlear stimulation has been shown to modulate the phosphorylation of CREB in the rat auditory brainstem (Illing et al., 2002). Further, a study comparing the C57 mouse, a strain which exhibits high frequency hearing loss beginning in early adulthood, to the CBA mouse, which exhibits normal hearing throughout life, showed that the tonotopic gradient of Kv3.1 across the MNTB medial-lateral axis was lost in aged C57 mice, and CREB immunoreactivity was almost 50% decreased in aged C57 mice compared to aged CBAs (von Hehn et al., 2004). As the genetic basis for C57 age-related hearing loss is a defect restricted to hair cells (Noben-Trauth et al., 2003), these experiments suggest that the normal distributions of Kv3.1 and CREB are dependent on normal levels of cochlear output. Deficits in Kv3.1 expression in the auditory brainstem could have detrimental effects on hearing, as the channel is important not only in sustaining high firing rates to rapidly modulated stimuli, but for maintaining the precision of responses to even a single pair of closely spaced stimuli (Macica et al., 2003).

According to histological analyses, nuclei along the ascending auditory pathway express Kv3.1 (Li et al., 2001). For the purposes of the current study, it is important to note that most cells of the inferior colliculus (IC) express Kv3.1, with relatively equal expression across the central nucleus, external and dorsal cortices of the IC. In the auditory cortex (ACtx), Kv3.1 is expressed predominantly in a population of GABAergic fast-spiking inhibitory interneurons (Li et al, 2001).

A novel positive modulator of the Kv3.1 channel has recently been developed and is currently being tested in animal models of hearing loss as well as in other potential applications (Autifony, Ltd). Cell culture and in vitro slice recordings have shown that the compound, heretofore referred to as AUT3, shifts the voltage dependent activation of Kv3.1 to more negative potentials (Brown and Kaczmarek, under review), and increases the number and temporal precision of action potentials of MNTB neurons with low Kv3.1 expression in response to rapidly modulated stimuli.
One model for the mechanism of channel modulation by AUT3 is that the compound may shield the voltage sensing element of the channel with a positive charge, effectively changing the potential that the channel ‘sees’ to more positive values. Several deficits in midbrain temporal encoding in the ouabain treated mouse were observed at 30 days post-treatment (see Chapter 2), including increased variability of spiking responses and a decreased capability of sustained spiking to trains of rapidly repeated pulses, which may contribute to degraded cortical ensemble classification of speech tokens and potentially, to degraded perception of rapidly modulated stimuli. AUT3 was administered systemically to ouabain-treated mice at +30 days of recovery, and extracellular responses in the IC and ACtx were recorded in response to chirp train stimuli and human speech tokens. The results described here indicate that positive modulation of the Kv3.1 channel could improve the temporal precision and classification accuracy of multiunit responses in the IC, and though its effects on ACtx processing are less clear, the therapeutic potential of the compound for humans with hearing loss, as well as the multiple avenues for continued investigation into the effects of the drug in the ouabain model, are discussed.

Methods

For methods regarding ouabain treatment and chronic electrode implantation, as well as information on acoustic stimuli, recording procedures and analysis, see Methods, Chapter 2.

AUT3 preparation and administration: The drug was prepared in the following vehicle for injection: Captisol 20% w/v, HPMC 0.5% w/v, Tween80 0.1% w/v in water. Two systemic dosages were
tested: 30 mg/kg and 60 mg/kg. Prior to recordings, animals were briefly anesthetized with isoflurane (5% induction, 2% maintenance) and an i.p. injection of the drug + vehicle, or vehicle only was given. While the animal was under anesthesia, a dense foam earplug was placed in the untreated ear, so that sound stimulation would be isolated to the ouabain-treated ear (contralateral to recording) during the experiment. The animal was allowed 15-20 minutes to recover from the isoflurane injection, and recording commenced. Experiments lasted roughly one hour to 90 minutes, well within the period of maximal blood concentrations of the drug (Autifony, personal communication). All experiments were carried out on animals that had undergone ouabain treatment at least 30 days prior.

Results

Ouabain treatment causes selective degeneration of Type 1 SGNs, IC and ACtx responses recover low thresholds and steep rate-level functions after one month of recovery

Recordings in the IC and ACtx were performed in animals that had received ouabain treatment contralateral to recording sites at least 30 days prior (see Chapter 2). The ouabain treatment selectively degenerated Type 1 auditory nerve fibers (Fig. 3.1A), thus substantially increasing auditory brainstem response (ABR) thresholds (Fig. 3.1B), while hair cells were left intact. Fig. 3.1C shows that DPOAE thresholds were normal in ouabain-treated ears (between groups ANOVA, F(2) = 1.36, p = 0.27). With 30 days of recovery, central compensatory plasticity restored low threshold responses in IC and ACtx. Overall suprathreshold sound-driven amplitudes remained significantly lower than control in the IC (between groups ANOVA, F(2) = 171.54, p < 0.001),
although they recovered to levels indistinguishable from control in ACtx (See Chapter 2, Figure 2.3).

Auditory nerve responses did not recover over the same time period (See Chapter 2, Figure 2.3).
FIGURE 3.1. Thirty days after unilateral spiral ganglion neuron degeneration, central recordings reveal a recovery (IC) or enhancement (ACtx) of sound-driven responses despite a profound loss of output from the cochlea.

(A) Adult CBA/CaJ mice were unilaterally treated with 1mM ouabain applied the round window. Cochlear histology performed 30+ days after ouabain treatment reveals a loss of inner hair cell (IHC) to SGN synapses, shown as the juxtaposition of IHC synaptic ribbons (CtBP2; red) and glutamate receptors (green). (B,C) Auditory brainstem response (ABR) wave 1 and distortion product otoacoustic emission (DPOAE) thresholds (red lines = individual measurements from the ouabain-treated ear, black lines = individual measurements from the control (untreated) ear) reflect a loss of SGNs and preservation of hair cell function. Recordings in the midbrain and cortex (D) were performed 30 days after ouabain treatment in awake adult mice.
Figure 3.1 (continued)
AUT3 administration increases IC multiunit firing rates in response to chirp train stimuli

Ouabain treated animals were briefly anesthetized with isoflurane in order to place a dense foam earplug in the control (untreated) ear in order to isolate sound stimulation to the ouabain-treated ear, contralateral to recording sites. In addition, the animals were given a systemic i.p. injection of either the AUT3 compound (with injection vehicle), or the vehicle alone. Dosages of AUT3 tested were 30 mg/kg and 60 mg/kg. The animals were then allowed to recover fully from isoflurane anesthesia, and awake, simultaneous recordings from IC and ACtx commenced approximately 20 minutes after the drug or vehicle injection.

Upon systemic administration of AUT3, recordings were taken from the IC and ACtx while animals were awake and passively listening to sound. The first stimulus tested was a chirp train (see Chapter 2), presented at 20 dB above threshold, of frequencies from 1-320 Hz. Fig. 3.2A shows the peri-stimulus time histogram of a response from a single multiunit site in IC from a single animal following vehicle administration (Fig. 3.2A, left), and increasing dosages of the AUT3 compound (Fig. 3.2A, middle, right) in response to a 45 Hz chirp train. In this example, the magnitude of the spiking response to each chirp, as well as the ability of the multiunit response to sustain with multiple chirp repetitions, are increased with increasing drug dosage. The PSTH was averaged across all sites in all animals in each drug condition, as well as across chirp train frequencies, and then smoothened with a 10 ms moving average (Fig. 3.2B, C). In the IC (Fig. 3.2B), the increase in response amplitude across the population trended toward an increase, but was not statistically significant. However, in ACtx, AUT3 application caused a significant decrease in the overall magnitude of the response (one-way ANOVA, F(2) = 3.17, p < 0.05). Notably, spontaneous firing rates were unchanged with drug administration in both brain areas (one-way ANOVA, IC: F(2) = 1.9, p = 0.15; ACtx: F(2) = 1.75, p = 0.18).
FIGURE 3.2. Administration of AUT3 increases the magnitude of average sound driven responses in the IC while suppressing responses in ACtx.

(A) PSTH from an example IC site, tested in the vehicle, 30 mg/kg AUT3, and 60 mg/kg conditions (drug administered via i.p. injection; stimuli were broadband pulse trains from 1-320 Hz, presented at 20 dB above threshold) in the inferior colliculus. (B) Average PSTHs from all sound-driven sites in the IC. (C) Average PSTHs from all sound-driven cortical sites, showing a decrease in average firing rate with increasing drug dosage.
Figure 3.2 (continued)
AUT3 administration significantly increases synchronization of IC responses to rapid chirp trains.

One month after ouabain treatment, IC multiunit responses to chirp trains remained significantly less synchronized, as measured via vector strength, relative to control (See Chapter 2). Therefore, the vector strength of IC multiunit responses to chirp trains was calculated in the vehicle and drug conditions to test whether shifting the activation of Kv3.1 channels to more negative potentials could restore temporal fidelity of responses in the ouabain model. Vector strength was calculated as described in Chapter 2.

Fig. 3.3A shows example raster responses of IC multiunits in each drug condition to chirp train stimuli at 40, 80, and 160 Hz. Two effects are exemplified in the raster plot: first, an increase in the precision of the response, with fewer desynchronized spikes contaminating the response with increasing dosage of the drug, and second, a prolongation of the response so that it sustains with continued chirp train stimulation.

Vector strength values across frequencies for the population of multiunits were higher with increasing dosages of the drug, and the effect of the drug was highly significant in the IC (one-way ANOVA, F(2) = 43.25, p < 0.001; Fig. 3.3B, bold lines). In the ACtx (Fig. 3.3B, lighter lines), a significant increase in vector strength was also observed (one-way ANOVA, F(2) = 37.72, p < 0.001), which was surprising given the synchronization values are typically very low in the ACtx, even under normal hearing conditions. Separate analysis of phase-projected and cycle-by-cycle vector strength cutoff values (the chirp train frequency where the vector strength is not significantly
higher than chance) revealed a significant effect of AUT3 administration at the 60 mg/kg dose in the IC (two-sample t-test, p < 0.05; Fig. 3.3C).
FIGURE 3.3. AUT3 administration improves synchronization to rapid pulse train stimuli in the IC

(A) Example rasters from IC sites with increasing drug dosage to chirp trains of three different rates, presented at 20 dB above threshold. (B) Average vector strength across all sound driven sites, with cutoff frequencies summarized in (C).
Figure 3.3 (continued)
AUT3 administration significantly improves the classification accuracy of neural responses to human speech tokens in the IC

As AUT3 administration significantly enhanced temporal synchrony in IC multiunits in response to rapid chirp trains, human speech tokens were presented in order to test the hypothesis that neural classification of consonant-vowel-consonant combinations (Fig. 3.4A-B), which differed from each other primarily in voice-onset-time (VOT), would also be significantly improved with the drug. VOT is a cue which distinguishes voiced from unvoiced stop consonants (see Chapter 2, Results). A classifier model which created templates from peri-stimulus time histogram (PSTH) responses and classified single trials based on Euclidean distances from template PSTHs (Fig. 3.4C), was used on IC responses 30 days after ouabain treatment with the vehicle and drug conditions. (see Chapter 2, Methods – an important difference in the AUT study is that averaged site-by-site, rather than ensemble, classification was used to avoid ceiling performance in IC multiunit classification).

AUT3 administration significantly improved speech token classification by IC responses (two sample t-test, p < 0.05; Fig. 3.4D). Increased average accuracy was seen with the 60 mg/kg dose relative to the 30 mg/kg dose. By contrast, AUT3 administration did not significantly improve ACtx classification (two sample t-test, p = 0.29). Rather, the trend in the ACtx was toward degraded classification with increasing drug dosage, however this effect was not statistically significant.
FIGURE 3.4. AUT3 administration improves the classification of speech tokens in the IC, impairs classification for ACtx sites.

(A) Example speech token recorded from a human speaker and pitch shifted to mouse hearing range (B). Formants are highlighted in yellow. (C) Schematic of confusion matrix indicating correct classification using a PSTH-based site-by-site (IC) or ensemble (ACtx) classifier. (D) Confusion matrices showing mean classification across vehicle and drug conditions for IC sites. Colormap along the diagonal indicates mean probability of veridical classification (E) Classification data for ACtx sites
AUT3 administration significantly improves chirp train classification in the IC, but not ACtx

IC and ACtx neurons may represent amplitude modulations via spike timing or spike rate, or a multiplexed code utilizing both response features (Krishna and Semple, 2000; Lu et al., 2001; Johnson et al., 2012). In order to further test the encoding capacity of multiunit responses in both brain areas, the PSTH-based classifier model was trained and tested on responses to chirp train stimuli of 1-320 Hz, presented at 20 dB above threshold. Because of their tendency towards synchronization versus purely spike rate-based encoding, single IC sites can typically classify many of the presented chirp rate frequencies with a high degree of accuracy. By contrast, ACtx units, if tuned at all, tend to show narrower spike rate-based tuning to a specific set of chirp rates, while responding little or not at all to chirp trains at other frequencies. Therefore, to avoid ceiling performance on classification, IC averaged site-by-site classification performance is shown, whereas to ensure some level of classification accuracy, ensembles of 20 neurons were utilized for ACtx classification.

As shown in Fig. 3.5, IC multiunits (Fig. 3.5A) perform significantly better at PSTH-based classification of chirp train frequency with increasing dosage of AUT3 (two sample t-test, p < 0.001). This improvement is manifested both as an increase in average classification accuracy, and an extension of accurate classification to higher frequencies which were poorly classified in the drug condition (Fig. 3.5A, upper right quadrant of confusion matrix). The improvement in classification can likely be attributed to the increase in synchronization shown in Fig. 3.3. Concurrently, simultaneously recorded multiunit ensembles in ACtx did not perform at significantly different levels
of accuracy with increasing drug dosage (Fig. 3.5B), although a trend towards less accurate classification at the 60 mg/kg dose was observed.
FIGURE 3.5. AUT3 improves classification of pulse trains in the IC, but does not significantly alter classification in ACtx.

(A) From left to right, confusion matrices representing mean classification for IC sites in the vehicle, 30mg/kg AUT3, and 60 mg/kg AUT3 conditions. Cortical data is shown in (B). (C, D) Bar plots showing mean probability of correct classification for IC (C) and ACtx (D) across drug conditions.
Figure 3.5 (continued)
Trial-by-trial reliability increases with increasing drug dosage in IC responses to chirp train stimuli

Cycle-by-cycle vector strength increases in the IC with administration of AUT3 suggested that the modulation of Kv3.1 currents allowed responses to synchronize more precisely and reliably to individual chirps within each 500 ms trial. In order to investigate the compound’s effect on IC spiking reliability across trials, the Fano factor (ratio of spike rate variance to the mean) was calculated in three time windows relative to the stimulus (Fig. 3.6A): (1) the onset response, or first 50 ms of the response, (2) the sustained portion of the response, from 51-500 ms and (3) the 100 ms pre-stimulus spontaneous portion of the response. In all three time windows, administration of AUT3 significantly decreased trial-by-trial variability, with a minimal variability in the (most variable overall) onset portion of the response with a 30 mg/kg dose (two sample t-test, p < 0.01; Fig. 3.6B), while progressive reductions with increasing dosage from 30 to 60 mg/kg were observed in the two other time windows (two sample t-test, p < 0.01; Fig. 3.6C-D). Notably, administration of the compound resulted in a reduction of the mean Fano factor of below 1 in the sustained and spontaneous time windows, indicating that significant variability was essentially eliminated with AUT3.
FIGURE 3.6. AUT3 administration lowers trial-by-trial variability during onset, sustained, and spontaneous portions of response to broadband pulse trains.

(A) Example PSTH with onset (0-50 ms), sustained (51-500 ms) and spontaneous (501-1000 ms) portions indicated. Orange line indicates presence of stimulus. (B) Mean fano factor (spike count variance divided by the mean) for IC sites in each of the three drug conditions, for the onset (B), sustained (C) and spontaneous (D) portions of the response.
Figure 3.6 (continued)
Ceiling classification performance is achieved in the IC with progressively smaller multiunit ensembles upon administration of AUT3.

As a final step, we explored how classification was performed by an ensemble of multiunit recording sites of varying size. After ouabain treatment, many response channels carrying cochlear output are eliminating, thus much of the redundant information carried across multiple auditory nerve fibers is lost. This loss of output channels could have a large impact on network-level encoding in higher brain regions, such that processing may have to be pooled over a larger number of neural responses in the IC and ACtx in order to overcome noise and transmit accurate information regarding external sound stimuli. By increasing the precision and reliability of IC multiunit temporal encoding, the convergence and pooling requirements across IC multiunits may be eased somewhat, so that a greater degree of information for accurate stimulus classification is encoded by a smaller number of sites.

This concept was investigated by repeatedly sampling ensembles of 5 – 30 randomly selected recording sites from the IC and ACtx and comparing changes in the mean probability of veridical classification across speech sounds (Fig. 3.7A) and pulse rates (Fig. 3.7B). These trends support and extend upon the classification accuracy estimated from individual recording sites. In the IC, classification accuracy improved with larger ensembles. AUT3 was associated with improved classification for pulse rate (n-way ANOVA; F(2) = 67.65, p < 0.001) but an overall impairment for speech sounds (n-way ANOVA; F(2) = 4.36, p < 0.05). Decoding either stimulus type from ACtx spiking was less successful and AUT3 was associated with further degradation (speech, F(2) = 15.55, p < 0.001; chirps, F(5) = 0.55, p = 0.741).
FIGURE. 3.7. Probability of correct classification increases with larger ensemble sizes, AUT3 allows growth function to saturate at smaller ensemble size for IC sites encoding pulse trains.

Example of single site (A) versus 20-site ensemble (B) for encoding CVC speech tokens in ACtx. (C) Probability of correct classification of speech tokens as a function of ensemble size, for IC (left) and ACtx (right) responses. Line color represents drug condition. (D) Same as in (C), but for responses to pulse trains.
Figure 3.7 (continued)

A. Single site classification, CVC speech tokens

B. Ensemble classification (20 sites), CVC speech tokens

C. Probability of correct classification for speech tokens:
   - Vehicle 30 m/k AUT3
   - Vehicle 60 m/k AUT3

D. Probability of correct classification for pulse trains:
   - Vehicle 30 m/k AUT3
   - Vehicle 60 m/k AUT3
Discussion

The experiments described here document the first time that a novel compound which positive modulates Kv3.1 currents via systemic administration (AUT3) has been tested in vivo with awake, simultaneous neurophysiological recordings performed in the IC and ACtx. Further, these experiments introduce the use of human speech token stimuli and machine learning analyses in order to probe the effect of AUT3 on neural encoding of complex stimuli beyond specific metrics such as vector strength and firing rate. The observed results largely agree with in vitro investigations into the effect of AUT3 in MNTB slice and cell culture (Brown and Kaczmarek, under review), in that the compound is capable of increasing trial-by-trial reliability, as well as temporal precision and synchronization, in cases where a lower level, or altered level, of Kv3.1 is inferred based upon previous studies linking auditory experience with Kv3.1 modulation (von Hehn et al., 2004). This inference will have to be confirmed in future histological analyses of brain tissue of ouabain-treated mice, to directly test the hypothesis that Kv3.1 expression levels are reduced in this particular hearing loss model.

Notably, the compound exerted the most obvious and significant effects in the IC, whereas in ACtx the effects of the compound either did not significantly affect encoding metrics tested here, or significantly suppressed the magnitude of response (Fig. 3.2). This result, however, makes sense within the context of differential Kv3.1 expression along the auditory processing pathway (Li et al., 2001). The IC and ACtx diverge significantly in terms of the firing rate and synchronization demands of individual neurons and thus, the specific complement of ion channels that different cell types must express in order to function normally within the information processing circuit. For example, most neurons of the central nucleus of the IC express some level of Kv3.1, whereas the
The experiments described here provide an intriguing starting point for questioning the role of AUT3 in adjusting physiological responses to sound, however the improvement in vector strength evidenced in the midbrain with AUT3 after ouabain do not necessarily indicate that the compound could have a therapeutic benefit in terms of perception. In order to more directly address this issue in the present ouabain mouse model, careful behavioral studies with discriminatory, rather than simple detection, behaviors would be necessary.

Although the different expression patterns and cell types of the midbrain versus the cortex may seem like a confounding variable in this study, the differences could point to the possibility of AUT3 conferring distinct benefits due to its actions in the distinct brain areas. For example, although precise timing of cortical responses was not improved with AUT3, the magnitude of cortical responses was suppressed, indicating that inhibition may have been increased. This suppression of cortical sound-evoked activity could implicate the compound in the treatment of
tinnitus, a common ailment thought to be connected to cortical hyperexcitability after hearing loss. Thus, the same diversity of cell types and channel expression patterns that cause an array of perceptual problems following hearing loss could also underlie a multiplicity of benefits from a single compound. Importantly, the compound did not increase cortical inhibition to the extent that created noticeable sedation or other behavioral deficits in our mouse model.
General Discussion

In order to develop a wider diversity of effective treatments for specific types of hearing loss in humans, the understanding of several aspects of central auditory encoding must be broadened. Among these, studying the transformation of precisely timed, isomorphic representations of stimulus waveforms at the periphery to poorly timed, nonisomorphic representations at the level of the cortex is particularly difficult because of the lack of convergence in the literature regarding the nature of cortical encoding of sound feature preference. This work has largely been hampered in the past by inconsistencies across studies in terms of anesthetic state and stimulus set. A more unified theory of central auditory encoding may be reached with less biased receptive field estimates, and a more holistic view of receptive field structure which takes into account the ability of auditory cortical neurons to integrate tuning to multiple parameters simultaneously in order to form robust, stable percepts of auditory objects. With these ideas in mind, I have presented in Chapter 1 a closed-loop, online stimulus optimization procedure which rapidly and reliably converged onto regions of the multidimensional sound feature space which consistently drove ACtx and IC single neurons at high rates. With this method, it is clear that the encoding of feature preference with spike rate is robust in the ACtx, contrary to prior studies which argued that ACtx neurons fire very few spikes in response to preferred sound stimuli, therefore carrying little information about stimulus identity in their firing rates.

I found that in awake ACtx, although responses are sluggish relative to peripheral responses, and do not precisely mimic the acoustic structure of sound stimuli, they retain valuable stimulus identifying feature tuning while discarding information that may hinder stable auditory object perception. For example, ACtx neurons were more level tolerant than IC neurons, a fundamental
transformation along the processing hierarchy that could conserve the efficient coding of identifying features of a stimulus while allowing the representation to be consistent across sound level. As sound sources in the everyday environment must be identified in a variety of sound level conditions (e.g. identifying a friend’s voice whether they are close by or far away), this transformation appears to be beneficial for auditory object formation.

Given the complexity of central computations that must take place in order to achieve these efficient hierarchical transformations, it is not surprising that a massive loss of cochlear output can wreak havoc on aspects of stable auditory perception. However, what is even more surprising is that many of those aspects appear to be robust in the face of peripheral insult. In an animal model of profound cochlear neuropathy (Chapter 2), I found that cortical rate encoding remained relatively intact after roughly 95% of Type 1 SGNs were selectively eliminated. Moreover, animals retained sound detection behavior after denervation. These results parallel many of the clinical findings in humans with profound neuropathy, indicating that the mouse ouabain model could be extremely useful in deconstructing the neural mechanisms behind suprathreshold hearing impairment, a class of hearing impairment for which there is currently no effective therapy.

In parallel with recent progress in the understanding of suprathreshold hearing impairment, avenues of treating central encoding deficits with compounds aimed at adjusting specific ion conductances in order to precisely control cell excitability are now emerging. One such compound, a novel positive modulator of the Kv3.1 delayed rectifier potassium channel, was tested in the experiments described in Chapter 3. After the persistent temporal encoding deficits in IC were identified in Chapter 2, I used the same mouse model to test whether systemic administration of the compound (AUT3) could restore IC multiunit synchronization and trial-by-trial variability. These metrics, as well as accurate classification of speech tokens and chirp trains by IC responses, were
significantly improved with administration of the compound, indicating its potential as a therapeutic
treatment after peripheral neuropathy, noise exposure or other forms of hearing loss which render
central temporal encoding mechanisms impaired. More studies must be done in order to elucidate
the compound’s effects in ACtx, which are likely to directly impact perception. Initial studies,
described here, from extracellular recodings of ACtx multiunits, indicate that the compound may
suppress responses in ACtx, which is not surprising given that the Kv3.1 channel is primarily
expressed in inhibitory interneurons. What has yet to be gleaned from these results is whether the
compound differentially affects intracortical computations or if suppressive effects are the result of
feedforward inhibition originating in the IC. In addition, it is unknown whether the network-level
effects of adjusting the Kv3.1 current ultimately aids subjects in perceptual discrimination tasks, or
in the case of human subjects, speech comprehension in challenging environments.
Bibliography


