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Stimulus change detection in phasic auditory units in the frog midbrain: frequency and ear specific adaptation

Abhilash Ponnath,

Neuroscience Center, Department of Otorhinolaryngology, Louisiana State University Health Sciences Center, 2020 Gravier St., New Orleans, LA 70112, USA

Kim L. Hoke, and

Department of Biology, Colorado State University, 1878 Campus Delivery, Fort Collins, CO 80523-1878, USA

Hamilton E. Farris

Neuroscience Center, Department of Otorhinolaryngology, Louisiana State University Health Sciences Center, 2020 Gravier St., New Orleans, LA 70112, USA

Hamilton E. Farris: hfarris@lsuhsc.edu

Abstract

Neural adaptation, a reduction in the response to a maintained stimulus, is an important mechanism for detecting stimulus change. Contributing to change detection is the fact that adaptation is often stimulus specific: adaptation to a particular stimulus reduces excitability to a specific subset of stimuli, while the ability to respond to other stimuli is unaffected. Phasic cells (e.g., cells responding to stimulus onset) are good candidates for detecting the most rapid changes in natural auditory scenes, as they exhibit fast and complete adaptation to an initial stimulus presentation. We made recordings of single phasic auditory units in the frog midbrain to determine if adaptation was specific to stimulus frequency and ear of input. In response to an instantaneous frequency step in a tone, 28 % of phasic cells exhibited frequency specific adaptation based on a relative frequency change ($\Delta f = \pm 16\%$). Frequency specific adaptation was not limited to frequency steps, however, as adaptation was also overcome during continuous frequency modulated stimuli and in response to spectral transients interrupting tones. The results suggest that adaptation is separated for peripheral (e.g., frequency) channels. This was tested directly using dichotic stimuli. In 45 % of binaural phasic units, adaptation was ear specific: adaptation to stimulation of one ear did not affect responses to stimulation of the other ear. Thus, adaptation exhibited specificity for stimulus frequency and lateralization at the level of the midbrain. This mechanism could be employed to detect rapid stimulus change within and between sound sources in complex acoustic environments.

Keywords

Stimulus specific adaptation; Auditory stream; Stimulus change detection; Binaural; Frequency

Introduction

The ability to process stimulus change is critical for the perception of many communication sounds (Bregman 1990). Indeed, changes in a signal's acoustic parameters are often used by receivers to interpret information from the sender (Bradbury and Vehrencamp 1998). For example, within signals from a single source, spectral change (e.g., frequency modulation, FM) may enable such processes as phoneme identification (Lieberman et al. 1952, 1957, 1961) and species recognition (Wilczynski et al. 1995). More broadly, a change in sound parameters could be used to segregate multiple sources (Bregman 1990; O'Connor and Sutter 2000; Carlyon 2004; Fay 2008; Demany and Semal 2008). One mechanism for detecting such changes is stimulus specific adaptation (SSA), neural adaptation to constant (ongoing), or high probability stimuli, while maintaining the ability to respond to stimulus change or low probability stimuli (Naatanen 1995; Ringo 1996; Ulanovsky et al. 2003; Ulanovsky et al. 2004; Nelken and Ulanovsky 2007; Reches et al. 2010). SSA often refers to the adaptive sensitivity exhibited in response to a change in repetitive or pulsed stimuli (Ulanovsky et al. 2004). It is commonly tested using a method similar to that for the mismatch negativity response (MMN), a negative voltage component of gross multiunit recordings in response to a novel stimulus (Naatanen et al. 2001; Nelken and Ulanovsky 2007). In this design, adapting stimuli may be presented hundreds of times with novelty defined by a percentage of presentations (e.g., Tikhonravov et al. 2010), such that a series of high probability pulses are interleaved with low probability, "oddball" pulses (Ulanovsky et al. 2003; Perez-Gonzalez et al. 2005; Malmierca et al. 2009; Szymanski et al. 2009). MMN is, thus, often limited to relatively long time scales based on its reliance on stimulus probability, and does not match the time frame of many rapid changes in the auditory scene, including those detectable in human psychoacoustic experiments, which show, for example, that frequency specific adaptation is sensitive to changes occurring over <700 ms (Viemeister and Bacon 1982). To determine the possible contributions of SSA to a greater variety of stimulus changes, all potential time scales in which SSA occurs and the extent to which such neural adaptation is restricted to particular stimulus parameters must be understood.

To address adaptive sensitivity to rapid stimulus changes that do not follow repetitive pulsed stimulation, an alternative experimental design to that for MMN is the use of a pair of pulses, an adapting pulse and a change pulse, which probes the minimum stimulus difference required to overcome adaptation to the preceding pulse. This latter design has shown that SSA in mammalian cortical and midbrain cells can detect changes over hundreds of milliseconds following only a single presentation of an adapting stimulus (Muller et al. 1999, 2001; Malone and Semple 2001), a speed potentially explaining the sensitivity found for SSA in behavioral tests (Viemeister and Bacon 1982). A potential neural substrate for rapid adaptive sensitivity is the rapidly adapting responses of phasic units that are common in central auditory nuclei. Such units, which may respond only to the onset of a stimulus (Gooler and Feng 1992; Bass et al. 2005; Brimijoin and O'Neill 2005; Yang et al. 2009), are often found to be well suited for processing changes in stimulus amplitude or temporal properties (Hall and Feng 1991; Feng and Lin 1994). Our study, however, extends these results to assess whether the fast adaptation in these cells is also sensitive to fast changes in stimulus spectrum and space (i.e., lateralization). Because these units are not typically studied in the context of SSA, our study complements measures of adaptive sensitivity using long-term repetitive stimulation.

In the acoustic spectral and spatial domains, the adaptation of cells in nuclei exhibiting convergent circuitry are potentially well suited to detect stimulus change, in that adaptation could exploit across-channel processing (e.g., Reches et al. 2010). This means that neurons

could receive separate channels of stimulus input, coding different stimulus information (Heinz et al. 1996), and thus, capable of independent (channel-specific) adaptation. Although much SSA work has focused on cortical SSA (Condon and Weinberger 1991; Malone et al. 2002; Ulanovsky et al. 2003; Taaseh et al. 2011), any site with convergent inputs could, in principle, contribute to channel-specific adaptation. Recent evidence shows that adaptive sensitivity to stimulus change occurs in subcortical nuclei in mammals (Perez-Gonzalez et al. 2005; Yu et al. 2009; Malmierca et al. 2009; Antunes et al. 2010; Lumani and Zhang 2010; Bauerle et al. 2011) that is later refined in the cortex (Szymanski et al. 2009). From an ethological perspective, understanding the specificity of adaptation in subcortical nuclei is important because processing stimulus change within and between sources in complex acoustic environments plays a crucial role in social communication across taxa, including non-mammals (Krebs 1976; Searcy 1992; Feng et al. 2009; Hemmi and Merkle 2009; Dong and Clayton 2009).

In our attempt to address the acoustic parameters that determine the stimulus specificity of rapid adaptation, we chose to examine a major sensory processing site with convergent input in a taxon in which the challenges of sensory processing during functionally relevant behavior are well-understood. Using recordings of single auditory units of the torus semicircularis (TS; homologue to the inferior colliculus; Bass et al. 2005; Wilczynski and Endepols 2007) in awake frogs, this study focused on the stimulus specificity of adaptation by measuring the extent to which the rapid and complete adaptation of phasic auditory units is sensitive to changes in frequency and ear of input. The results show that stimulus information coded separately in the periphery produces separate adaptation on a time scale of <200 ms. Furthermore, in the frequency domain, the specificity of adaptation is based on relative not absolute stimulus differences, potentially allowing for flexible processing of stimulus change.

Methods

Animals

Frogs, *Rana pipiens* ($N = 200$ yielding 149 analyzed cells), were supplied by licensed commercial vendors. All animal care and experimental procedures were performed in strict accordance with the protocols established by the veterinarians on the Louisiana State University Health Sciences Center Animal Care and Use Committee (protocol to HEF).

Stimulus production

Acoustic stimuli were generated using a TDT II DA3 16 bit D-A converter (20 μ s sample period) and amplified using a Harmon/Kardon integrated amplifier. Stimulus amplitude was controlled with TDT PA2 programmable attenuators. For free field experiments testing frequency specific adaptation (FSA), acoustic stimuli were presented from a single Fostek #FE127 broadband speaker positioned at 0° normal to the front of the frog (30 cm distance). Thus, stimuli from this speaker evaluated responses in monaural or binaural units. For dichotic acoustic stimuli testing ear specific adaptation (ESA) in binaural units, sounds were presented at 1 mm from each tympanum using Etymotic (ER 4 Micropro) headphones attached to 1 cm length plastic tubes. All speakers were calibrated using a Bruel and Kjaer B&K 2608 measuring amplifier with a B&K model 4133 1/2-in. microphone. The calibration system was checked using a B&K 4220 pistonphone calibrator. Free field calibration was done at the position of the recording site, directly between and dorsal to the two tympana. Each headphone was independently calibrated 1 mm from the tube. Because the tubes were not sealed to the head, headphones were also calibrated at the contralateral ear, showing that external propagation resulted in >21 dB attenuation at the contralateral

tympanum. Symmetry between the two headphones was ± 1.5 dB at all frequencies. All frequency components of the ambient noise were measured to be 21.5 dB SPL (peak ambient noise range 120–230 Hz), the lower limit of the microphone's dynamic range.

Preparation and recording

To assess the breadth of stimuli to which adaptation was generalized, we used in vivo extracellular electrophysiology. Following general anesthesia (2.5 % urethane immersion, 20 min), the midbrain of adult *R. pipiens* was exposed by resecting a small piece of skull dorsal to the optic tectum. Topical analgesia of the surgical area (from the start of surgery to the end of testing) was accomplished using dibucane cream (0.9 %). The head wiping reflex was monitored as an indicator of anesthesia effectiveness. After 24 h recovery, frogs were immobilized by intramuscular injection of succinylcholine chloride (22 μ g/g body weight; Glasgow and Ewert 1997). Cutaneous respiration was maintained using a moist towel. Temperature was monitored with a digital thermometer. Immobilized frogs were mounted dorsal side up on an air table in a foam (Tecnifoam 4 in.; NRC 1.21 at 500 Hz) lined Faraday cage that was closed on all sides. The frog's mouth was propped open >0.5 cm, so that directional sensitivity was limited to binaural neural mechanisms (Feng and Shofner 1981; Pinder and Palmer 1983). Extracellular electrophysiological activity was recorded using thin-walled glass micropipettes filled with 4 M NaCl (10–100 M Ω ; but broken to change resistance ad hoc). Neural responses were amplified (GRASS P511 with high impedance head stage) and digitized (100 μ s sampling period) using a TDT AD3 and System II array processor with custom written software. Auditory units were isolated using a series of search stimuli that cover the range of frequency sensitivity and call spectrum (~ 0.1 –4 kHz) in *R. pipiens* (Liff 1969; Mecham 1971; Mudry et al. 1977; Fuzessery and Feng 1982, 1983; Ronken 1991; Goense and Feng 2012). Search stimuli included (but were not limited to) a 60-ms Gaussian noise, a 30-ms tone and a 20-ms band limited noise centered at 2 kHz. Units were considered isolated if they initially showed >20 dB signal to noise ratio in the voltage recording (peak action potential voltage/noise), although this ratio sometimes changed during the course of a recording.

After isolating a unit, its frequency tuning between 0.2 and 5 kHz (200 Hz steps) was characterized before proceeding to FSA or ESA stimulus sets. Neural thresholds to 200 ms pulses were measured using an adaptive procedure with five stimulus presentations at each amplitude ($3/5$ down, $2/5$ up; ± 3 dB resolution). Thus, a unit's best frequency (BF; frequency with lowest threshold) was measured for later use in testing adaptation sensitivity. This single tone method did not allow measuring of any inhibitory bands. After completing all experiments, the flanks of the tuning curve were modeled using the rounded exponential equation (roex) so that their equivalent rectangular bandwidths (ERB) could be calculated. This method of auditory bandwidth analysis has been used across numerous taxa (Patterson et al. 1982; Hartmann 1998; Witte et al. 2005; Ponnath and Farris 2010), and was chosen so that the entire tuning curve and its dynamic range would contribute to comparisons between cells (cf. filter Q, which would have been limited to a particular cross section of the curves, for example). All roex fits were statistically significant ($P < 0.00024$).

Following measurement of tuning curves, a series of stimuli was used to measure either frequency or ear specific adaptation, as detailed below. In all tests, stimulus period was 2 s to prevent interstimulus adaptation (Zhao et al. 2011). When all recordings were completed, we injected current at the recording site (or at a particular depth) to produce electrolytic lesions, which were subsequently resolved using Nissl staining (Fig. 1a) to calibrate the stereotactic position of the recording site (i.e., electrode depth) measured with a calibrated micromanipulator (Narashige). Animals were euthanized for tissue fixation and staining using the external anesthesia agent (urethane immersion).

Frequency specific adaptation

Adaptation is defined as the reduction in response to a constant or maintained stimulus (Adrian and Zotterman 1926). In this study, adaptation is the discontinuation of action potentials to an ongoing acoustic stimulus. Change detection is the subsequent reappearance of action potentials in response to stimulus change. Change detection was easily determined by application of the experimental design below because we only included isolated units exhibiting phasic excitation: 1–2 action potentials to a particular phase of an auditory stimulus (e.g., onset; Table 1) with very low spontaneous rate (Joermann 1988; Gooler and Feng 1992).

Whether or not phasic cells exhibited frequency specific adaptation (FSA) was characterized using three forms of frequency change: (1) tonal stimuli with an instantaneous frequency step; (2) continuous frequency modulation (FM); and (3) tonal stimuli with transient frequency change. The latter two regimes determine whether our FSA categorization using tonal steps reflected a general propensity of cells to respond to frequency changes. All stimuli in each test were presented at a minimum of 20 replicates.

Frequency specific adaptation: frequency steps

Using a sequence of two tones with different frequencies, FSA in response to frequency steps was determined by measuring whether adaptation to the first tone was maintained for the subsequent one. Confirmation that responses to the frequency change were not due to differences in sensitivity to the first and second frequencies was accomplished by presenting a single tone with an amplitude step. FSA cells were defined as those exhibiting a release from adaptation in response to a frequency change, but not amplitude change. Details of stimulus sets and statistical analyses follow.

Stimulus set 1—A 200 ms pure tone (0.1 ms ramps; a constant amplitude of 35 ± 13 dB re. threshold, this range was 72–90 dB SPL across all cells) near each unit's best frequency (± 200 Hz) confirmed the phasic response. Stimulus set 2: The 200 ms tone was then altered to create a two-tone sequence in a single pulse with a single frequency step change (Malone and Semple 2001) at the 0 crossing closest to 100 ms (i.e., the middle of the pulse), preventing transients or clicks (Formby and Forrest 1991). This stimulus tests whether adaptation produced by the first frequency is overcome by the frequency change. The first frequency (F1) is that presented in stimulus set 1. The second frequency (F2) varies, producing an FSA curve, which shows the probability of overcoming adaptation as a function of the frequency change from F1 to F2 (Fig. 1b, c). The frequency resolution of F2 sampling varied due to the tuning curve of each cell and our use of a quasiadaptive procedure: F2 started at one edge of the tuning curve and was adjusted toward F1 (near the BF) until there was a change in the adaptation response (i.e., action potentials in response to the change). The size of these initial F2 adjustments depended on the bandwidth of the tuning curve and usually started at ~25 % of the half bandwidth. The FSA boundary was then measured by halving subsequent steps. The procedure was then repeated on the other side of the tuning curve.

Stimulus set 3—Tuning curves are not flat, meaning a change in frequency is correlated to a change in sensitivity (i.e., position on the I/O curve). Because F1 and F2 were presented at the same amplitude, release from adaptation could be due to a difference in sensitivity (rather than frequency) to the F1 and F2 stimuli (i.e., the level above threshold differs for F1 and F2). Thus, to ensure that change detection was not due to a subjective amplitude change, we presented a 200-ms stimulus at the F1 frequency with an amplitude step over the final 100 ms that started at the nearest 0 crossing. The amplitude step ranged over 36 dB (± 6 , 12

or 18 dB; Fig. 1d) to cover much of the dynamic range of many frog auditory units (Feng 1982). For cells in which F1 was exactly at the best frequency, FSA was confirmed if the negative amplitude step did not elicit adaptation release. In cases where F1 differed from the best frequency by 200 Hz, confirmation of FSA required the failure of both positive and negative amplitude steps in eliciting adaptation release.

Action potentials were characterized as being in response to either F1 or the stimulus change by simply splitting the response buffer into two windows divided at 100 ms of the stimulus, the Onset Response Window (ORW) including 0–100 ms from onset of F1, and the change response window (CRW), encompassing the next 100 ms after the stimulus change (onset of F2 or amplitude shift) (Fig. 1c). This was possible because no cells exhibited false alarms in response to pure tone control stimuli (i.e., action potentials in the CRW in stimulus set 1, when there was no stimulus change). Note, to make sure that offset responses (13 of 96 cells) were due to the frequency change and not simply to the offset of the adapting stimulus, an offset response had to be elicited to every F2 stimulus as well. This confirmed that the F2 was detected (see example in results).

A conservative statistical approach confirmed FSA using a combination of two criteria: the response rates to both the frequency and amplitude change stimuli. Using the Fisher exact test (Zar 1999), sensitivity to the frequency change was confirmed if the response rate in the CRW to frequency steps was not statistically different from 100 % (e.g., 17/20 stimulus presentations). Conversely, cells were excluded as FSA neurons if their responses to amplitude change did not differ from 0 % response at any of the amplitude levels (e.g., 3/20 stimulus presentation). Thus, cells which failed to significantly respond to any F1–F2 change (<17/20) or responded to the amplitude step (>3/20) were deemed non-FSA cells. For some cells, the three stimulus sets were repeated in random order several times, creating >20 replicates per set.

Frequency specific adaptation: continuous frequency modulation

The previous experiment used instantaneous frequency steps to elucidate FSA. But, in natural stimuli, changes in frequency are often produced as part of continuous modulation. Thus, these experiments tested whether FSA enabled sensitivity to continuous frequency change, confirming that data collected using step changes in tonal stimuli predicted a general tendency to detect frequency change. Specifically, we tested the prediction that, when compared to a tone, an FM stimulus elicits a greater increase in action potentials in cells exhibiting FSA compared to non-FSA cells. Only onset cells (FSA and non-FSA) were used in this test. Furthermore, cells deemed non-FSA due to their failure of the amplitude test (i.e., they detected both the frequency step change and the change in amplitude) were not included. This limited the assay to sensitivity based on frequency change alone. Following determination of FSA versus non-FSA using frequency steps (above), cells were presented (minimum 20 reps) with 200 ms linear FM sweeps (descending) across the 90 dB SPL bandwidth of a unit's tuning curve, such that the stimulus only included frequencies in the excitatory band. Note that different tuning bandwidths meant that FM slopes varied between cells. The response change was normalized to the number of action potentials elicited to the control tone stimulus at the best frequency [(spikes FM—spikes tone)/spikes tone]. The normalized change in action potentials was then compared between FSA and non-FSA cells (*t* test). Note that ascending sweeps were not used to maximize efficiency of recordings, as the descending sweeps were sufficient to test the hypothesis.

Frequency specific adaptation: frequency transients produced by gaps

By creating stimuli that differed in the amount of spectral and/or temporal change, we simultaneously tested the hypotheses that FSA cells are more sensitive than non-FSA cells to stimulus change in the form of transient (fast) spectral changes; and that this sensitivity is not based on faster temporal processing (e.g., ramp sensitivity). Transients are a product of fast amplitude modulation of tones, such as silent gaps in sinusoids (Formby and Forrest 1991). When these gaps interrupt a tone at a phase other than a 0 crossing, it provides a transient spectral cue along with the temporal cues of gap ramps and silence. These cues were uncoupled by presenting stimuli that either varied in the amount of transients or duration of a gap, thus independently testing sensitivity of adaptation to spectral and temporal cues.

These tests included phasic units that were previously characterized as FSA and non-FSA. Each cell was presented with three different sets of stimuli (at least 20 repetitions). Stimulus set 1: A best frequency (± 200 Hz) tone in which the gap generates both temporal (i.e., ramps and silent gap) and transient spectral (splatter) cues. Gap parameters were: 100 % depth, 0.01 ms ramp, and centered in a 200 ms stimulus. Stimulus set 2: A best frequency tone in which the gap is filled with noise to prevent 100 % gap depth (reducing the envelope or temporal cue) and leave predominantly a spectral cue. Stimulus set 3: A Gaussian noise with a gap generating a temporal cue only (Green and Forrest 1989; Formby and Forrest 1991). Onset and offset ramps of the entire stimulus were 0.1 ms, which did not produce transients. Gap durations were chosen based on sensitivity in the auditory nerve (Feng et al. 1994). For tonal stimuli, they were (in ms): 50, 20, 10, 5, 3, 2, 1, and 0.5; for the noise stimuli: 75, 50, 30, 20, 10, 5, 2, 1, and 0.5. Note that presenting a gap within a constant duration stimulus (Green and Forrest 1989; Wilson and Walton 2002) generates an inescapable tradeoff in stimulus control, as it causes the duration of gap markers (i.e., the sound pulses preceding and following the gap) to vary with gap duration. This has been shown to affect gap detection, but in a limited set of conditions (Grose et al. 2001): when the duration of the leading marker is short, < 50 ms (Phillips et al. 1998; Eggermont 2000). Thus, the shortest gap marker used here was 62.5 ms. This design was chosen to prevent a change in interstimulus interval, overall stimulus duration, and stimulus period, which could also affect adaptation (Zhao et al. 2011).

An action potential was scored as being in response to a gap (a hit) if it occurred in the change response window: from the start of the gap to the end of the trailing gap marker. At least 20 repetitions with control stimuli lacking a gap enabled measurement of false alarm rates (a response in the CRW when there was no gap), which was more important for noise stimuli. Gap detection was quantified by calculating the corrected hit probability, a measure from signal detection theory that is appropriate for sensory data (Swets 1986). A separate analysis of variance (SAS software) for each stimulus set tested the hypothesis that FSA cells and non-FSA cells differed in gap detection across all gap durations. The analysis (blocked design; Zar 1999) controlled for the fact that individual cells were represented in only one cell type (FSA vs. non-FSA). Based on our hypothesis that FSA cells respond to frequency changes in many forms, it was predicted that the FSA cells should show greater gap detection sensitivity than non-FSA cells whenever spectral transients are present.

Ear specific adaptation

From a systems point of view, at least one potential mechanism of SSA is that adaptation to different stimuli is separated through anatomically separate processing in the periphery, such as separate positions on the auditory epithelium or separate ears. Although the FSA experiments were consistent with tests of separate positions on the auditory epithelium,

responses to frequency steps could have arisen by other mechanisms. The ear specific adaptation (ESA) experiments described here were designed to directly test whether adaptation is specific to peripheral sensory channels: comparing information from the two ears involves distinct peripheral channels.

ESA was inferred by evaluating responses from four stimulus sets. Whereas FSA experiments used free field stimuli, these used earphones, separating the adapting and change stimuli to different ears. Because of this different setup, different animals were used in FSA and ESA experiments. We selected units ($N = 53$) for these experiments only if they exhibited excitatory—excitatory binaural phasic onset responses. After isolation, frequency tuning was measured for both the ipsilateral and contralateral (re. recording site) ears separately. Subsequently, a series of stimuli characterized whether adaptation was ear specific. All stimuli were presented at 90 dB (SPL) with 0.5 ms ramps at 20 repetitions. Stimulus set 1: Binaural and monaural presentation of a 200-ms tone (± 200 Hz from ipsi and contralateral BF) confirmed the unit was phasic for both ears and determined the rate of false alarms (if any) in the change response window (CRW) starting in the middle of the stimulus at 100 ms. Stimulus set 2: Both ipsi- and contralateral presentation of the same tone, but with only 100 ms duration. This confirmed there was no difference in response for the shorter stimuli. Stimulus set 3: Dichotic stimuli consisting of a 100-ms tone presented in ipsi-then-contralateral (and vice versa) sequence, totaling 200 ms duration. The interpulse interval was 0 ms, as the end of the preceding pulse in one ear was coincident with the start of the following pulse in the other (the onset and offset ramps were 0.1 ms). Thus, this stimulus presents an adapting pulse to one ear, followed immediately by a test pulse to the other ear. Due to propagation around the head (although >21 dB attenuation with our headphones), there is a potential detection cue that is not ear specific and based on monaural amplitude modulation. Thus, to confirm that any responses to change in stimulus set 3 were due to ear rather than amplitude differences or ramp sensitivity, stimulus set 4 simultaneously presented an identical 200 ms stimulus with ramped amplitude modulation (0.1 ms offset and onset) at 100 ms to both ears. The stimulus sets were presented in pseudorandom sequence, as stimulus set 1 was always first. As with FSA, statistical determination of release from adaptation due to the dichotic change in stimulation was based on whether response rates in the CRW of stimulus set 3 differed from 100 % (Fisher exact test). Cells that responded ($>3/20$ repetitions) to the ramps at 100 ms in stimulus set 4 were conservatively deemed not to exhibit ESA.

To test if ESA was limited to processing narrow band sounds (i.e., tones), the experimental sequence was repeated using Gaussian noise pulses. This sequence, however, did not include stimulus set 4 because the noise envelope was random, removing the necessary control against responses due to envelope modulation at 100 ms.

Results

Frequency specific adaptation: frequency steps

Ninety-six phasic cells were recorded in the torus semicircularis (83 onset; 11 onset-offset; 2 offset). Consistent with recordings in the frog midbrain, these cells represented approximately 30 % of the auditory units encountered (Narins and Capranica 1980; Gooler and Feng 1992; Penna et al. 2001). Their frequency sensitivity was typical for *Rana pipiens*, best frequency: 1071 (± 506) Hz; mean best threshold: 55 (± 14) dB SPL; mean lower and upper tuning curve boundaries: 529 Hz (± 348) and 2491 (± 1257) Hz. In 27 cells (28.1 %; 24 onset; 3 onset-offset), adaptation was clearly sensitive to frequency change in the form of a single step. Figure 2a, c shows an onset-offset unit that maintains adaptation throughout the presentation of a tone, but overcomes adaptation for an instantaneous frequency change.

Analysis of the frequency change required to elicit FSA in this cell (i.e., frequency change in which the response rate is not significantly different from 100 %; see methods) revealed that frequency steps of 15 or –45 Hz overcome adaptation (Fig. 2c). This means that sensitivity in the excitatory bandwidth of the cell (Fig. 3f) was not uniform: stimulation at one frequency did not cause adaptation of the entire excitatory band. In contrast, cells that did not significantly respond to any F1–F2 frequency were deemed non-FSA (Figs. 2b, d, 4j–l). Although the shapes of the excitatory bands in FSA cells exhibited the varied tuning that is typical of the torus (Fig. 3; Fuzessery 1988), there were no significant differences from non-FSA cells in their overall tuning or voltage responses (Table 1).

FSA curves (Fig. 4) fell in three categories relative to a unit's best frequency: two types which responded to only one direction of frequency change (low or high frequency steps elicit significant response rates) and those, that responded to both step directions. The size of the frequency step required to elicit FSA depended on the best frequency of the unit, as the mean relative frequency increase and decrease were 1.15 ± 0.12 and 0.84 ± 0.12 , respectively (Fig. 5). This constant sensitivity to ± 16 % change showed that for these phasic cells, spectral change was detected based on relative, not absolute differences.

Frequency specific adaptation: frequency modulation

The failure of a single frequency (F1) to cause adaptation of the entire excitatory band in FSA cells suggested that FM sounds could continuously stimulate un-adapted regions of the tuning curve. Thus, experiments tested the hypothesis that stimuli with continuously changing frequency would elicit greater excitation in FSA versus non-FSA cells. Before presenting FM stimuli, units were first characterized as FSA or non-FSA using frequency steps (above). Figure 6 shows that compared to responses to a tone at the best frequency, FM stimuli ranging in frequency within a unit's excitatory band elicited a greater relative increase in the number of action potentials in FSA versus non-FSA cells (Table 1). In each cell class, there was initial excitation to the stimulus onset for both tone and FM stimuli. However, whereas non-FSA cells remained largely adapted throughout the FM stimulus (as in their response to the tone), FSA cells exhibited further excitation, increasing their response by 56 %. This increase in excitation is interesting. Based solely on the shape of the tuning curves, this FM sweep is predicted to elicit a reduced response relative to the tone due to the reduction of stimulus energy at the best frequency. Therefore, the increased response to FM is interpreted as further evidence that adaptation was overcome due to the frequency change.

Because the absolute amplitude of the FM stimulus was constant, the amplitude relative to threshold changed as the frequency modulated across the tuning curves (Fig. 6). This potentially provided an amplitude (re. threshold) rather than spectral cue for release from adaptation. Amplitude control experiments (stimuli in Fig. 1) showed that amplitude change did not cause release from adaptation in FSA cells, however. Note that data are not shown for the amplitude control tests, as adapted cells simply remained adapted for the amplitude step. Furthermore, if amplitude (re. threshold) did cause release from adaptation, significant difference in action potential number between FSA and non-FSA cells would not have been expected, as both classes would have changed responses. Thus, release from adaptation was, again, due to frequency change, which, in this case, was continuous.

Frequency specific adaptation: frequency transients produced by gaps

Stimulus frequency change can take many forms, including spectral transients produced by fast temporal modulation. Thus, these experiments exploited the transients produced by the onset and offset ramps of silent temporal gaps (Fig. 7) to test: (1) whether FSA allowed for

transient detection in an ongoing stimulus, and (2) that this detection was not based on the speed of temporal sensitivity. Three sets of stimuli that produced spectral and/or temporal cues as to the presence of a gap (Fig. 7) were presented. For all stimuli, the corrected hit probability of detecting a silent gap decreased with shorter gap durations for non-FSA cells (Figs. 8, 9). For FSA cells, however, when spectral cues were available, no such effect was observed: for gaps in tones (i.e., gap produces splatter) and tones in which the gap was filled with noise, gap detection probability was nearly constant as gap duration decreased (Fig. 9; supplementary material 1). For these tone stimuli, gap detection probability in FSA cells was significantly greater than that for non-FSA cells (FSA vs. non-FSA tones: $F = 38.07$, $P < 0.0001$; noise in tones: $F = 25.15$, $P < 0.0001$). In contrast, for gaps in noise stimuli, there was no difference in gap sensitivity ($F = 0.05$, $P = 0.82$) (Fig. 9c), as both cell types exhibited decreasing responses as gaps got shorter. Thus, the difference in response between FSA and non-FSA cells was generated by sensitivity to spectral cues in FSA cells, as the noise stimuli provide temporal cues only, removing the FSA advantage. This means that FSA cells were not simply faster than non-FSA cells.

Taken together, the FM and gap tests showed that adaptation was sensitive to spectral change in the form of continuous modulation and transients, confirming that the method of categorizing cells as FSA or non-FSA using frequency steps revealed/implied a general tendency to respond to a change in frequency.

Ear specific adaptation

Whereas the previous experiments tested change detection in the spectral domain, stimuli here assessed whether adaptation was sensitive to changing binaural cues. In a new set of recordings (i.e., different from those used to measure FSA), 24 out of 53 binaural phasic onset units exhibited ESA. Figure 10a–e shows the responses of one such cell. It exhibited adaptation for ongoing monaural stimuli, but overcame adaptation when the stimulus alternated in an ipsi-then-contralateral sequence. This sensitivity was not due to sensitivity to amplitude modulation (Fig. 10e). In contrast, non-ESA cells exhibited similar monaural responses, but either failed to respond to the dichotic stimuli (Fig. 10h, i) or responded to the amplitude modulation control (Fig. 10o). Tests of whether any monaural response characteristics were predictive of ESA and non-ESA sensitivity revealed very little difference in sensitivity between the two cell categories. Pairwise comparisons of ipsi- and contralateral sensitivity (i.e., monaural) within ESA and non-ESA cells showed similar responses with only one exception: more action potentials were generated in contra- versus ipsilateral stimulation in ESA cells, but not non-ESA cells (Table 2). This difference was also realized when comparing these response characteristics between the two cell groups (Table 3). Thus, the lack of distinguishing monaural response characteristics meant that binaural adaptation properties were unlikely to be identified without explicit dichotic testing.

To test whether ESA was limited to within frequency comparisons, the dichotic test was repeated using noise stimuli. Of the 24 cells showing ESA to best frequency tones, 15 exhibited ESA in response to noise stimuli (Fig. 11): four units did not respond to noise, four did not exhibit significant rates of adaptation release, and one data set was incomplete. Likewise for the 29 non-ESA tone cells, seven switched to ESA responses for noise stimuli due to an increase in rates of adaptation release. Thus, although within frequency comparisons between the ears were not necessary, dichotic change detection was affected by frequency content in some cells.

Although adaptation was sensitive to dichotic stimulation, directional (sequence) sensitivity of ESA was not symmetrical for tone or noise stimuli. Whereas all ESA cells exhibit

excitation to the ipsi-then-contralateral stimulus change, only 13 and 18 % of cells detected the change in the contra-then-ipsi sequence for tones and noise, respectively.

Discussion

Across-channel adaptive sensitivity

Studies of audition have revealed several examples of across-channel processing, including comodulation masking release, profile analysis, and modulation discrimination interference (Hall et al. 1984; Green 1988; Yost and Sheft 1993; Moore 2008). All implicate circuitry employing a decision or “voter” module that integrates separate peripheral channels (Heinz et al. 1996). Central nuclei such as the torus semicircularis (and its homologue the inferior colliculus) are well known for integrative and convergent circuitry (Roth et al. 1978; Brunso-Bechtold et al. 1981), as evidenced by tuning curves with greater bandwidth than that in the periphery (Fuzessery 1988; Winer and Schreiner 2005). But broadband (i.e., multiple channel) tuning alone does not indicate the ability to compare information across the integrated channels. The sensitivity of adaptation described here, thus, elucidates a form of across-channel comparisons (e.g., change in frequency or location of stimulation) that is based solely on input within each unit’s excitatory band, as opposed to input from inhibitory sidebands. Our FSA data suggested that the acuity of these comparisons was based on frequency resolution in the periphery, as adaptation was overcome by a relative change in frequency ($\pm 16\%$), rather than an absolute frequency step size. This result is predicted by the increasing relationship between critical bandwidth and center (i.e., best) frequency in the auditory nerve (Ehret and Capranica 1980). Furthermore, the resolution of FSA at particular carrier frequencies matched that in the periphery. This was true even for the extremely acute case in which there was -45 and 15 Hz resolution (at 1.0 kHz; Fig. 2), as the critical bandwidths at that F1 in the auditory nerve range from 10 Hz to 1 kHz (Ehret and Capranica 1980).

Gap detection tests provided additional data consistent with separate adaptation for separate frequencies in FSA cells. In addition to the difference in FSA and non-FSA responses to tones with silent or noise-filled gaps, evidence of multiple adaptation channels is also revealed when gaps were presented in noise. Here, the ongoing information in all frequency channels was the same, with each channel receiving the same excitation and/or adaptation. Under these conditions, we propose that FSA cells became a single channel system like non-FSA cells, limiting processing to only temporal cues and thus, forcing the two types of cells to show similar responses. That is, for noise stimuli, FSA cells no longer could use adaptation to compare information across channels. This means that excitation, adaptation, and its release were not simply faster in FSA cells, as detection of gaps in noise was identical in the two cell classes. Thus, while both FSA and non-FSA cells receive convergent input (e.g., similar width ERBs), only one group shows evidence for treating those inputs independently.

Because critical bandwidths in the auditory nerve vary in size and may overlap, our FSA data are consistent with, but not conclusive for, across-channel processing. Thus, we used dichotic stimuli to control for peripheral overlap so that overcoming adaptation could only be accomplished by central circuitry (interaural mechanical processing was eliminated by keeping the mouth open; Rheinlaender et al. 1981). Thus, independence of adaptation across peripheral channels was confirmed through ESA. Note, however, that this independence of adaptation was not universal, as responses were not elicited to all frequency and directional changes. For example, like for similar tests in gerbil IC (Malone and Semple 2001), FSA responses were asymmetrical around the adapting frequency (F1). Furthermore, for ESA, responses were largely limited to the ipsi-contralateral sequences of ear stimulation.

Potential frequency and ear specific adaptation functions in phasic neurons

Adaptation or habituation over longer time scales (seconds) likely mediates certain auditory behavior in frogs (Megela and Capranica 1983). However, given the phasic neurons' rapid and specific responses, how might the adaptive sensitivity found here function in the auditory scene of *R. pipiens*? With regard to FSA, the neuroethology of segregating species specific calls in choruses likely includes processing rapid frequency changes (Ryan and Rand 2001), as the overlapping calls in multi-species aggregations may contain species specific frequency bands (Bee and Micheyl 2008). In addition, such processing could also be relevant to intraspecific call discrimination. Male *Rana pipiens* produce broadband calls of at least three types (Mecham 1971). There is significant within- and between-male variance in the frequency content of each call type (Larson 2004). The ability to quickly detect changes in frequency potentially enables receivers (male or female) to detect changes in call type and source identity during the complex sequences of calling found in the male chorus (Larson 2004).

When moving through a chorus, adaptation would, of course, be useful in comparing the position of sources relative to that of the head. Using stimuli that create interaural phase differences, previous studies have shown that adaptation in mammalian IC neurons is sensitive to changes in stimulus azimuth (Spitzer and Semple 1991; McAlpine et al. 2000; Ingham and McAlpine 2004), with phase sensitivity based on microsecond to millisecond scales (Yin and Chan 1988). An important difference between such tests and our data is the fact that stimuli used to test interaural phase difference require ongoing binaural stimulation with the same frequency, creating beats in the ongoing coding of phase at the two ears. When a source moves (relative to the head), there is a change in interaural phase difference causing excitation in the adapted cell. The stimulus change used to measure ESA, however, was never presented to the two ears at the same time, and thus, did not provide any ongoing phase cues. Furthermore, besides tones, a noise sequence was presented separately to the two ears, which further reduced phase differences at any frequencies. Thus, the stimuli in our study more closely modeled two different sources 180° apart. We propose that the ear specific adaptation measured here could potentially function in source segregation: adapting to one source, while remaining excitable to sound from a different direction.

Comparison to typical stimulus specific adaptation methodology

Our results complement previously published SSA studies by measuring the specificity of adaptation at fast time scales important to auditory processing, but not tested in experiments using long-term repetitive presentations of stimuli. Indeed, the MMN or oddball design is often limited to testing adaptive responses to only a subset of sounds found in the auditory scene: pulsed or repetitive auditory streams (Micheyl et al. 2005; Bee et al. 2010; Schul et al. 2012). Whether measured in gross recordings (Naatanen 1995; Naatanen and Alho 1995) or in single cells of the midbrain, thalamus and cortex, the experiments show that adaptation to common repetitive sounds may be overcome with presentation of a rare one (Ulanovsky et al. 2003; Perez-Gonzalez et al. 2005; Szymanski et al. 2009; von der BW et al. 2009; Antunes et al. 2010). These tests of SSA are different from those used here. Our use of fast adapting phasic neurons means that there was no build up of adaptation with repeated presentation of pulses, as the excitatory response (action potentials) was fully adapted following the onset of a single maintained stimulus. Furthermore, the phasic response distinguishes our data by avoiding an effect of response history (i.e., conditioning), in which variance in sensitivity to stimulus change is potentially explained by the history of a unit's activity, rather than stimulus history (McAlpine et al. 2000). Using phasic cells, response history in our dataset is identical for all stimuli (1 or 2 action potentials). For example, before being probed with a stimulus change, all adapting stimuli elicited the same number of

action potentials and all reach 100 % adaptation (Table 1, 3). This important control in our dataset notwithstanding, it is likely true that activity history that is below action potential threshold may still be varying in these cells.

Another control in our FSA experiments, the use of continuous rather than pulsed stimuli, meant that stimulus change was not set apart by any amplitude modulation as in other common SSA methods. As demonstrated by the gap detection data, silence (between pulses) by itself changes the maintenance of adaptation (Sun and Wu 2008). Thus, tests of change sensitivity that include silent intervals between stimuli may not be able to exclusively attribute responses to the parameter change of interest. In other words, two things may be changing a cell's adaptive state: the silence, which allows the cell to return to its preadapted state; and the subsequent stimulus change. We controlled for the need to measure the effect of silent intervals before stimulus change using an instantaneous change.

Although our focus on adaptation in general and acrosschannel independence, in particular, was supported by the data, other underlying mechanisms could be involved. Indeed, the auditory midbrain (but also more peripheral nuclei) exhibits complex circuitry with electrophysiology that can be mediated by a variety of mechanisms in addition to adaptation (Winer and Schreiner 2005). For example, models of complex IC responses incorporate across-channel (binaural) excitation and inhibition in addition to adaptation to integrate ascending auditory information (Cai et al. 1998a, b). Such integration can produce change (FM) sensitivity similar to that found here (Pollak et al. 2011), and potentially underlies the different sensitivities in FSA and non-FSA cells. Thus, independence of adaptation, while simple, functions as a parsimonious hypothesis for how such change detection could be accomplished. By focusing on the phenomenon of change detection, our data now form the fundamental basis for such tests at the intracellular and computational levels.

Conclusion

This study reached three important conclusions with respect to adaptive sensitivity in the frog auditory midbrain. (1) Adaptation to single frequencies in phasic cells did not necessarily adapt a cell's entire excitatory band, as excitation was clearly maintained for other stimuli. (2) Adaptive sensitivity in phasic cells appeared to be based on separate channels of peripheral input, which in the frequency domain resulted in adaptation based on relative frequency differences. (3) The sensitivity of adaptation shown here required no build up with repetitive stimulation and was not activity (action potential)-dependent, as all phasic cells showed the same activity prior to release from adaptation. The lack of build up means that these phasic cells are not only well suited for detecting fast temporal changes, but also fast changes associated with frequency modulation and source position, potentially important to processing varying communication sounds in a multi-source environment (Feng and Ratnam 2000).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BF	Best frequency
CRW	Change response window
ESA	Ear specific adaptation
ERB	Equivalent rectangular bandwidth
F1	First frequency presented
F2	Second frequency presented
FM	Frequency modulation
FSA	Frequency specific adaptation
MMN	Mismatch negativity
ORW	Onset response window
SSA	Stimulus specific adaptation

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Fig. 1.

Example of recording localization and schematics of stimuli used to test adaptation sensitivity to frequency step changes. **a** Coronal slice (20 μm thick) of midbrain with Nissl stain showing a recording site (*arrow*), which is enlarged in *inset*. **b** Schematic of a tuning curve and two frequencies used in frequency step tests. F1 is at the best frequency (BF), and F2 can range above and below the BF. **c** Schematic of stimuli used to test sensitivity to a frequency step. Stimulus set 1 comprised a constant 200 ms tone with frequency F1. Stimulus set 2 began with 100 ms at F1, then transitioned to F2, with the frequency change

occurring at the 0 crossing closest to 100 ms, as depicted in the higher time resolution sine wave below. Action potentials were sorted into either the onset response window (ORW) or the change response window (CRW). **d** To ensure responses in FSA cells were due to frequency change and not sensitivity change, stimulus set 3 included amplitude steps at the F1 frequency over a 36 dB amplitude range (± 18 dB) with no change in frequency. Cells were considered FSA if they had action potentials in the CRW after frequency changes in stimulus set 2, but not spontaneously in set 1 or after amplitude changes in set 3 (data for these amplitude control experiments not shown)

Fig. 2.

Example of FSA and non-FSA in single cells. **a** Post stimulus time histograms (PSTHs) for an onset/offset phasic cell in response to a tone. Adaptation was not maintained for frequency step changes. **b** PSTHs for an onset cell in which adaptation was maintained for frequency changes. **c–d** Curves showing the probability of detecting the F1 to F2 change as a function of F2–F1 (or F2/F1). *Dashed lines* represent significant change detection. Histogram bin width is 10 ms. Number of repetitions were 106, 76, 22 and 21 in **(a)**. **b** stimulus repetitions are 20 for each histogram. Stimulus amplitude in **a** and **b** were 82 and 80 dB SPL, respectively

Fig. 3.

Examples of tuning curves for several FSA and non- FSA cells. *Letters* correspond to FSA curves shown in Fig. 4. There was no difference in tuning characteristics between

Fig. 4.

FSA curves showing the probability of overcoming adaptation in response to an F1-to-F2 step as a function of F2/F1. Letters correspond to tuning curves shown in Fig. 3. **a–i** FSA cells. *Left, middle and right columns* show cells that detected frequency change in *downward, upward or both directions*. **j–i** Non-FSA cells did not exhibit significant frequency change detection. *Dashed lines* represent hit probability for significant change detection. Because F1 is at the best frequency (± 200 Hz), the actual F1-to-F2 frequency change can be determined using Fig. 3

Fig. 5. Frequency required to elicit FSA as a function of F1. *Solid and open symbols* are for frequency increases and decreases, respectively. The mean relative increase and decrease were 1.15 ± 0.12 and 0.84 ± 0.12 , respectively. Curves show a $\pm 16\%$ change as a function of F1

Fig. 6.

FSA versus non-FSA responses to continuous frequency modulated stimuli. **a** Tuning and voltage response of an FSA cell presented with a tone at the best frequency and an FM stimulus across the excitatory bandwidth (at 90 dB) of the tuning curve. **b** non-FSA cell for the same stimuli. Compared to non-FSA cells, there was a significant increase in action potential number to FM stimuli (re. tone stimuli) in FSA cells (Table 1)

Fig. 7.

a–d. Gap stimuli and their spectra used to test sensitivity to transient frequency change. The example tone is at 700 Hz. Action potentials (Fig. 8; Supplementary Fig. 1) were analyzed as occurring in the onset response window (ORW) or the change response window (CRW), the latter beginning at the start of the gap and ending at the end of the entire stimulus. The response windows varied with gap duration. False alarms were action potentials in the CRW for control stimuli without a gap (e.g., 200 ms tone or noise). **b** the gap produces both transient spectral and temporal cues in the tone; **c** a noise filled gap in a tone largely removes envelope fluctuations and produces spectral cues; **d** the gap in noise produces only temporal cues as the spectrum is the same as that for a noise without a gap (not shown)

Fig. 8.

Post stimulus time histograms showing responses of an FSA and a non-FSA cell to three different gap stimuli. **a** Gap in a tone which had both spectral and temporal cues. **b** Noise fills gap in a tone producing only spectral cues. **c** Gap in noise, producing only temporal cues. The FSA cell exhibited better gap detection than the non-FSA cell when spectral cues were present

Fig. 9.

Summary of gap detection for three different stimuli with different cues for stimulus change. **a–b** For FSA cells (*closed squares*), gap duration had no effect on corrected hit probability when spectral cues were present. There is a significant difference between FSA and non-FSA (*open squares*) hit probability for these two stimuli (FSA vs. non-FSA tones: $F=38.07$, $P<0.0001$; noise in tones: $F=25.15$, $P<0.0001$). **c** When only temporal cues were present, gap detection was similar in the two cell classes ($F=0.05$, $P=0.82$)

Fig. 10.

Example PSTHs and voltage traces of ESA and non-ESA responses to dichotic stimuli. Ipsi- and contralateral stimuli (*blue* and *red*, respectively) are shown above each PSTH. **a–e** ESA cell showing adaptation was not maintained for the ipsi-then-contralateral stimulus. **f–j** non-ESA cell in which adaptation was maintained, as the cell did not respond to dichotic stimulus change. **k–o** non-ESA cell which responded to dichotic change, but also responded to amplitude modulation. This test (**e, j, o**) controlled for the possibility that dichotic responses could have been mediated by sensitivity to amplitude modulation. Each stimulus is presented 20 times. The entire sequence of responses to all test and control stimuli (along with cell tuning) are shown in supplementary Figs. 2–4

Fig. 11.

Post stimulus time histograms for a cell showing ESA response to noise stimuli. Ipsi- and contralateral stimuli (*blue* and *red*, respectively) are shown above each PSTH. **a–b** Monaural response to 100 ms control stimuli. **c–d** Monaural response to 200 ms control stimuli; response after 100 ms revealed false alarms. **e–f** Dichotic stimuli revealing ESA for ipsi-then-contralateral stimuli

Table 1

Comparison of general and FM sensitivity in FSA and non-FSA cells

Parameter	FSA	Non-FSA	<i>P</i> value
N cells	27	69	
Best frequency (Hz)	948 ± 353	1,101 ± 559	0.069
BF thresh. (dB SPL)	54 ± 13	55 ± 14	0.360
ERB (Hz)	572 ± 293	563 ± 353	0.452
Action potentials per stim.	1.7 ± 0.5	1.6 ± 0.8	0.288
Latency 1st action potential (ms)	23.55 ± 7.26	24.23 ± 11.62	0.389
FM test (<i>N</i> cells)	14	36	
Proportional change in action potential count (FM vs. tone)	0.56 ± 0.44	0.19 ± 0.70	0.037

Columns are the parameters and samples sizes of cells determined to exhibit FSA and non-FSA response characteristics. *P* values are for *t* test comparisons of the means (FSA vs. non-FSA). Two non-FSA cells were not included in the latency measure because they exhibited offset responses (*N* = 67). FSA and non-FSA cells differed in their relative change in action potential count (re. a BF tone) in response to FM stimuli

Table 2

Comparison of monaural sensitivity: stimulation of the ipsilateral versus contralateral (re. recording site) ear within ESA and non-ESA cells

Sensitivity parameter	N	Ipsilateral side	Contralateral side	P value
<i>ESA cells</i>				
Best frequency (Hz)	21	1,185.7 ± 571.2	1,147.6 ± 428.5	0.7522
BF thresh. (dB SPL)	21	55.0 ± 9.5	45.9 ± 8.7	0.0030
ERB (Hz)	21	817.9 ± 366.7	818.9 ± 359.1	0.9893
Action potentials per stimulus	24	1.3 ± 0.6	2.1 ± 1.1	0.0009
Latency 1st action potential (ms)	24	23.7 ± 10.8	19.9 ± 6.5	0.0252
<i>Non-ESA cells</i>				
Best frequency (Hz)	24	1,233.3 ± 579.9	1,091.7 ± 408.5	0.1624
BF thresh. (dB SPL)	24	51.1 ± 11.6	43.3 ± 9.7	0.0031
ERB (Hz)	24	687.8 ± 358.4	706.0 ± 289.9	0.7711
Action potentials per stimulus	29	1.4 ± 0.5	1.5 ± 0.8	0.3038
Latency 1st action potential (ms)	29	19.5 ± 11.4	18.0 ± 10.7	0.0178

Columns are the: sensitivity parameter of the cells; number of observations; mean values for stimulation of the Ipsilateral and Contralateral sides (re. recording site) and twotailed *p* value of the paired *t* test. Although means are shown, tests were pairwise comparison within cells

Table 3

Comparison of sensitivity between ESA and non-ESA cells

Sensitivity parameter	N _{ESA}	ESA	N _{non-ESA}	Non-ESA	P value
<i>Ipsilateral side</i>					
Best frequency (Hz)	21	1,185.7 ± 571.2	25	1,264 ± 588	0.6509
BF thresh. (dB SPL)	21	55.0 ± 9.5	25	50.9 ± 11.4	0.196
ERB (Hz)	21	817.9 ± 366.7	25	712.1 ± 371.3	0.3385
Action potentials per stimulus	24	1.3 ± 0.6	29	1.4 ± 0.5	0.5526
Latency 1st action potential (ms)	24	23.7 ± 10.8	29	19.5 ± 11.4	0.1768
<i>Contralateral side</i>					
Best frequency (Hz)	23	1,126.1 ± 424.5	24	1,091.7 ± 408.5	0.7782
BF thresh. (dB SPL)	23	45.5 ± 8.5	24	43.3 ± 9.7	0.4038
ERB (Hz)	23	812.9 ± 349.5	25	706.0 ± 289.9	0.2588
Action potentials per stimulus	24	2.1 ± 1.1	29	1.5 ± 0.8	0.0341
Latency 1st action potential (ms)	24	20 ± 6.5	29	18.03 ± 10.74	0.4688

Columns are the: sensitivity parameter; number of observations of ESA cells; mean values of the ESA cells; number of observations of non-ESA cells; mean values of the non-ESA cells; and two-tailed *p* value of the two sample *t* test