Responses of Midbrain Auditory Neurons in Rats to FM and AM Tones Presented Simultaneously

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Abstract

Speech and other communication signals contain components of frequency and amplitude modulations (FM, AM) that often occur together. Auditory midbrain (or inferior colliculus, IC) is an important center for coding time-varying features of sounds. It remains unclear how IC neurons respond when FM and AM stimuli are both presented. Here we studied IC neurons in the urethane-anesthetized rats when animals were simultaneously stimulated with FM and AM tones. Of 122 units that were sensitive to the dual stimuli, the responses could be grossly divided into two types: one that resembled the respective responses to FM or AM stimuli presented separately (“simple” sensitivity, 45% of units), and another that appeared markedly different from their respective responses to FM or AM tones (“complex” sensitivity, 55%). These types of combinational sensitivities were further correlated with individual cell’s frequency tuning pattern (response area) and with their common response pattern to FM and AM sounds. Results suggested that such combinational sensitivity could reflect local synaptic interactions on IC neurons and that the neural mechanisms could underlie more developed sensitivities to acoustic combinations found at the auditory cortex.

Key Words: combinational sensitivity, frequency modulation, amplitude modulation, inferior colliculus, speech recognition

Introduction

Most natural sounds have components that are modulated in frequency and amplitude (frequency modulation, FM and amplitude modulation, AM). FM and AM sounds are considered building blocks of communication signals and their coding mechanisms are linked to speech recognition (12). Neural sensitivities to FM or AM sounds have been reported at various levels of the auditory pathways, and the responses typically change with stimulus parameters altered in a variety of ways (7, 11, 21-23, 28).

FM sensitivity, when defined as the selective response to frequency sweeps of one direction but not both (upward and downward changes in the tonal frequency), involves a central mechanism as it is not found at the auditory nerves (24). In an attempt to understand the basic response determinants and to unify the various stimulus parameters affecting FM responses, a simple classification of FM sensitivities was proposed for auditory midbrain neurons based on differential responses to FM and steady tones (19), namely: (a) FM “specialized” that respond to FM but not steady tone, (b) FM “insensitive” that respond to steady but not FM tone, and (c) FM “mixed” that respond to both FM and steady tones. FM “specialized” sensitivity emerges at the midbrain (or inferior colliculus, IC), as its incidence is low at the cochlear nucleus (24).
parallel classification for AM responses was also proposed (2, 20): (a) AM “specialized” with response during the rising phase, but not the steady phase of the AM envelope; (b) AM “insensitive” with response during the steady phase but not the rising phase; (c) AM “mixed” with response during both the steady and the rising phases.

Evoked potential studies in human showed that when FM and AM sounds are presented together, responses are larger in amplitude as compared to presentation of the sounds individually (10). Results of neuroimaging also support the presence of combined sensitivity to FM and AM as cortical fields activated by FM or AM stimuli appear overlapped (8). In auditory specialized animals like bats and songbirds, combinatorial sensitivities to multiple FM sounds are reported at single cell level in the cortex (14, 26). Similar sensitivity is also found at their midbrain (15). But combinatorial sensitivities to FM and AM sounds in the less specialized animals (like rodents) have not been studied. Furthermore, to what extent cortical responses may reflect what have been accomplished at the subcortical centers is unclear. Here we investigated at single cell level at the rat’s auditory midbrain, how responses change when FM and AM tones are both present. Results supported the presence of combination sensitive cells at the midbrain of rodents.

Materials and Methods

Animal Preparation

A total of 107 adult rats (Sprague-Dawley, 150-350 gm) were studied under urethane anesthesia (Sigma, 2 gm/kg i.p.). Detailed procedures were reported earlier (2). In brief, skull was opened over the occipital cortex for electrode entry to the underlying midbrain and cerebrospinal fluid drained at the foramen magnum to reduce brain pulsation. Skin incision was sutured to restore pinnae to the resting position for free-field sound stimulation. A screw was cemented onto the cranium for head fixation to a modified stereotaxic frame in a sound-attenuated room (IAC). Rectal temperature was controlled at 38°C with a thermal regulator. Sounds were delivered free-field from a loud speaker (Pioneer SP77, QUAD 306) placed 70 cm away, 30° in azimuth contralateral to the surgical side. Stimulus sound levels were calibrated at the site of the animal (B&K microphone 4191). The procedure was approved by the Animal Ethics Committee, NCKU.

Acoustic Stimuli

Slow Frequency Sweep for Characterizing Response Area (RA)

An exponential signal (0.5 cycle/sec) was digitally synthesized (TDT system II) and fed to the voltage-control-frequency input of a function generator (Tektronix FG280 Manufactory, Beaverton, OR, USA) to generate a glide tone of slowly-varying frequency to induce spikes from the cell. The range of frequency sweep was first adjusted to cover the cell’s best frequency and minimum threshold, and tone levels dropped systematically across 60 repeated trials (0.5-1.0 dB steps from high to low; 2-sec/trial; details see 3).

FM Stimuli

Two types of FM tones were generated by feeding digitally synthesized modulating waveforms to the function generator:

(a) Exponential FM: They were tones modulated by a family of exponential ramps (n = 3 to 6), each of different FM velocities, with ramps appearing intermittently in each stimulus trial. Tone levels dropped systematically across repeated trials in a session. To best activate the cells, three different ranges of ramp rates were used: (a) 2 to 5, (b) 4 to 20, and (c) 6.6 to 100 cycle/sec.

(b) Pseudo-random FM: The modulating envelope was generated digitally by low-pass filtering a white noise signal (at 50 or 100 Hz). The peaks and valleys in the filtered signal were reconnected digitally with an exponential envelope before input to the function generator.

AM Stimuli

Two types of AM tones were generated by feeding a digitally synthesized modulating waveform to the function generator through its voltage-control-amplitude input (at 40 dB dynamic range):

(a) Exponential AM: They were tones modulated by a family of exponential ramps (n = 3 to 6), each of different AM velocities, with ramps appearing intermittently in each stimulus trial. To best activate the cell, three different ranges of ramp rates similar to FM were used. In a given stimulus session, unlike FM, AM stimulus trials were repeated mostly at a fixed level (instead of being systematically varied).

(b) Pseudo-Random AM: The envelope was generated in a way similar to FM stimuli.

Dual FM-and-AM Stimuli

Two types of envelopes similar to the description above for FM and AM stimuli were used: (a) exponential and (B) pseudo-random. An audio-mixer com-
bined the FM and AM signals before input to the speaker. The direction of sweeps was opposite between AM and FM sweeps as that was empirically found to be more effective in exciting IC cells when presented together.

**Electrophysiological Recording**

Single-units were recorded extracellularly from a microelectrode (3M KCl-filled glass micropipette, 30-60 MΩ; Dagan) advanced into the IC using a motorized microdrive (Narishige 5113). Amplified and band-pass filtered spike waveforms (Axonprobe-1A, PARC; 0.3-3.0 kHz) were conditioned into rectangular pulses (duration 0.5 ms) with a level discriminator and spike times stored in a computer for off-line analysis.

An intensive click (0.1 msec pulse, ~95 dB SPL) was first used to search for sound responsive units. After identifying an auditory unit, steady tones were then presented, followed by the random FM stimulations to determine audio-visually the cell’s response characteristics, i.e., best frequency (BF) and minimum threshold (MT). Responses to the battery of time-varying sounds were determined through the computer controlled system. Carrier frequency of the stimulus tone was adjusted to cover the unit’s BF and the stimulus level set at 30 to 50 dB above MT.

**Statistical Analyses**

To determine dependence of the categorical variables (response area, FM or AM sensitivity, and combinational sensitivity), the chi-square test or Fisher’s exact test was used wherever appropriate. To confirm the categorical classification of response types, results from another observer were compared using Cohen’s kappa coefficient. In all statistical tests, significance level was set at \( P < 0.01 \).

**Results**

A total of 939 click-sensitive single units were recorded from 107 rats. Distributions of BFs and MTs were consistent with earlier reports from this laboratory (BF range: 0.5 to 50 kHz; lowest MT: -10 dB SPL at 11 kHz), suggesting a comprehensive sampling of the midbrain and the relative stability of response within the range of body weight. FM responses were tested on 837 units (31% “insensitive”, 44% “specialized”, 25% “mixed”), and AM responses of 286 were further characterized (38% “insensitive”, 30% “specialized”, 22% “mixed”). The breakdowns of both FM and AM sensitivities were moderately consistent across two observers (Cohen’s kappa coefficient = 0.60, \( P < 0.001 \)), and results are comparable with previous reports (2, 19, 20).

**Response Areas (RAs)**

Frequency tuning characterized as response area (RA) was determined in 683 units (Fig. 1). The majority (73%, \( n = 497 \)) responded to the slow glide tone and displayed dot-raster patterns that were classified as: “simple”, “multi-band” or “asymmetric”. A minority (27%, \( n = 186 \)) of them responded to other sounds in the stimulus battery but not to this slow glide tone, and the cells were classified to have “no response” RA. Results are in general comparable with literature (3).

**FM and AM Responses**

A total of 261 units were fully characterized in terms of their response to the FM or AM stimuli that were presented separately (Fig. 2). For the same cell, the FM and AM sensitivities are highly correlated (\( P < 0.001 \), chi-square test; Table 1). Similarly a high correlation was found between RA type and the cell’s sensitivity to the modulated sounds (\( P < 0.001 \), chi-square test; Table 2). For example, “simple” RA is associated more often with “insensitive” types (93%); “no response” RA exclusively with “specialized” types (100%); and “multi-band” RA with both “specialized”
or “mixed” types.

**Responses to Dual FM-and-AM Stimuli**

A total of 122 units were characterized in terms of their response to the dual FM-and-AM stimuli. For the sake of description, the combinational sensitivity is classified into two gross categories: (a) “simple” sensitivity: one that resembled the cell’s respective AM or FM responses (45% of 122 units); and (b) “complex” sensitivity: one that did not (the remaining 55%). Figs. 3 to 5 show some representative examples of the two types of responses. The category of combinational sensitivity is also highly correlated with the cell’s RA type (P < 0.001, Fisher’s exact test; Table 3). Specifically “simple” combinational sensi-

### Table 1. FM and AM sensitivity types are highly correlated (P < 0.001, chi-square test)

<table>
<thead>
<tr>
<th>Response type</th>
<th>FM “insensitive” (n = 102)</th>
<th>FM “mixed” (n = 66)</th>
<th>FM “specialized” (n = 93)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM “insensitive” (n = 91)</td>
<td>80 (31% of 261)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>AM “mixed” (n = 61)</td>
<td>15</td>
<td>33 (13% of 261)</td>
<td>13</td>
</tr>
<tr>
<td>AM “specialized” (n = 109)</td>
<td>7</td>
<td>22</td>
<td>80 (31% of 261)</td>
</tr>
</tbody>
</table>

### Table 2. Response area type and the FM-and-AM sensitivity type are highly correlated (P < 0.001, chi-square test)

<table>
<thead>
<tr>
<th>Response Area type</th>
<th>“Insensitive” to both FM, AM</th>
<th>“Specialized” to both FM, AM</th>
<th>“Mixed” to both FM, AM</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Simple” RA (n = 69)</td>
<td>64 (93%)</td>
<td>2 (3%)</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>“Asymmetrical” RA (n = 20)</td>
<td>5 (25%)</td>
<td>7 (35%)</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>“Multi-band” RA (n = 66)</td>
<td>11 (16%)</td>
<td>32 (49%)</td>
<td>23 (35%)</td>
</tr>
<tr>
<td>“No response” RA (n = 40)</td>
<td>0 (0%)</td>
<td>40 (100%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Fig. 2. Types of responses to pseudo-random FM or AM tone (A, D: “insensitive”, B, E: “mixed” and C, F: “specialized”) as showed by 6 units. The modulating waveform for AM represents the instantaneous level of the sound. Note the striking similarity in selective responses of the same type to the changing or steady phases to FM and AM.
Fig. 3. “Simple” combinational responses to FM and AM sounds as showed by 2 units (A, B).

Fig. 4. “Complex” combinational responses to FM and AM sounds as showed by 2 units (C, D).

tivity is associated more often with “simple” RA (92% out of 24 units), and “asymmetrical” RA (89% out of 9 units), and “complex” combinational sensi-
tivity on the other hand is associated more with “no response” RA (87% out of 37 units). “Multi-band” RA is associated with both combinational sensi-
Discussion

We reported for the first time that combination sensitive cells to FM and AM sounds are present at the level of midbrain of the rat, a species that is considered not as specialized in hearing as the bat or songbird. Our division of the combinational sensitivities into “simple” and “complex” patterns is likely more of operational than functional value at this point. The most interesting finding is that some cells (in particular those with “complex” combinational sensitivity) need the presence of both FM and AM to generate strong spike responses (e.g., Fig. 5). It is likely that the proportion of IC cells with “complex” combinational sensitivity could be even higher than the current percentage of 55%, should the hunting procedure be more efficient, i.e., a random combination of the dual sounds without requiring response of the cell to one or both stimuli in the first instance.

One primary difficulty of studying combinational sensitivity is the provision of a complete dual stimulus set. The number of ways FM and AM stimuli can be combined for presentation is larger than what had been tested in the present study based on stereotyped modulations. For technical reasons, we were only able to vary a limited number of stimulus parameters. Hence, responses to dual FM-and-AM stimuli were mostly studied with one stimulus parameter systematically varied while keeping the other constant. Similar findings of “simple” and “complex” combinational sensitivities using the more extensive pseudo-random dual stimuli supported the validity of the results. More advanced stimulus strategies for hunting these units are required for a deeper understanding of the effects of other stimulus parameters on combinational responses (e.g., intensity level, modulation rate, and modulating envelope).

The “simple” combinational sensitivity, by virtue of its resemblance to FM and AM respective responses suggested minimal or simple interactions between the two inputs, like summation of stimulus energy from FM and AM inputs, post-excitatory inhibition or lateral inhibition that are known to take place in the IC (1, 21, 25). The “complex” combinational sensitivity on the other hand suggested non-linear interactions between the two inputs. Furthermore, the “complex” combinational sensitivity associated more often with the “specialized” responses types. Since FM-“specialized” cells in the IC have been found to be larger neurons (18), the interactions underlying the “complex” combinational sensitivity could potentially take place over their extensive dendritic fields. The complex inhibitory and excitatory synaptic neurotransmitter systems found in the IC is also in support of complex interactions in the midbrain (6, 9, 16) in addition to other non-linear response properties of neurons that exist as low as the level of cochlear nucleus (17). There has been growing evidence that some of the non-linear response properties found at the cortex are built upon mechanisms lower in the brainstem (4, 5, 13, 27). Our present finding of “complex” combinational sensitivity as low as the midbrain is in line of this view.

<table>
<thead>
<tr>
<th>Response Area type (n = 122 units)</th>
<th>Combinational FM-and-AM sensitivity</th>
</tr>
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<tbody>
<tr>
<td>“Simple” RA (n = 24)</td>
<td>“Simple” (45%, n = 55)</td>
</tr>
<tr>
<td>“Asymmetrical” RA (n = 9)</td>
<td>“Simple” (45%, n = 55)</td>
</tr>
<tr>
<td>“Multi-band” RA (n = 52)</td>
<td>“Complex” (55%, n = 67)</td>
</tr>
<tr>
<td>“No response” RA (n = 37)</td>
<td>“Complex” (55%, n = 67)</td>
</tr>
</tbody>
</table>

Table 3. Combinational sensitivity and response area type are highly correlated ($P < 0.001$, Fisher’s exact test)

![Fig. 5. Example of a unit with “complex” combinational sensitivity, with its response strongly depended on the simultaneous presentation of FM and AM sounds.](image-url)
The highly correlated FM and AM response types for the same cell suggested strongly that time-variance is a common denominator of response for neurons in the auditory midbrain. Consistent with this finding, similar co-variance of FM and AM response types has been reported at auditory cortex (7). The high correlation we found between FM and AM sensitivities and RA type and combinational sensitivities further supported the usefulness of our three-type classification on time-varying sensitivity of midbrain auditory neurons.

Acknowledgments

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References