The Personalized Auditory Cortex of the Mustached Bat: Adaptation for Echolocation

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SUMMARY AND CONCLUSIONS

1. In the mustached bat, Pteronotus parnellii, the "resting" frequency of the constant-frequency component of the second harmonic (CF2) of the orientation sound (biosonar signal) is different among individuals within a range from 59.69 to 63.33 kHz. The standard deviation of CF2 resting frequency is 0.091 kHz on the average for individual bats. The male's CF2 resting frequency (61.250 ± 0.534 kHz, n = 58) is 1.040 kHz lower than the female's (62.290 ± 0.539 kHz, n = 58) on the average. Females' resting frequencies measured in December are not different from those measured in April when almost all of them are pregnant. Therefore, the orientation sound is sexually dimorphic.

2. In the DSCF (Doppler-shifted CF processing) area of the auditory cortex, tonotopic representation differs among individual bats. The higher the CF2 resting frequency of the bat's own sound, the higher the frequencies represented in the DSCF area of that bat. There is a unique match between the tonotopic representation and the CF2 resting frequency. This match indicates that the auditory cortex is "personalized" for echolocation and that the CF2 resting frequency is like a signature of the orientation sound.

3. If a bat's resting frequency is normalized to 61.00 kHz, the DSCF area overrepresents 60.6–62.3 kHz. The central region of this overrepresented band is 61.1–61.2 kHz. This focal band matches the "reference" frequency to which the CF2 frequency of a Doppler-shifted echo is stabilized by Doppler-shift compensation.

4. Since DSCF neurons are extraordinarily sharply tuned in frequency, the personalization of the auditory cortex or system is not only suited for the detection of wing beats of insects, but also for the reduction of the masking effect on echolocation of conspecific's biosonar signals.

5. Because the orientation sound is sexually dimorphic and the auditory cortex is personalized, the tonotopic representation of the auditory cortex is also sexually dimorphic.

INTRODUCTION

The shape and size of the sensory maps in the cerebral cortex show differences among individuals as well as common features for a species (e.g., 12, 17, 21, 24 for the auditory cortex; 7 for the somatosensory cortex; 28 for the visual cortex). Such differences have been considered as random variation and rarely have been correlated with behavioral differences. Since large amounts of data accumulated by several neurobiologists indicate that the functional organization of sensory systems is altered by postnatal experience (e.g., 7, 8, 27), variations in the shape and size of cortical sensory maps are likely to have behavioral correlates. In the mustached bat, Pteronotus parnellii, certain areas of the auditory cortex differ among individuals, not only in shape and size (12, 24), but also in tonotopic representation (21). The tonotopic representation in the auditory cortex is unique, and its unique aspects are directly related to the amplitude spectrum of the bat's orientation sound, which is also called a biosonar signal (18). Therefore, this species of bat offers us an excellent opportunity for the detailed examination of whether a small
difference in the functional organization of an auditory cortex or system among bats is correlated with a small difference in biosonar signal among them.

The mustached bat has a "velocity-sensitive" echolocation system. It emits orientation sounds each consisting of two major portions: a long constant frequency (CF) portion and a short frequency-modulated (FM) portion. Each portion consists of four harmonics, so that there are eight components (CF$_{1-4}$, FM$_{1-4}$) in each orientation sound. (The third portion, a short initial FM, has been ignored, because the frequency sweep in it is very small.) The CF components are suited to obtaining velocity information. To optimize the analysis of velocity information, the bat performs an auditory behavior called Doppler-shift compensation, by which the CF$_2$ component of Doppler-shifted echoes is stabilized at the "reference" frequency. This is 0.1-0.2 kHz higher than the bat's CF$_2$ "resting" frequency, which is the frequency of the CF$_2$ component of the orientation sound emitted by the bat not in flight (2, 3, 15).

In the orientation sounds emitted by mustached bats from Jamaica and Panama, the CF$_2$ component is always predominant. Its frequency differs among individuals within a range between 59 and 64 kHz, averaging ~61 kHz (24). The auditory system of the mustached bat is highly specialized for fine frequency analysis of the CF$_2$ component of biosonar signals: 1) the auditory periphery has an array of neurons extremely sharply tuned to 60-62 kHz (19, 25); 2) the central auditory system produces 60-62 kHz tuned neurons, which show "level-tolerant" frequency tuning because of lateral inhibition (10, 11, 21, 25, 26); and 3) among several areas of the auditory cortex, the DSCF (Doppler-shifted CF processing) and CF/CF (CF/CF combination sensitive) areas are highly specialized for processing the information carried by the CF$_2$ component of a Doppler-shifted echo. The DSCF area over-represents sounds between 61 and 62 kHz (18, 21). The CF/CF area extracts velocity information by comparing the CF components of the emitted orientation sound with those of Doppler-shifted echo (20, 22, 24). The tonotopic representation in the DSCF and CF/CF areas is sufficiently detailed that it reflects the small differences in CF frequency among orientation sounds produced by different individuals (23, 26). However, the data supporting this last statement is limited.

The aim of the present paper is to present data indicating that the distribution of the best frequencies of neurons in the DSCF area varies according to the CF$_2$ resting frequency of a bat's own orientation sound and that females' resting frequencies are significantly higher than the males'. We conclude that tonotopic representation of the auditory cortex is "personalized" for echolocation and is sexually dimorphic.

METHODS

The methods of the present research were nearly the same as those previously described (21), so that only the main portions of these are summarized below.

Materials, surgery, and recording of neural activity

Six mustached bats, $P$. panellii rubiginosus, from Panama were used for electrophysiological experiments. Only one of these was sexed. In preparation for surgery, each bat was injected with a 1:50 mixture of Fentanyl-droperidol (Innovar-Vet 4.08 mg/kg body wt). The dorsal part of the skull was exposed, and the flat head of a 1.5-cm-long nail was attached with glue and cement. The bat was kept separately for 2-3 days in the animal room to allow for recovery from surgery. At the beginning of each day-long experiment, the resting frequency of the CF$_2$ component of orientation sounds emitted by each animal was expressed by a voltage signal with a frequency-to-voltage converter. The voltage signals for ~20 orientation sounds were stored on the screen of a storage oscilloscope, and the center voltage of these, i.e., approximate average resting frequency, was measured. During an experiment, an unanesthetized bat was put in a Lucite restraint, which was suspended with an elastic band in the center of an echo-attenuated soundproof room. To immobilize the bat's head, the nail was locked into a metal rod with setscrews. The animal was given water and sometimes crushed mealworms during the experiments, and its surgical wound was treated with local anesthetic (Xylocaine) and antibacterial nitrofurazone ointment (Furacin). The temperature of the soundproof room was maintained at 30-32°C. After the experiments the animal was returned to the animal room. The same bat could be used for up to 2 wk (4 8-h sessions) for studies of the response properties of neurons in
the DSCF area. Throughout this period, special care was given to the animal by application of antibiotic nitrofurazone ointment to the surgical wound as well as maintenance on antibiotic (tetracycline)-laced water.

For electrode penetration into the DSCF area, holes of ~50-μm diam were made in the skull with a sharpened needle. During this procedure, animals showed no sign of distress, yet responded vigorously to accidental touching of the face. This indicated their awareness and ability to express pain if so needed. A vinyl-coated tungsten wire electrode with a tip diameter of 5-7 μm was inserted obliquely or orthogonally through the holes into the DSCF area for recording action potentials from single neurons or clusters of a few neurons. An indifferent tungsten wire electrode was placed on the dura mater near the recording electrode. Action potentials were fed into a level detector to isolate action potentials of one or two neurons. The instruments used for recording were the same as previously described (21).

Acoustic stimulation

A CF tone was delivered at a rate of 4.5/s from a condenser loudspeaker placed 72.5 cm in front of the bat. The duration and rise-decay time of the CF tone were 30 and 0.5 ms, respectively. The frequency and amplitude of the tone burst were manually varied to measure best frequencies (BFs) for excitation of neurons. The instruments used for delivering acoustic stimuli were the same as previously described (21).

Data acquisition and processing

Action potentials of a single neuron or a cluster of a few neurons were amplified and displayed on the oscilloscope screen. Action potentials exceeding the threshold of a level detector triggered uniform electric pulses that were fed into an audiomonitor and also to the beam intensification of the oscilloscope for monitoring the action potentials exceeding the trigger level. The BF of an isolated neuron was measured by observing its response on the oscilloscope screen and by listening to the response on the audiomonitor.

Measurement of resting frequency

To study the distribution of the CF2 resting frequency within a bat population, the resting frequencies of 116 Jamaican mustached bats, *P. parnellii parnellii*, were measured by a computer (PDP 11/23+). Each bat was isolated from the colony and was placed in a small cage, in which it produced trains of orientation sounds spontaneously. The signal from a microphone placed 15–30 cm from the bat was amplified and filtered (50- to 65-kHz band pass, 24 dB/octave slope). The voltage reference level of the filtered signal was set by inspection with an oscilloscope to detect only the vocalized signal. Every 10 positive-going crossings produced by a comparator gated a TTL signal first high then low. The times of transition of the TTL pulse were measured with a 1-MHz real-time clock (Data Translation 2769). By measuring groups of 10 rather than individual transitions, the interrupt rate was reduced from ~60 to 6/ms, and the precision of measurement was increased. Each orientation sound consists of a short initial FM component, a long CF component, and a short FM component. The resting frequency of each orientation sound was estimated from the average of the TTL pulse transition times occurring during a 10-ms window, which opened with a 10-ms delay from the onset of the sound to exclude the initial FM component from

![Image](image_url)

**FIG. 1.** Orientation sounds (biosonar signals) emitted by mustached bats at rest. A: sounds emitted by 4 bats (a–d). B: 3 trains of sounds (a–c) emitted by one individual bat. The upper and lower traces, respectively, represent the oscillograms of the sounds and the frequencies of the second harmonics of these sounds, i.e., the outputs of the frequency-to-voltage converter. The frequency of the CF2 component of each sound is represented by the flat portion of the lower trace.
the measurement. The CF component of the orientation sound typically lasts for 15 ms, but to guarantee that only the CF component was measured, trials in which any single point dropped below 55 kHz (i.e., trials with a contribution from the terminal FM component of the orientation sound) were automatically rejected. The final resting frequency assigned to the animal was the average of 200 sounds that were usually emitted by a bat within 5 min. During the course of handling the animals, the sex was ascertained by inspecting the genitalia.

RESULTS

CF$_2$ resting frequency

Orientation sounds emitted by mustached bats each consist of a short upward sweeping initial FM component, a long CF component, and a short downward sweeping terminal FM component. Figure 1A shows examples of orientation sounds emitted by four different bats. The CF$_2$ resting frequencies were 60.59, 61.28, 61.60, 61.69, 61.70, and

![Graph showing differences in CF$_2$ resting frequency among mustached bats.](image)

FIG. 2. Differences in CF$_2$ resting frequency among mustached bats. A: distribution of CF$_2$ resting frequencies of orientation sounds emitted by 58 males (filled circles) and 58 females (open circles). The mean ± SD of CF$_2$ resting frequency were 61.250 ± 534 Hz for 58 males and 62.290 ± 588 Hz for 58 females. B: CF$_2$ resting frequencies of 3 females (a-c) and 2 males (d and e) measured over 32–38 days. Each data point indicates an average of CF$_2$ resting frequencies of 200 orientation sounds emitted by a bat within 5 min. The means ± SD of CF$_2$ resting frequencies of these bats are listed in Table 1.
62.14 kHz for the six Panamanian mustached bats, which were used for the present electrophysiological experiments. The standard deviation in CF$_2$ resting frequency ranged between 0.08 and 0.14 kHz, i.e., 0.13 and 0.23% of the CF$_2$ resting frequency, when 200 sounds were sampled (4). This within-animal variation in resting frequency was so small that the differences among these six mean resting frequencies were all significant except for the difference between 61.69 and 61.70 kHz.

The bats, when excited, often emitted trains of orientation sounds. The CF$_2$ resting frequency of the first sound of each train tended to be slightly lower than that of the rest (Fig. 1B). The standard deviation of CF$_2$ resting frequency would be 50–100 Hz, if the first sound of each train of orientation sounds was excluded from the measurement.

To examine the distribution of CF$_2$ resting frequencies within the population, the CF$_2$ resting frequency was measured in 116 Jamaican mustached bats (Fig. 2). In these
bats, the CF₂ resting frequencies ranged between 59.692 and 63.334 kHz. The distributions of the male's and female's resting frequencies overlapped each other by 33%. The mean and standard deviation of CF₂ resting frequency were 61.250 ± 0.534 kHz for the males (n = 58) and 62.290 ± 0.588 kHz for the females (n = 58). The 1.040 kHz difference in resting frequency was significant, $P < 0.001$ ($\chi^2$ test). The high resting frequency of the females was not due to pregnancy, because there was no significant difference in resting frequency between pregnant bats collected and tested in early April and nonpregnant bats collected and tested in the middle of December (Table 1A). Therefore, the orientation sound was sexually dimorphic.

In two males and three females, the CF₂ resting frequency was measured every late afternoon over 31–38 days between late February and early April (Fig. 2B). The average resting frequencies and standard deviations of these bats are listed in Table 1B. The standard deviations ranged between 67 and 117 Hz, averaging 91 Hz. The resting frequency tended to drift lower or higher over 10–20 days. An unusually large drift was observed in one male within the initial 11 days of the measurements (Fig. 2Be).

**CF₂ resting frequencies and BFś of neurons in the DSCF area**

The size and shape of the DSCF area were slightly different among individual bats. Tonotopic representation in this area was highly systematic in some bats, but less so in others. Iso-BF contour lines were commonly eccentric, as previously described (18, 21). In addition to these variations, the range of frequencies represented in the DSCF area was also different among different bats. For instance, BFś were predominantly between 60.5 and 61.5 kHz in bat P4-23-81 (Fig. 3C) and between 62.0 and 63.0 kHz in bat P7-15-81 (Fig. 3B). P7-15-81 was a female and P4-23-81 was presumably a male because of its very low CF₂ resting frequency. The CF₂ resting frequencies of P4-23-81 and P7-15-81 were 60.59 and 62.14 kHz, respectively. The difference in resting frequency is 1.55 kHz, which is similar to the difference in frequency representation between the DSCF areas of these two animals.

Across individual bats, a consistent relationship between CF₂ resting frequency and BFś of DSCF neurons was observed. This is clearly shown by BF histograms (Fig. 4). Since the standard deviation in CF₂ resting frequency was about ±0.1 kHz on the average (4; 0.091 kHz in Table 1B), the histograms were plotted with a 0.20-kHz class-width. The center frequency of one of the classes for each bat was at the CF₂ resting frequency of that bat. In Fig. 4A, the modes of histograms a, b, and e are 0.2–0.4 kHz higher than the resting frequency. This was also true in the data obtained from bat P12-4-78, which are not shown in Fig. 4A. In histograms c and d, the mode is at the same frequency as the bat's own resting frequency. In addition to the difference in frequency between the resting frequency and the mode of BF histograms, the shape of BF histogram was also different among individual bats.

Among the data obtained from different bats, there were some variations in relationship between a resting frequency and a histogram. However, there were two common features shared by all the data: 1) the higher the resting frequency, the higher were the

**TABLE 1. Resting frequencies of CF₂ components of orientation sounds of Pteronotus pannelli parnellii from Jamaica**

<table>
<thead>
<tr>
<th>A. Sexes</th>
<th>Resting Frequencies, Hz</th>
<th>No. of Bats</th>
<th>Month Measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>61.388 ± 543</td>
<td>29</td>
<td>April</td>
</tr>
<tr>
<td>Males</td>
<td>61.111 ± 525</td>
<td>29</td>
<td>December</td>
</tr>
<tr>
<td>Females</td>
<td>62.278 ± 646</td>
<td>30</td>
<td>April</td>
</tr>
<tr>
<td>Females</td>
<td>62.302 ± 431</td>
<td>28</td>
<td>December</td>
</tr>
<tr>
<td>(Mean SD:536)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Bat ID No.</th>
<th>Resting Frequencies, Hz</th>
<th>No. of Sounds</th>
<th>No. of Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig. 2Ba</td>
<td>62.067 ± 109</td>
<td>200 × 37</td>
<td>37</td>
</tr>
<tr>
<td>Fig. 2Bb</td>
<td>61.556 ± 83</td>
<td>200 × 38</td>
<td>38</td>
</tr>
<tr>
<td>Fig. 2Bc</td>
<td>61.418 ± 77</td>
<td>200 × 37</td>
<td>37</td>
</tr>
<tr>
<td>Fig. 2Bd</td>
<td>60.885 ± 67</td>
<td>200 × 31</td>
<td>31</td>
</tr>
<tr>
<td>Fig. 2Be</td>
<td>60.691 ± 117</td>
<td>200 × 37</td>
<td>37</td>
</tr>
<tr>
<td>(Mean SD:91)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. CF₃, constant frequency of the second harmonic.
BFs of DSCF neurons; and 2) the population of neurons with BFs higher than the resting frequency was much larger than that of neurons with BFs lower than the resting frequency. These data indicate that the functional organization of the DSCF area matches the properties of the bat’s own bio-

sonar signal to process the information carried by the CF$_2$ component of positively Doppler-shifted echoes.

To obtain a composite BF histogram for six bats, individual BF histograms were normalized to 61.00 kHz, which is close to the mean resting frequency of the population of

FIG. 4. Distributions of best frequencies (BFs) of DSCF neurons recorded from mustached bats. A: data obtained from 5 different bats (a–e) were separately plotted. The numbers and arrows indicate the CF$_2$ resting frequencies of these bats and their BF histograms. B: a composite BF histogram. The BF histograms for 6 different bats were normalized by shifting these along the frequency axis by a difference between 61.00 kHz and the bat’s own resting frequency. Then these were added to produce the composite BF histogram.
bats. That is, each histogram was shifted along the frequency axis by the difference between the CF$_2$ resting frequency of each bat and 61.00 kHz. Then the histograms were added together (Fig. 4B). The composite BF histogram is peaked at a 61.1- to 61.3-kHz band. It shows that the DSCF area represents sounds from 60.6 to 62.3 kHz, applying a criterion of more than four neurons per 0.2-kHz band and that the distribution of DSCF neurons tuned to different frequency bands is highly skewed: 50, 23, and 8% of all neurons are tuned to the first, second, and third 0.5-kHz bands above the resting frequency, respectively, and only 18 and 1% are tuned to the first and second 0.5-kHz bands below the resting frequency. The DSCF area represents the sound between the CF$_2$ resting frequency and 0.5 kHz above it with a disproportionately large region.

In one animal, sufficient data were obtained for a comparison in BF between the DSCF areas in the left and right hemispheres. The CF$_2$ resting frequency of this bat was 61.69 kHz on the average. The distributions of BFs measured in the left and right DSCF areas were nearly the same (Fig. 5). Both modes of the distributions were 61.7 kHz, and both the DSCF areas predominantly contained neurons tuned to sounds between 61.5 and 62.0 kHz.

**DISCUSSION**

**The personalized auditory system**

During flight, the mustached bat performs Doppler-shift compensation: the bat reduces the frequency of orientation sounds to stabilize a positively Doppler-shifted echo at a preferred frequency (reference frequency), which is 0.1-0.2 kHz higher than the bat’s own CF$_2$ resting frequency (2, 3, 15). Because of this compensation, a bat with a CF$_2$ resting frequency at 61.0 kHz, for example, predominantly receives Doppler-shifted echoes with a CF$_2$ component at 61.1-61.2 kHz and a CF$_3$ component at 91.7-91.8 kHz.

The mustached bat has a “velocity-sensitive” echolocation system. Its auditory system is specialized for extracting velocity information from both the CF$_2$ and CF$_3$ components of Doppler-shifted echoes (22, 24, 26). The peripheral auditory system contains two groups of neurons extraordinarily sharply tuned to either 60-62 kHz or 90-93 kHz (19, 25). The primary auditory cortex, which is tonotopically organized, overrepresents these two frequency bands (1, 18, 21). In particular, the over-representation of 61-62 kHz by the DSCF area is phenomenal (18, 21).

The histogram displaying the distribution of BFs in the DSCF area, normalized to a 61.0-kHz resting frequency, indicates that the DSCF area overrepresents a sound between 60.6 and 62.3 kHz. The histogram is peaked at a 61.1- to 61.2-kHz band (Fig. 4B). This “focal” band matches the band for the reference frequency. Eighteen, 50, and 23% of DSCF neurons represent 60.5- to 61.0-, 61.0- to 61.5-, and 61.5- to 62.0-kHz bands, respectively. Therefore, 91% of DSCF neurons represent sounds at around the bat’s own reference frequency and resting frequency. The DSCF area is undoubtedly specialized to represent biosonar information carried by the CF$_2$ component of “stabilized” Doppler-shifted echoes. [The echoes from flying insects also fall near the reference frequency, but not at the reference frequency because of their own flight speed (16).] Since
the focal band varies together with CF₂ resting frequency, the functional organization of the DSCF area matched the bat’s own biosonar signal.

In the auditory cortex of the mustached bat, there is the CF/CF area, where neurons are tuned to combinations of the CF₁ of the orientation sound and the CF₂ or CF₃ of a Doppler-shifted echo to extract target velocity information (22, 24, 26). The functional organization of this area matches the CF₂ and CF₃ frequencies of the bat’s own orientation sound (26). Located anterior to the DSCF area, the anterior primary auditory cortex overrepresents 92–94 kHz (1). The tonotopic representation of this area is expected to match the bat’s own CF₃ reference frequency.

The match between the properties of a bat’s own orientation sound and the functional organization of its auditory cortex indicates that each bat has a private line for echolocation, which is “communication with the environment.” In other words, both the orientation sound and the auditory cortex of the mustached bats are personalized. This match also indicates that the frequency of the CF components of the orientation sound is like a signature. It has not yet been examined whether this personalization of the auditory system (setting of the bat’s tuner) is because of a genetic code and/or auditory experience.

The personalization of the auditory cortex apparently originates from the periphery. The best frequency of cochlear microphonic response (CM) is sharply tuned at ~61 kHz and slightly differs from bat to bat (14). In two bats studied, it has been demonstrated that the CM best frequency was found to be 0.2 kHz higher than the CF₂ resting frequency (2, 3). Neurons sharply tuned at ~61 kHz are disproportionately large in population at the periphery (19, 25), in the inferior colliculus (13), and in the auditory cortex (18, 21). In two bats studied, it has been demonstrated that the population of inferior collicular neurons is disproportionately large at the CM best frequency (13). These observations indicate that the peripheral and central auditory systems are specialized for the detection and fine frequency analysis of sounds at ~61 kHz, i.e., the CF₂ frequency of biosonar signals. However, none of these papers shows the data demonstrating that the CM best frequency or focal band or tonotopic representation at ~61 kHz vary significantly from bat to bat, according to the bat’s own CF₂ resting frequency. No data demonstrating the personalized auditory system has previously been published, although this has been stated in the past (2, 16, 23). The papers cited in this paragraph, however, are very consistent with the view that the auditory periphery and subcortical auditory nuclei are personalized.

The horseshoe bat, Rhinolophus ferrumequinum, emits an orientation sound consisting of a long CF component and a short FM component. As reviewed by Schnitzler and Ostald (16), its auditory system is apparently personalized according to the CF₂ resting frequency in the bat’s own orientation sound. However, it is not yet demonstrated that the CM best frequency or focal band or tonotopic representation varies significantly from bat to bat, according to the bat’s own CF₂ resting frequency.

Variation in CF₂ resting frequency and the range of tonotopic representation in the DSCF area

The CF₂ resting frequency is quite constant in some animals, but it varies significantly in others, as shown in Fig. 2B and Table 1B. Over the 40 days when CF₂ resting frequencies were measured, the bat’s health appeared to be good, and they were alert and emitted a lot of orientation sounds when they were placed in a small cage. The health and alertness were unlikely sources to the observed drifts/variations in CF₂ resting frequency. The room temperature was nearly the same over those days. Further, since the directions of the drifts in frequency differed among the bats studied over the same days, the room temperature could not be the cause for the drifts.

We don’t know the cause of the observed drifts in CF₂ resting frequency. However, these present intriguing problems: whether there is a positive correlation between the range of tonotopic representation in the DSCF area and the magnitude of drift/variation in CF₂ resting frequency over a prolonged time period, and whether tonotopic representation changes together with a drift in CF₂ resting frequency. One may speculate
that the difference in frequency tuning of the DSCF area among bats (e.g., a difference in shape between BF histograms a and c of Fig. 4A) is significant and that the amount of drift/variation in CF2 resting frequency is positively correlated with the frequency tuning of the DSCF area.

The personalized echolocation system and reduction of masking

In radio communication, a specific carrier frequency within a particular band is assigned to each station, and a tuner is adjusted to receive signals from a particular station. The assignment of a different carrier frequency to each station and an array of sharp filters in each tuner are essential for communication and reduction of masking. In contrast, in the mustached bat, the tuner is fixed and the emitter is adjusted so that the returning signal (Doppler-shifted echo) is maintained at the reference frequency to which the tuner is set. The central auditory system contains groups of neurons with sharp level-tolerant frequency-tuning curves at and around the bat's own resting and reference frequencies (10, 11, 13, 21, 24, 25, 26). Therefore, the personalization of the biosonar signal and auditory system is not only suited for the detection of wing beats of insects, but also for the reduction of the masking effect on echolocation of sounds produced by conspecifics, as discussed previously (20, 22) and below in detail.

The CF2 resting frequencies of the 116 bats studied ranged from 59.69 to 63.34 kHz. That is, they spread over 3.64 kHz. The standard deviation in CF2 resting frequency is 0.091 kHz on the average for individual bats. This implies that each bat has not a particular single frequency, but a particular narrow frequency band that overlaps with other's. How many different bats can theoretically be discriminated in terms of CF2 resting frequency? A 1.16 SD on either side of the mean corresponds to a 75% confidence level. Assuming that the distribution of bat's CF2 resting frequencies is uniform over the 3.64-kHz band, 3.64 kHz is divided by 0.21 kHz. The result, 17.3, is the number of bats to be discriminated 75% of the time using the CF2 resting frequency as the only cue. If there are additional acoustic cues such as the harmonics and envelope of orientation sounds, a much greater number of bats could be discriminated. However, what is primarily important to each bat is not the discrimination of these 17 bats but to discriminate its own biosonar signals from the sounds produced by conspecifics. The distribution of bat's CF2 resting frequency is not uniform over the 3.64-kHz band (Fig. 2A). Therefore, a bat emitting orientation sounds at either the lower or higher end of the 3.64-kHz band could easily discriminate its own biosonar signals from those produced by conspecifics. On the other hand, a bat emitting orientation sounds at the center of the band would face a harder discrimination. The personalization of the echolocation system would reduce the masking effect of conspecific's sounds on echolocation, but it alone is apparently not sufficient to make the echolocation system tolerant to masking, and other mechanisms should be considered together with this, as previously described (20, 22).

The personalized echolocation system and prey detection

Johnson et al. (6) first pointed out the following: the "flight-speed" Doppler-shift compensation adjusts the carrier frequency of an insect echo to the center of the particular bat's sharp sensitivity peak. Then the periodic frequency modulation (FM) in the echo due to the wing beats of the insect occurs as the frequency sweeps back and forth through the sensitivity peak. Because of the sharp tuning of the cochlea, the FM is converted into large-amplitude modulation to stimulate periodically auditory neurons. Johnson et al. clearly pointed out that the auditory system with sharp tuning at the CF2 frequency is a specialization for the detection of wing beats of insects. This theory has been well accepted. The mustached bat is extremely sensitive to the wing beat (5). Its peripheral neurons with a BF at ~61 kHz are extraordinarily sharply tuned and are sensitive to very small periodic FMs (19). Neurons in the DSCF and CF/CF areas of the auditory cortex are also sharply tuned at ~61 kHz, and many of them are also sensitive to small periodic FMs (22).

The horseshoe bat, R. ferrumequinum, emits orientation sounds, each of which consists of a long CF component followed by a short FM component. As reviewed by
Schnitzler and Ostwald (16), behavior and coding related to wing-beat detection have been more extensively studied in this species than in the mustached bat.

Sexual discrimination with CF$_2$ frequency

Because the CF$_2$ resting frequency is significantly different between males and females, there is a possibility that CF$_2$ resting frequency serves as an acoustic cue to identify sex. The distribution of male's resting frequency overlaps with that of female's by 33% (Fig. 2.4). If mustached bats use the CF$_2$ resting frequency for sexual discrimination, the percent correct level of discrimination would be 67%.

Sexual dimorphism of the auditory system

In prior studies, the CF$_2$ resting frequency was 60.87 ± 0.48 kHz for 77 P. p. rubiginosus from Panama (24) and 61.21 ± 0.68 kHz for 34 P. p. parnelli from Jamaica (26). The exact male-to-female ratio is not known in these samples but it was probably about 4:1, because they were imported at this ratio. There is no significant difference in resting frequency between these two subspecies. The present study of CF$_2$ resting frequency revealed sexual dimorphism of orientation sound in the Jamaican mustached bats. Of the six Panamanian mustached bats used for the electrophysiological experiments, we had sexed only one (P7-15-81), which was female and emitted orientation sounds at the highest resting frequency among the six. There is no reason to doubt that orientation sounds of Panamanian mustached bats are also sexually dimorphic. Since the orientation sound is sexually dimorphic and the auditory cortex is personalized, the auditory system of the mustached bat is sexually dimorphic. The mustached bat is the first example of sexual dimorphism of tonotopic representation in a certain range of frequencies, in spite of the fact that both males and females hear sounds in a wide range of frequencies from <10 kHz to >100 kHz. As in many different species of mammals, the male mustached bats tend to be slightly larger than the females and their sounds are slightly lower in frequency than those of females.

Sounds produced by males and females in many species are commonly different in frequency and/or structure. But a difference in the auditory system between males and females has been found only in the tree frog, *Eleutherodactylus coqui*. The communication sound of the male tree frog consists of two notes: "Ko" and "Ki," which are respectively emitted for territorial maintenance and female attraction. The male's ear is sensitive to the low-frequency note Ko, but not to the high-frequency note Ki. In contrast, the female's ear is sensitive to Ki, but not to Ko (9). This is the best example of sexual dimorphism of the auditory system. It should also be noted that in the zebra finch, the auditory response of the vocal system is quite different between males and females (29).

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