Combination-Sensitive Neurons in the Primary Auditory Cortex of the Mustached Bat

Douglas C. Fitzpatrick, Jagmeet S. Kanwal, John A. Butman, and Nobuo Suga

Department of Biology, Washington University, St. Louis, Missouri 63130

In the mustached bat, *Pteronotus parnellii*, neurons in the primary auditory cortex (AI) have been thought to respond primarily to single frequencies, as in other mammals. However, neurons in the Doppler-shifted constant-frequency (DSCF) area, a part of the mustached bat's AI that contains an overrepresentation of the prominent CF₂ component of the biosonar signal, were found to show facilitative responses to combinations of different frequencies in the pulse and echo. The essential components for facilitation were the pulse FM₁ and the echo CF₂. The FM₁-CF₂ facilitation was sensitive to echo delays, indicating that DSCF neurons respond better to targets within particular ranges. On average, the longest discriminable echo delay, based on increased impulse counts due to facilitation, corresponded to a target range of 4.3 m, and the most discriminable delay corresponded to a target 3.6 m distant. Since mustached bats first show a behavioral response to a target at a distance of 3–4 m, DSCF neurons are suited to signal the presence of an insect within this behaviorally important range. DSCF neurons were broadly tuned to echo delay, with the average minimum discriminable echo delay corresponding to a target range of 1.9 m, and the delay tuning of the neurons followed (tracked) changes in pulse duration, indicating that facilitation occurs during much of the approach phase of insect pursuit when target characterization is presumably occurring. These results show that AI neurons in the mustached bat are specialized to respond to complex, behaviorally relevant stimuli during the search and approach phases of insect pursuit.

[Key words: biosonar, echolocation, neuroethology, cerebral cortex, auditory system, facilitation, overrepresentation]

In bat biosonar, accurate estimation of information-bearing parameters such as echo delay (for measuring the distance to objects) and amount of Doppler shift (for measuring relative target velocity) are critical for successful foraging. The auditory cortex of the mustached bat, *Pteronotus parnellii*, contains multiple

areas specialized for extracting different information-bearing parameters from biosonar signals (Fig. 1A). The FM-FM area, for example, contains neurons tuned to particular echo delays that are systematically arrayed across the area (Suga and O'Neill, 1979; O'Neill and Suga, 1982; Suga et al., 1983b). Similarly, the CF/CF area contains neurons tuned to particular Doppler shifts, and the amount of Doppler shift is systematically expressed on the cortical surface (Suga et al., 1983). FM-FM and CF/CF neurons are combination sensitive, in that they respond poorly to single tones and single elements in the biosonar signal, but are facilitated by combinations of two signal elements.

The primary auditory cortex (AI) of mustached bats contains an expanded representation of a behaviorally significant frequency band, about 60–64 kHz, that corresponds to the second-harmonic constant-frequency component (CF₂) of the biosonar signal (Fig. 1B). While flying, mustached bats lower the frequency of the emitted pulse to compensate for Doppler shifts in echoes, so that the “Doppler shift-compensated” echo CF₂ is stabilized to a frequency (called the reference frequency) that is typically between about 61.0 and 61.5 kHz (Schnitzler, 1970a,b). An overrepresentation of this important frequency band begins at the periphery and is maintained throughout the auditory system (Pollack et al., 1972; Suga et al., 1975; Suga and Jen, 1976; Pollack and Bodenhamer 1981; Kossel and Vater, 1985; Olsen, 1986; Zook and Leake, 1989; Covey et al., 1991). In the auditory cortex, the area containing the overrepresentation of the echo CF₂ is called the Doppler-shifted CF (DSCF) processing area.

Within the mustached bat auditory cortex, there is a main tonotopic representation (Fig. 1A) that receives topographic projections from the ventral division of the medial geniculate body (MGB), and is therefore presumably homologous to AI of other species (Suga and Jen, 1976; Olsen, 1986). The DSCF area occupies approximately 50% of the mustached bat's AI. In other species, a large proportion of AI neurons respond well to pure tones and have a single characteristic frequency to which they respond at lowest threshold (see Brugge, 1985, for a review). This is also true of mustached bat AI neurons, including those in the DSCF area (Suga and Jen, 1976; Suga, 1977; Manabe et al., 1978; Suga and Manabe, 1982; Suga et al., 1987). However, we noticed that in addition to the well-characterized response to the CF₂, DSCF neurons are facilitated when the CF₂ is paired with frequencies contained in the first harmonic of the biosonar signal (H₁; see Fig. 1B). Here we describe this combination sensitivity and show that the facilitation is sensitive to echo delay, and hence to target range. This is the first report that demonstrates specialized responses of AI neurons to behaviorally relevant combinations of species-specific sounds.

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Correspondence should be addressed to N. Suga, Department of Biology, Box 1137, Washington University, St. Louis, MO 63130.

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Figure 1. *A*, A dorsolateral view of the functional organization of the mustached bat's auditory cortex. The dots enclose the AI where low frequencies are represented posteriorly and high frequencies anteriorly. Within AI, *area a* is the DSCF area, which overrepresents frequencies of 60–63 kHz. *Area b* is the CF/CF area, which contains combination-sensitive neurons that encode target velocity. *Areas c, d, and e* are the FM-FM, dorsal fringe (DF), and ventral fringe (VF) areas, respectively, that contain combination-sensitive neurons encoding target range. *Area f* is the H_1 – H_2 area, which contains neurons that respond to all combinations of the first and second harmonics of the biosonar signal. *Areas g and h* are the dorsomedial and ventroposterior areas, respectively, that contain neurons with multi-peaked tuning curves that are sensitive to changes in sound locations. *B*, A schematicized biosonar signal. The signal consists of an emitted pulse (solid lines) and echo (dotted lines). The pulse has four harmonics (H_1 – H_4) and each harmonic has a CF and an FM component (CF_{1-4} and FM_{1-4} , respectively). The echo is shown delayed and Doppler shifted (DS) from the pulse.

Materials and Methods

Surgery and recording of action potentials. Recordings were made in seven bats, using methods described previously (Suga et al., 1983b). Under Innovar analgesia (0.08 mg/kg Fentanyl and 4 mg/kg Droperidol, i.m.) the skull was exposed and a metal post mounted with cyanoacrylate gel. Three days after surgery, the unanesthetized bat was placed in a styrofoam restraint suspended by an elastic band at the center of a soundproof, echo-attenuated room maintained at 30–32°C. The head was immobilized by fixing the post mounted on the skull into a metal tube with set-screws, and adjusted so that the eye–nose line was centered at 0° in front of condenser loudspeakers located 68 cm away. Insulated tungsten-wire electrodes with tip diameters of 5–10 μ m were inserted into the DSCF area through small holes (~50 μ m diameter) made in the skull with a sharpened needle. An indifferent electrode was placed anterior or posterior to the auditory cortex. Neuron activity was recorded in the middle layers (300–600 μ m depth). A window discriminator was used to isolate the responses of single neurons, and conditioned pulses from the discriminator were fed to a high-speed event recorder (MI², Philadelphia, PA) for the recording of spike arrival times.

Acoustic stimuli. Synthesized components of biosonar pulses and echoes were delivered from separate condenser loudspeakers. Each pulse and each echo consisted of CFs followed by FMs or combinations of these. The durations of the CFs and FMs were 20 and 3.0 msec, respectively, except where otherwise noted. The frequency range swept by the FM_1 was 6 kHz; the FM_n s ($n = 2, 3$, or 4) swept 12, 18, or 24 kHz. The rise and decay times of the signals were 0.5 msec, and the stimuli were delivered at a rate of 5/sec. Stimulus amplitudes were controlled with manual attenuators. All stimulus levels reported are in dB SPL (sound pressure level re 20 μ Pa).

Stimuli for examining the echo delay sensitivity of neurons consisted of trains of pulse–echo pairs with decrementing echo delays, generated with computer-controlled pulse generators and timers (MI²). The trains were 3450 msec long and divided into 23 blocks, each of 150 msec duration. Blocks 2–21 contained pulse and echo components that decremented in echo delay at the rate of 2.5 msec/block, starting from 47.5 msec delay (block 2) to 0 msec delay (block 21). Blocks 1, 22, and 23 contained a pulse H_1 alone, echo H_2 alone, and no stimulus, respectively. The silent period between trains was 200 msec. Each stimulus within a train was presented at the best frequency and amplitude for facilitation.

Data collection and analysis. When action potentials of a single DSCF neuron were recorded, we first identified the best frequencies or best FMs, best amplitudes, and best delay for facilitation, the minimum thresholds to each facilitatory component, and the upper threshold (if any) of each component. The frequency of the best FM was expressed

by the initial frequency of the sweep. Stimuli presented at the the best frequencies and amplitudes for facilitation were called the best stimuli (e.g., best CF_2). The best facilitative frequencies of each neuron were normalized to account for differences in the frequency of CF_2 emitted while at rest among individual bats, according to the formula $y = x(61.00/RF)$, where y is the normalized and x the measured best frequency for facilitation, RF is the measured resting frequency, and 61.00 kHz is taken as the approximate population mean for resting frequency. The methods used to measure RF were identical to those described in Suga et al. (1987).

Based on the recorded spike arrival times, we constructed peristimulus time histograms (PSTHs) and cumulative PSTHs, and determined latencies. Receiver operating characteristic (ROC) analysis was used to determine objectively whether different stimuli could be discriminated on the basis of impulse count. The probability distributions of impulse counts (i.e., the number of stimuli that yielded a given number of spikes divided by the total stimuli presented) were determined from the responses to 150 stimuli at each of 20 different echo delays. Responses were counted over a time window of 150 msec. Based on these impulse count distributions, ROC curves (see Fig. 5A) were constructed to estimate the discriminability of each delay from the longest delay tested (47.5 msec), to which no facilitation was ever observed. To construct the ROC curves, the minimum impulse number (or criterion level) for deciding that the short delay occurred, the criterion level was varied from 0 to the maximum observed, and the probability of “hits” was plotted against the probability of “false alarms.” A hit occurred when the impulse count exceeded the criterion level when the short delay stimulus was presented, and a false alarm occurred when the response to the long delay stimulus exceeded the criterion level. Thus, when the criterion level was 0 impulses it was exceeded by all stimuli, and the probability of hits and false alarms was equal to 1. When the short-delay stimulus caused reliable facilitation and the criterion level was such that it was rarely exceeded in the absence of facilitation, then the probability of hits approached 1 while the probability of false alarms approached 0. The probabilities of hits and false alarms were plotted for all criterion levels, and the area under the resulting ROC curve gave the probability of distinguishing the two stimuli based on spike count (Cohn et al., 1975).

Results

Responses of DSCF neurons are facilitated by the FM_1 and CF_2 of the biosonar signal

DSCF neurons were sharply tuned to the echo CF_2 , as reported in previous studies (Suga and Jen, 1976; Suga, 1977; Suga and

Manabe, 1982; Suga et al., 1987). In addition to the CF₂ response, however, DSCF neurons were facilitated by the pulse H₁, and specifically by the FM₁. By facilitation it is meant that the neuron's response to the paired stimuli is greater than the sum of the responses to individual stimuli. To obtain maximum facilitation to the H₁-H₂ or FM₁-CF₂ combinations, some echo delay was required (as will be fully described in a later section); the best echo delay for the neuron in Figure 2 was 26.0 msec. As can be seen, the neuron responded weakly to the best H₁ alone (Fig. 2a) and modestly to the best H₂ alone (Fig. 2b). When the H₁ was paired with the H₂ (Fig. 2c), the response was facilitated. When the FM₁ was removed, the response declined to the level of the H₂ only (Fig. 2d). When only the FM₁ and CF₂ were presented, the facilitation was as strong as that to the complete harmonics (Fig. 2e). Thus, the essential components for facilitation of this neuron were the FM₁ and CF₂, as was true of all ($n = 142$) DSCF neurons examined in this study.

Tuning to pure tones

When a "best" pure tone within the frequency range of the FM₁ was substituted for the H₁ and presented together with the CF₂, facilitation of DSCF neurons occurred without the necessity of introducing echo delay (Fig. 3). We called this pure tone the best "FM₁ frequency." For the neuron in Figure 3A, the best FM₁ frequency for facilitation was 25.98 kHz delivered at 72 dB SPL and the best CF₂ was 62.70 kHz at 47 dB SPL. This neuron had a good response to the CF₂ alone (120 spikes to 100 stimuli in a 100 msec time window), as did most neurons tested, but was clearly facilitated when both stimuli were present (202 spikes). The best CF₂ measured when the CF₂ was presented alone typically had the same frequency as the CF₂ presented as part of a facilitatory pair. The neuron in Figure 3B shows a response similar to that in 3A, except that it responded well to both the best FM₁ frequency alone and the best CF₂ alone (25.80 kHz at 77 dB SPL and 61.60 kHz at 12 dB SPL, respectively). Like the neuron in Figure 3A, however, facilitation occurred when both components were presented together. For the 60 neurons tested, the average best FM₁ frequency for facilitation was 26.34 ± 0.824 kHz at 71 ± 5.5 dB. This average best frequency is well below that of the lowest CF₁ emitted by the bats (about 28 kHz), but is within the range of the FM₁ (which sweeps from 28–31 kHz to 22–25 kHz). The average best CF₂ was 61.56 ± 0.670 kHz at 36 ± 11.9 dB, which is typical of the Doppler shift-compensated echo CF₂. The average minimum threshold to the FM₁ frequencies at the best frequency for facilitation was 52 ± 5.3 dB, and for the CF₂s was 16 ± 11.7 dB. The large best amplitude and minimum threshold differences between the components indicate that in nature the H₁ response is to the loud outgoing pulse while the CF₂ response is to the much fainter echoes.

A complete description of excitatory, inhibitory, and facilitative frequency tuning curves will be the subject of a later report, and so will be only highlighted here. In 15 neurons in which the best FM₁ frequency was fixed and the CF₂ was varied in frequency, the CF₂ tuning widths (to the 50% maximum response contour measured at the best CF₂ amplitude for facilitation and with 0 msec echo delay) averaged $11 \pm 7.4\%$ broader than when the FM₁ frequency was not present. The minimum threshold to the CF₂ was lowered by an average of 9.8 ± 10.1 dB. Thus, facilitation by the best FM₁ frequency can both broaden the frequency tuning and lower the threshold to the CF₂, but by relatively small and variable amounts.

Figure 2. Demonstration of the essential components for DSCF neuron facilitation. The response of this neuron was small to the H₁ or H₂ presented alone (*a* and *b*, respectively), but facilitation occurred to the H₁-H₂ pulse-echo pair (*c*). When the FM₁ was eliminated (*d*) the facilitation disappeared, but the facilitation to the FM₁-CF₂ pair (*e*) was as strong as to the complete harmonics (compare *c* and *e*). The pulse FM₁ and echo CF₂ are therefore the essential components for facilitation. The best frequencies and amplitudes for each component and the best echo delay for this neuron are indicated. Small numbers to the right of each histogram are the spike counts.

Magnitude of the facilitation

The magnitude of the facilitatory interactions between two stimulus components can be expressed as a facilitation ratio (FR), defined as the paired response (number of impulses) divided by the sum of the responses to each component alone, for stimuli at the best frequencies and amplitudes for facilitation and with spontaneous activity subtracted. For 60 facilitated DSCF area neurons tested with FM₁ frequencies and CF₂s, the FRs ranged from 1.2 to 5.7, with a median of 1.7. For most neurons, the response to the FM₁ frequency alone was weak or absent, but in 14 (23%) the FM₁ frequency alone response was as large or larger than the response to the CF₂ alone. For an additional 55 neurons tested with H₁-H₂ pairs at the best delay, the FRs ranged

Figure 3. Facilitation of DSCF neurons to the best FM₁ frequency and CF₂. **A**, In this neuron, the response to the best FM₁ frequency alone (*a*) was small, and the response to the best CF₂ alone (*b*) was modest. When both were presented together (*c*), facilitation occurred; for pairs of pure tone stimuli such as this echo delay was not required for facilitation. **B**, For this neuron, the response to the FM₁ frequency alone (*a*) was nearly equal to that of the CF₂ alone (*b*), but facilitation still occurred when both were presented together (*c*). *Small numbers* to the right of each histogram are the spike counts.

from 1.0 to 10.1, with a median value of 2.4. Because the median and maximum FRs were higher to the best H₁s at the best delay than to the best FM₁ frequencies at 0 msec delay, the complete natural stimuli appear more facilitative than single frequencies, although both induce facilitation with a roughly twofold increase in the response magnitude.

With few exceptions, DSCF neurons showed clear facilitation. In a sample of 50 neurons from two hemispheres, 38 (76%) were facilitated, that is, had FRs greater than 1.2 (an FR of 1.20 was used as the criterion level for facilitation to discount variability in the responses not related to facilitation). Of the 12 neurons with FRs less than 1.2, 8 responded reliably to the paired stimuli and only occasionally to the CF₂ alone. However, the occasional CF₂ alone response consisted of a burst of up to 10 impulses, whereas when both components were present the response typically had only one to five impulses, resulting in FRs less than 1.2. Thus, most DSCF neurons changed their firing pattern to the CF₂ in the presence of an FM₁ frequency.

The facilitation of DSCF neurons is sensitive to echo delays

As mentioned above, H₁-H₂ pairs evoke maximum facilitation at a particular echo delay. Since echo delay is used by bats to estimate target range (Simmons, 1973), we examined the effect of varying echo delay on the responses of 55 DSCF neurons (see Materials and Methods). For the neuron in Figure 4, no facilitation was evident for the longest echo delay tested of 47.5 msec (Fig. 4*Aa-e*). As the delay was shortened, facilitation first occurred at 27.5 msec delay (Fig. 4*Ab*) and reached a peak at 22.5 msec echo delay (Fig. 4*Ac*). Further decreasing the echo delay caused the facilitation to decline (Fig. 4*Ad*), but it rebounded at short delays, causing a second peak at 7.5 msec echo delay (Fig. 4*Ae*). The response then declined again as echo delay

was further shortened to 0 msec (not shown). At the short delay peak, the CF₂ arrived before the FM₁, so that the FM₁ appeared to facilitate an off-response to the CF₂ or an on-response to the FM₂ that was not otherwise apparent. Compared to the long delay response at 22.5 msec echo delay, the short delay response was temporally more dispersed.

Neurons with a two-peaked response in which the short delay peak was at least 50% of the long-delay peak (as in Fig. 4*A*) were called type I (45%, $n = 20$). Those which had only a single major peak in their response were classed as type II (55%, $n = 30$). In type II neurons, the major peak always occurred to echo delays longer than 15 msec. In five type I neurons, the short delay peak was higher than the long delay peak.

To estimate the population response of DSCF neurons to changing delays, we summed the response of the 55 neurons and normalized and plotted the result (Fig. 4*Be*). The maximal summed response occurred to an echo delay of 22.5 msec. The short delay peak of type I neurons was hardly apparent; however, the type I and very broadly tuned type II neurons served to maintain a wide (4.3–28.7 msec) delay response width measured at 50% of the maximal firing rate.

For individual type I and type II neurons, the 50% delay-tuning widths varied considerably. The neuron in Figure 4*Bc* had a 50% delay-tuning width of 17.5 msec, which was close to the population mean of 20.2 ± 9.03 msec for the 55 neurons. The neurons in Figure 4*B, c* and *d*, are near the extremes in terms of delay-tuning widths, at 7.6 msec and 39.9 msec, respectively. In Figure 4*C* the distribution of 50% delay-tuning widths for the 55 neurons is plotted as a function of best delay. The wide range of delay widths encountered is evident. This figure also indicates that there was no correlation between best delays and 50% delay-tuning width. Such a correlation might

Figure 4. Responses of DSCF neurons to H₁-H₂ pairs of varying echo delay. **A**, PSTHs for pulse-echo pairs with different delays. For this neuron (J9-5-18-23), the pulse and echo responses are distinct at the longest delay tested (47.5 msec). As the delay shortens and the responses merge, facilitation occurs, reaching a maximum at an echo delay of 22.5 msec. At a delay of 15.0 msec, the facilitation is less and the responses to each component are again separate but now the H₂ response precedes the H₁ response. As the delay was shortened to 7.5 msec, the facilitation increased again, with the increased spikes likely due to a facilitation of a CF₂ offset response not otherwise apparent. *Small numbers* to the right of each histogram are the total number of spikes at that delay. **B**, Differential tuning to changing echo delay among DSCF neurons. In **a**, the *open circles* represent the delays corresponding to the histograms in **A**. Neurons with this double-peaked delay-dependent response were called type I, and neurons without the short-delay peak were called type II neurons (**b**). Both type I and type II neurons had variable delay-tuning widths, examples of the extremes observed are shown in **c** and **d**. In **e**, the summed response of the population is given. In each curve, the *dotted lines* indicate the sum of the pulse and echo alone responses, and the *slashes through the curves* indicate points of 50% maximum response. The *arrow* in **c** indicates the direction that delay was changed in each stimulus train. All stimuli were delivered at the best frequencies and amplitudes for facilitation. **C**, The distribution of 50% delay response widths versus best delay for the population. The width of delay tuning was highly variable and did not correlate with echo delay.

be expected because of Weber's law, which says that the just-noticeable difference between stimuli should scale with the magnitude of the stimuli, and because in the FM-FM and dorsal fringe (DF) areas the width of delay tuning is in fact a function of the best delay, conforming to Weber's law (Suga and Horikawa, 1986). This result represents an important distinction between DSCF neurons and other delay-tuned neurons, because it indicates that although DSCF neurons are active in responses to echoes from targets at particular ranges, range is not the information-bearing parameter extracted by the neurons.

ROC analysis of the responses to changing echo delays

Since the facilitation occurs to particular echo delays, that is, to targets within particular ranges, targets within these ranges should be detectable by means of the increase in spike count relative to the weaker responses to closer or more distant targets. To quantify the delays at which increased impulse counts due to facilitation signal the presence of a target, we constructed ROC curves from responses at each echo delay in comparison to the longest delay tested (47.5 msec). An example of ROC

curves obtained for two delays in one neuron are shown in Figure 5A. The points on the curve represent the probability of "hits" versus "false alarms" for criterion values of zero spikes to the maximum number obtained in response to either the long (47.5 msec) or short (<47.5 msec) delay stimulus (see Materials and Methods). The area under each curve represents the probability of correct discrimination $p(CD)$ that the short delay stimulus was presented. For the 35.0 msec stimulus, the $p(CD)$ was essentially chance at 0.55, and for the 22.5 msec stimulus the $p(CD)$ was near maximum for this neuron at 0.88. The complete delay-discrimination curve for the neuron is shown in Figure 5B, with the points derived from the ROC curves in Figure 5A indicated with open circles. From curves like those shown in Figure 5B, we measured the longest discriminable delay (LDD), the most discriminable delay (MDD), and the shortest discriminable delay (SDD), using the 75% correct discrimination level as the basis for defining the LDD and SDD, and the point of maximum discrimination as the MDD.

The distributions of LDDs, MDDs, and SDDs for the 55 neurons tested are shown in Figure 5C, and statistics for each

Figure 5. Delay discriminability determined by ROC analysis. *A*, Examples of ROC curves for stimuli with two different echo delays. The area under the curves (shaded) provides the probability that an ideal observer of this neuron's (J9-5-91-31) response could correctly discriminate that the echo delay presented was shorter than the longest tested (47.5 msec). The 35.0 msec delay stimulus could not be discriminated [$p(CD) = 0.55$ or only slightly better than chance], while the 22.5 msec delay stimulus could be correctly discriminated approximately 90% of the time [$p(CD) = 0.88$]. Dashed line indicates the chance [$p(CD) = 0.5$] level. *Co*, criterion level (i.e., a spike number from 0 to the maximum observed); $p(hits)$, probability that the short delay stimulus was presented and the criterion level was equaled or exceeded; $p(false\ alarms)$, probability that the 47.5 msec echo delay stimulus was presented and the criterion level was equaled or exceeded. *B*, The complete delay discrimination curve. Points derived from the ROC curves in *A* are shown as open circles. The 75% correct discrimination line, used to determine the LDD, MDD, and SDD (see text), is shown. The direction of the changing delay in the stimulus trains is shown by the arrow. *C*, The distributions of LDDs, MDDs, and SDDs for the 55 neurons tested.

Table 1. Echo delays and target ranges associated with the delay-dependent responses of DSCF neurons

	Echo delay (msec)	Target range (m)
LDD	25.6 ± 3.26 (19.4–32.8)	4.4 ± 0.56 (3.3–5.6)
MDD	20.9 ± 5.55 (5.0–30.0)	3.6 ± 0.95 (0.9–5.2)
SDD	11.5 ± 6.51 (1.4–24.5)	2.0 ± 1.12 (0.2–4.2)

Values are mean ± SD for the 55 neurons tested; those in parentheses are the extremes observed. Target ranges were calculated using 344 m/sec as the speed of sound in air at 25°C. LDD, Longest discriminable delay; MDD, most discriminable delay; SDD, shortest discriminable delay.

cause a detectable increase in the response of DSCF neurons as compared to more distant targets was about 5.5 m, and most neurons would be facilitated by echoes from targets closer than 4.3 m. The maximum point of detectability for the population occurred to targets about 3.6 m distant.

Response latencies

Because the latencies to the different facilitative components have proven useful for understanding mechanisms of delay tuning in FM-FM neurons (Olsen and Suga, 1991a), latencies of DSCF neurons were measured to the best H_1 alone, the best H_2 alone, and the best H_1 - H_2 pair at best delay. The H_1 -alone and the H_1 - H_2 latencies were measured from the beginning of the pulse FM_1 ; the best H_2 -alone latency was measured from the beginning of the CF_2 . On average, latencies to the best H_1 alone were 7.8 msec longer than to the best H_2 (Table 2). However, the echo delays that caused the latencies of pulse and echo to coincide was rarely the best delay (or MDD); rather, the latency at the MDD occurred 3.2 msec prior to the pulse latency on average. For the neurons in Figure 6*A*, it can be seen that at the MDD (22.5 msec), the facilitation occurred prior to the onset of the H_1 -alone response. The delay that would cause the excitatory responses to the pulse and echo to coincide (27.5 msec) was not the best delay. Similarly, for the neuron in Figure 6*B* it can be seen that facilitation to the best FM_1 frequencies and CF_2 s occurred prior to the pulse only response.

The effect of changing CF duration on the delay tuning of DSCF neurons

The delay-response and ROC curves presented above were obtained to stimuli of constant duration (20 msec CF and 3 msec FM). Signals of this duration are typical of those emitted during the search phase of echolocation (Novick and Vainsys, 1964;

Table 2. Response latencies

	FM_1 alone latency (msec)	CF_2 -alone latency (msec)	Facilitated latency at best delay (measured from the FM_1) (msec)
Mean	18.1	10.3	14.9
Range	11.7–23.5	5.7–16.5	8.1–24.5
SD/SE	2.57	3.01	3.31
N	31	48	55

Data were obtained from 31 neurons with pulse-alone responses vigorous enough for the latency to be measured. An acoustic delay of 2.3 msec has been subtracted.

are given in Table 1. The LDDs of the neurons occurred from about 19 to 33 msec echo delay, and the MDDs from 5 to 30 msec delay. MDDs less than about 16 msec occurred rarely (14.5%, $N = 8$) and only in type I neurons where the short delay peak was higher than the long delay peak or in very broadly tuned type II neurons. The SDDs were much more variable than the LDDs. In Table 1, the target ranges corresponding to the means and extremes for the LDDs, MDDs, and SDDs are indicated. It can be seen that the maximum target range that would

Figure 6. *A*, Cumulative histograms of the responses of a DSCF neuron (J9-5-91-33) to the H_1 -alone, H_2 -alone, and two H_1 - H_2 pairs with different delays (22.5 and 27.5 msec). Best delay (22.5 msec) occurred when the H_2 response preceded the H_1 response by about 5 msec (point *b*), rather than at the delay (27.5 msec) where the H_1 and H_2 responses coincided (point *a*). The H_2 -alone response (broken line) was shifted along the x-axis and superimposed on the response at best delay to show that facilitation occurred prior to the onset of the pulse-alone response. *B*, Responses to H_1 tones and CF_2 s. In this neuron (J9-5-91-12), the pulse latency was 17.2 msec and the echo latency was 11.6 msec. Facilitation began earlier than the observed response to the pulse alone, as indicated by arrows.

Henson et al., 1987). Once an insect is detected, however, the duration of the CF initially lengthens to 25–30 msec and then progressively shortens to less than 10 msec as the target is approached (Novick and Vaisnys, 1964; Henson, 1987). Because the FM_1 occurs after the CF_1 while the echo delay is measured from the pulse onset, shifts in echo delay tuning are expected with changing CF duration. Such shifts are illustrated for two neurons in Figure 7, where the effects of changing pulse duration on the LDDs and SDDs are plotted. It can be seen that the LDDs “tracked” changes in pulse duration, while the SDDs remained constant. Similar shifts in LDDs with pulse duration were found in all neurons examined ($n = 5$).

Discussion

We have demonstrated that DSCF neurons show facilitative responses to FM_1 - CF_2 combination in biosonar signals, and that

the facilitation is echo delay dependent. In the following discussion, we focus on echolocation tasks to which DSCF neurons are likely to contribute, and consider implications of the results for the organization of auditory and sensory cortex in other species.

Behavioral significance of combination sensitivity and delay tuning of DSCF neurons

Detection of background objects. Doppler shift compensation is thought to be a strategy by which the relative velocity of flying insects and modulations from insect wingbeats produce echoes distinguishable from those produced by nonmoving background objects (Schnitzler, 1970a,b; Simmons, 1973; Johnson, 1974; Schnitzler et al., 1983). The horseshoe bat, *Rhinolophus ferrumequinum*, is an old-world species not closely related to mustached bats but that uses similar CF-FM biosonar signals and

Figure 7. The effect of changing CF duration on the delay-dependent response of two DSCF neurons (closed and open circles, respectively). The LDDs but not the SDDs increased with pulse duration.

shows Doppler shift compensation. Horseshoe bats Doppler shift compensate only to echoes from background objects and not insect targets (Trappe and Schnitzler, 1982). In mustached bats, most DSCF neurons are sensitive to the reference frequency (Suga et al., 1987) and lesion experiments have shown that an intact DSCF area is necessary for complete Doppler shift compensation (Gaioni et al., 1988). Thus, many DSCF neurons can be expected to respond to echoes from background objects. Our experiments suggest that most DSCF neurons will be facilitated by echoes from objects within a range of about 1.9–4.3 m, and the greatest distance at which a target would be detectable by the facilitation is about 5.5 m. Because of the considerable spread of best delays found among DSCF neurons and the typically broad delay tuning, the neurons can respond to targets located over a wide range of distances. Since mustached bats hunt in the acoustically cluttered, dense foliage of the new-world neotropics (Bateman and Vaughn, 1974), DSCF neuron facilitation may be important for maintaining an appropriate distance from background objects for optimal foraging.

Detection of insect targets by DSCF neurons. Both mustached bats and horseshoe bats attack only flying insects, that is, those that are beating their wings (Goldman and Henson, 1978; Schnitzler and Henson, 1980). Insect wingbeats produce modulations of echo amplitude and frequency, and these modulations cause the echoes from the wings to sweep through the response areas of DSCF neurons in a periodic fashion. Approximately 50% of the DSCF neurons show periodic responses to sinusoidally frequency modulated (SFM) stimuli that mimic echoes from beating wings (Suga et al., 1983a). Thus, DSCF neurons presumably respond to echoes from flying insects as well as background targets. The delay tuning of DSCF neurons observed in this study coupled with previous behavioral observations suggest a role for DSCF neurons in detecting insect targets. That is, mustached bats first pursue flying insects when they come within a range of 3–4 m (Novick and Vaisnys, 1964; Henson et al., 1987). For search phase pulses of 20–25 msec this distance closely corresponds to LDDs and MDDs typically obtained from DSCF neurons (Fig. 5C). Thus, the delay tuning of DSCF neurons correlates with the range at which the bats appear to detect insect targets. Possibly, DSCF neuron facili-

tation is instrumental in initiating the transition from the search to the approach phase of echolocation.

Characterization of insect targets by DSCF neurons. Several response properties of DSCF neurons indicate they may have a role in target characterization. These include periodic responses to SFM stimuli with frequency modulation depths in behaviorally relevant ranges (Suga et al., 1983a), and regular mapping of echo amplitude, which is a measure of target subtended angle (Suga, 1977; Manabe et al., 1982). During the approach phase, the bat initially increases the pulse duration (to 25–30 msec) and then decreases it until the pulses are about 10 msec long at the end of the phase (Novick and Vaisnys, 1964; Henson et al., 1987). The reason for the initial pulse lengthening may be to increase the number of wingbeat cycles in the echo, presumably to aid in characterization (Schnitzler and Flieger, 1983). Since the bat moves closer to the insect while this lengthening occurs, it is significant that most DSCF neurons are widely tuned to delay and remain facilitated even in the presence of considerable pulse-echo overlap (Fig. 7). Later in the approach phase, the best delay of DSCF neurons shifts with the decreasing CF duration, which should serve to maintain the neurons near their maximal degree of facilitation during most of the phase. Thus, DSCF neurons would be facilitated during much of the approach phase, when target characterization is presumably occurring.

Possible mechanisms of facilitation and delay tuning for DSCF neurons

The facilitation of DSCF neurons begins prior to any apparent pulse response (Figs. 3, 6; Table 2), so the pulse FM₁ appears to produce an EPSP that does not reach spike threshold for several milliseconds. No correlation between pulse and echo latency and the best delay, such as described for FM-FM neurons (Olsen and Suga, 1991a), was observed in DSCF neurons. The long-latency responses to the pulse, however, suggest that pulse channel delay lines are important for DSCF neurons, as for FM-FM neurons (Olsen and Suga, 1991a).

Anatomically, there are no intracortical connections that could account for the FM₁ and CF₂ frequencies becoming combined in the DSCF area (D. C. Fitzpatrick and N. Suga, unpublished observations). By contrast, fibers from the H₁ representation in the inferior colliculus are widespread in the MGB, including the ventral division (Wenstrup and Winer, 1987). FM-FM and CF/CF neurons are found in the MGB and cortex (Olsen and Suga, 1991a,b) but not in the inferior colliculus (O'Neill, 1985), suggesting that these types of combination-sensitive neurons are first created in the MGB. In the MGB, some CF/CF neurons show facilitated responses to frequencies typical of the FM₁ rather than the CF₁ (Olsen and Suga, 1991b). Thus, rather than the cortex, it is probably the MGB where FM₁ and CF₂ signals first converge to create FM₁-CF₂ combination-sensitive neurons.

Comparison of DSCF neurons with other combination-sensitive neurons in the mustached bat's auditory cortex

The FM₁-CF₂ combination sensitivity of DSCF neurons represents the fourth class of combination-sensitive neurons described in this cortex. FM-FM neurons, found in the FM-FM, DF, and ventral fringe (VF) areas (O'Neill and Suga, 1982; Suga and Horikawa, 1988; Edamatsu et al., 1989) are facilitated by FM₁-FM_n combinations ($N = 2, 3$, or 4) and are more sharply tuned to echo delays than are DSCF neurons. CF/CF neurons

are facilitated by the CF_1/CF_N ($n = 2$ or 3) combinations and often respond maximally to synthesized echoes with 0 msec delay and considerable pulse-echo overlap, which is why the abbreviations for the essential components are separated by a slash rather than a dash (Suga et al., 1983a). H_1 - H_2 neurons that respond to various combinations of H_1 and H_2 components have been localized to a small area just ventral and anterior to the DSCF area (Tsuzuki and Suga, 1988). However, the combination-sensitive and delay-tuned responses of FM_1 - CF_2 neurons described in this study are similar to those reported for H_1 - H_2 neurons, so whether the H_1 - H_2 area is in fact a discrete area, separate from the DSCF area, is questionable.

The present results suggest that functional areas in the auditory cortex are sequentially activated during insect pursuit. DSCF neurons are most active to echo delays between 11 and 26 msec, that is, to targets about 2–4 m distant. For echo delays less than 10 msec, about 50% of DSCF neurons no longer respond, and by 5 msec delay only about 10% respond. By contrast, 95% of neurons in the FM-FM area have best delays of 10 msec or less (Suga and O'Neill, 1979; O'Neill and Suga, 1982), as do all FM-FM neurons in the DF and VF areas (Suga and Horikawa, 1986; Edamatsu et al., 1989). Likewise, CF/CF neurons are in general broadly tuned to echo delay, but the responses typically decline for delays longer than about 10 msec (Suga et al., 1983b; Olsen and Suga, 1991b). Thus, the FM_1 - CF_2 neurons in the DSCF area would be most active during the early and middle stages of echolocation, while neurons in the FM-FM and CF/CF areas would become active as insects or other objects of interest were neared.

A feature of primary importance in the FM-FM, DF, and CF/CF areas is the regular mapping of an important information bearing parameter, that is, target range or velocity. At present, our data do not suggest a mapping of echo delay in the DSCF area, but the question has not been systematically addressed. Most DSCF neurons are facilitated by a broad range of echo delays and "track" changes in pulse duration so that they cannot encode range unambiguously, suggesting that they are not important for ranging per se and that a delay map in the DSCF area would not be useful. Echo frequency and amplitude are systematically represented in the DSCF area (Suga and Jen, 1976; Suga, 1977; Suga and Manabe, 1982; Suga et al., 1987).

Significance for the organization of the auditory cortex in other species

In the FM bat *Myotis lucifugus*, as many as 70–80% of the total neurons in the auditory cortex are delay sensitive (Sullivan, 1982; Wong et al., in press). In the mustached bat, delay-sensitive neurons (found in the FM-FM, DF, DSCF CF/CF, VF, and VA areas) occupy about 62% of the auditory cortex (Fitzpatrick and Suga, 1990). Neurons in the DSCF area account for about 26% of the delay-tuned neurons. In *Myotis*, about 30% of delay-tuned cortical neurons are "tracking" neurons whose peak of delay sensitivity changes during the insect pursuit sequence (Tanaka et al., 1992). By analogy with DSCF area neurons it may be that the delay sensitivity of *Myotis* "tracking" neurons is indicative of roles in target detection or characterization rather than ranging.

Unlike the combination-sensitive neurons previously described in the CF/CF, FM-FM, DF, VF, and H_1 - H_2 areas, the DSCF area is clearly part of AI, as shown by its location within the main tonotopic array of the auditory cortex (Suga and Jen, 1976) and by its connections with the ventral division of the

MGB (Olsen, 1986). Occasional selective responses to species-specific sounds (i.e., responses to some sounds but not others) have been reported in AI of non-bat species, including squirrel monkey (Wollberg and Newman, 1972; Newman and Wollberg, 1973a,b; Winter and Funkenstein, 1973), and humans (Creutzfeldt et al., 1989), but no studies have yet shown combination sensitivity of cortical neurons in a non-bat species to particular information-bearing parameters in behaviorally significant sounds. Previous results from the mustached bat, however, suggest that neurons in auditory cortex of other species may be specialized to represent information-bearing parameters contained within complex sounds, and the present results indicate that such neurons can even occur in AI.

Similar to the DSCF area, the primate visual and somatosensory cortices contain expanded representations of densely innervated regions of the sensory surface. Such expanded representations are generally regarded as a passive consequence of the dense peripheral innervation. Thus, in primates there are as yet no suggestions of processing mechanisms unique to the expanded portions of sensory cortex. This is surprising, since many visual and textural details of objects cannot be resolved until the objects are foveated or manipulated by the fingers, and much neural machinery is devoted to bringing these sensory surfaces to bear on objects of interest. In the DSCF area, great sensitivity and high resolution appear essential for roles in the detection and characterization of targets, but additional specializations such as combination sensitivity and delay tuning also occur. Preliminary experiments indicate that such specializations do not occur in posterior AI (Fig. 1A), where frequencies below 61 kHz are represented. We might expect to find neural response specializations in addition to high resolution within expanded representations in visual and somatosensory cortex of other species.

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