Plasticity of Bat’s Central Auditory System Evoked by Focal Electric Stimulation of Auditory and/or Somatosensory Cortices

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Ma, Xiaofeng and Nobuo Suga. Plasticity of bat’s central auditory system evoked by focal electric stimulation of auditory and/or somatosensory cortices. J Neurophysiol 85: 1078–1087, 2001. Recent findings indicate that the corticofugal system would play an important role in cortical plasticity as well as collicular plasticity. To understand the role of the corticofugal system in plasticity, therefore, we studied the amount and the time course of plasticity in the inferior colliculus (IC) and auditory cortex (AC) evoked by focal electrical stimulation of the AC and also the effect of electrical stimulation of the somatosensory cortex on the plasticity evoked by the stimulation of the AC. In adult big brown bats (Eptesicus fuscus), we made the following major findings. 1) Electric stimulation of the AC evokes best frequency (BF) shifts, i.e., shifts in frequency-response curves of collicular and cortical neurons. These BF shifts start to occur within 2 min, reach a maximum (or plateau) at 30 min, and then recover ~180 min after a 30-min-long stimulus session. When the stimulus session is lengthened from 30 to 90 min, the plateau lasts ~60 min, but BF shifts recover ~180 min after the session. 2) The electric stimulation of the somatosensory cortex delivered immediately after that of the AC, as in fear conditioning, evokes a dramatic lengthening of the recovery period of the cortical BF shifts but not that of the collicular BF shift. The electric stimulation of the somatosensory cortex delivered before that of the AC, as in backward conditioning, has no effect on the collicular and cortical BF shifts. 3) Electric stimulation of the AC evokes BF shifts not only in the ipsilateral IC and AC but also in the contralateral IC and AC. BF shifts are smaller in amount and shorter in recovery time for contralateral collicular and cortical neurons than for ipsilateral ones. Our findings support the hypothesis that the AC and the corticofugal system have an intrinsic mechanism for reorganization of the IC and AC, that the reorganization is highly specific to a value of an acoustic parameter (frequency), and that the reorganization is augmented by excitation of nonauditory sensory cortex that makes the acoustic stimulus behaviorally relevant to the animal through associative learning.

INTRODUCTION

The auditory, visual, and somatosensory systems, respectively, have cochleotopic, retinotopic, and somatotopic maps in their central neural pathways. These sensory epithelial maps are modified by deprivation, injury, and experience in young and adult animals (Clark et al. 1988; Gao and Suga 1998, 2000; Hubel et al. 1977; Irvine and Rajan 1996; Jenkins et al. 1990; Kaas et al. 1990; Merzenich et al. 1984; Pettet and Gilbert 1992; Recanzone et al. 1993; Snyder et al. 1990, 1991; Weinberger et al. 1993). Such plasticity has been explained by changes in divergent and convergent projections of neurons in the ascending sensory system. However, recent findings indicate that the cerebral sensory cortex and the descending (corticofugal) system play an important role in plasticity. In the motor (Nudo et al. 1996), somatosensory (Recanzone et al. 1993; Spengler and Dinse 1994), and auditory systems (Chowdhury and Suga 2000; Maladoñado and Gerstein 1996; Yan and Suga 1996, 1998; Zhang et al. 1997), focal electrical stimulation of a particular portion of the sensory or motor map in the cortex evokes changes in the cortical map around the stimulated portion and also in the subcortical map corresponding to the cortical map where changes are evoked.

Recent studies indicate that cortical neurons of the mus-tached bat (Pteronotus parnellii) mediate, via corticofugal projection, a highly focused positive feedback to subcortical neurons “matched” in the tuning of a particular acoustic parameter and a widespread lateral inhibition to “unmatched” subcortical neurons. This function, named egocentric selection, improves the neural representation of frequently occurring signals in the central auditory system (Yan and Suga 1996; Zhang and Suga 1997). In the big brown bat, Eptesicus fuscus, egocentric selection shifts the best frequencies (BFs) of collicular neurons not only toward the BF of electrically stimulated cortical neurons but also toward the frequency of a repetitively delivered acoustic stimulus (tone burst). BF shifts mean a reorganization of the frequency map in the inferior colliculus: IC (Yan and Suga 1998). Focal electric stimulation of the auditory cortex (AC) also evokes reorganization of the cortical frequency map (Chowdhury and Suga 2000). However, the time courses of collicular and cortical BF shifts have not yet been studied. The first aim of the present studies was to measure the time courses of collicular and cortical BF shifts evoked by focal electric stimulation of the AC.

The somatosensory cortex plays an essential role in plasticity of the IC (Gao and Suga 1998) and the AC (Gao and Suga 2000) evoked by fear conditioning with acoustic stimuli followed by electric leg stimulation. Inactivation of the somatosensory cortex during the conditioning blocks both collicular and cortical BF shifts that otherwise would be evoked by the conditioning. The second aim of the present studies was to investigate whether electric stimulation of the somatosensory cortex following electric stimulation of the AC augments collicular and cortical BF shifts.

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METHODS

Materials, surgery, recording of neural activity, acoustic stimulation, cortical electrical stimulation, data acquisition, and data processing were basically the same as those described in Yan and Suga (1996) and Chowdhury and Suga (2000). Therefore only the essential portion of the methods are summarized in the following text. Fifteen adult big brown bats (body weight: 18–24 g) were used for the present experiments. Under neuroleptanalgesia (fentanyl-droperidol mixture, 4.08 mg/kg body wt), a 1.5 cm-long metal post was glued on the dorsal surface of the bat’s skull. The physiological experiment was started 3–4 days after the surgery. The animal was placed in a polyethylene-foam body mold and was hung at the center of a sound-proof room that was maintained at 31°C. The bats used were neither anesthetized nor tranquilized. They were not going into hibernation. The temperature monitored with a thermistor placed between the bat and body mold was 37°C. The metal post mounted on the skull was fixed on a metal rod with set screws to immobilize the animal’s head, and the bat’s head was adjusted to face directly at a loudspeaker located 74 cm away. Holes 50–100 μm in diameter were made in the skull covering the AC or the inferior colliculus (IC). Tungsten-wire electrodes for recording action potentials or for electrically stimulating cortical neurons were inserted into the brain through these holes (see following text). The bats were monitored on a video monitor screen during the experiments. The protocol for this research was approved by the animal studies committee of Washington University.

Acoustic stimulation

Acoustic stimuli were 20-ms-long tone bursts with a 0.5-ms rise-decay time. They were generated by a voltage-controlled oscillator and an electronic switch and were delivered at a rate of 5/s with a leaf tweeter. The frequency and amplitude of the tone bursts were varied manually or computer-controlled. The amplitude was calibrated with a Bruel and Kjaer microphone and was expressed in dB SPL.

The frequency-tuning curve of a single collicular or cortical neuron was first manually measured. Then the amplitude of a tone burst was fixed at 10 dB above minimum threshold of the neuron, and a computer-controlled frequency scan was delivered. [A frequency-threshold or -tuning curve indicates that threshold varies as a function of the frequency of sound. The lowest threshold of a frequency-tuning curve has been called minimum threshold (Suga 1964).] The frequency scan consisted of 21 time blocks. In the first 20 blocks, frequency was changed in 0.3- or 0.5-kHz steps, and in the 21st (last) block, no stimulus was presented to count background discharges. The duration of each block was 200 ms, so that the duration of the frequency scan was 4,200 ms. An identical frequency scan was repeated 50 times, and the responses of a single neuron were displayed as an array of peri-stimulus-time (PST) histograms or PST cumulative (PSTC) histograms (Figs. 1 and 2).

Electrical stimulation

Electrical stimuli were delivered through a pair of tungsten-wire electrodes, the tips of which were 6–8 μm in diameter and were separated by 150 μm, one proximal to the other. These electrodes were used first to record auditory responses of cortical neurons at the depths of 200–900 μm, i.e., at cortical layers III–VI, then to measure the BF and minimum threshold of these neurons, and finally to electrically stimulate them. The electrical stimulation was a 6-ms-long train of four monophasic electric pulses (100 nA, 0.2-ms duration, 2.0-ms interval). The train of electric stimuli was delivered at a rate of 10/s for 2–90 min (hereafter, ES). The train of 0.2-ms-long, 100-nA electric pulses was estimated to stimulate neurons within a 60-μm radius around the electrode tip (Yan and Suga 1996). Therefore electrical stimulation of the AC is quite focal. The bat showed no behavioral response at all to such a weak electrical stimulation delivered to the AC.

To mimic trace conditioning with a train of tone pulses followed by an electric leg stimulation, a train of electric stimuli delivered to the AC (hereafter ESar) was paired with an electric stimulation of the primary somatosensory cortex (hereafter ESs). ESar was 1.0 s long and consisted of 33 trains. Each train was 6 ms long and consisted of four electric pulses, as in ESs. ESar was delivered twice per minute for 30 min. ESs was 50 ms long and consisted of 20 0.2-ms-long, 100-μA electric pulses. It was also delivered twice per minute for 30 min. When paired, ESs was delivered 1.0 s after ESar. To mimic backward conditioning, ESs was delivered 1.0 s before ESar.

Data acquisition and processing

Cortical responses were recorded at depths between 200 and 600 μm. The central nucleus of the IC is big and shows a simple and systematic tonotopic organization (Casseday and Covey 1992). The dorsal surface of the IC is directly visible through the skull. In dorsoventral electrode penetrations through the dorsal surface, the electrode passed across the main nucleus of the IC so that BFs of neurons systematically became higher exactly as expected from the tonotopic map (e.g., Yan and Suga 1998). The neurons were recorded at depths between 200 and 2,000 μm in the central nucleus of the IC.

The responses of a neuron to tone bursts in the frequency scan repeated 50 times were recorded before, during, and after ESar, ESs or ESs and ESs were displayed as arrays of PST or PSTC histograms. The waveform of an action potential was stored on a digital storage oscilloscope at the beginning of the data acquisition and was used as a template. Action potentials discharged by the neuron were continuously monitored together with the template on the screen of the digital storage oscilloscope during data acquisition: before, during, and after the cortical stimulation. Data acquisition was continued as far as action potentials visually matched the template. Data were stored on a hard drive of a personal computer and were used for off-line analysis.

Off-line data processing included plotting PST or PSTC histograms displaying responses of a collicular or cortical neuron to 50 identical acoustic stimuli and frequency-response curves based on 50 frequency scans obtained before, during, and after focal cortical stimulation. The magnitude of auditory responses was expressed by a number of impulses per 50 identical stimuli after subtracting background discharges counted in the last block of the frequency scan. A t-test was used to test the difference between the auditory responses obtained before and after the focal cortical stimulation and to test the difference between the responses of collicular and cortical neurons.

The following criteria were used for a shift in the frequency-response curve or BF of a neuron evoked by a focal cortical stimulation. If a shifted frequency-response curve or BF did not recover by 50%, the data were excluded from the analysis. In stable long recording conditions, all curves shifted by the cortical stimulation recovered by 50%. This recovery itself helped prove that the shift was significant. When a BF shift was small and its significance was not obvious, a weighted average frequency (i.e., BF) was calculated for the summed responses to five consecutive frequency scans (Blaisdell 1993). Then the mean and standard deviation of these weighted averages were computed, and a two-tailed paired t-test was used to determine whether or not the weighted-average frequencies obtained for control and stimulus conditions were significantly different for P < 0.05. The criterion for an increase or decrease in response magnitude (number of impulses per 50 stimuli) was a change of 20% from a response magnitude in a control condition, i.e., in a 20-min-long time period immediately prior to electrical stimulation of the AC.
RESULTS

Shifts in the best frequencies of collicular and cortical neurons evoked by focal electric stimulation of the AC

The effects of focal electric stimulation of the AC (ES_ar or ES_at) were studied on the auditory responses of 257 collicular and 212 cortical ipsilateral neurons (469 in total) and on those of 31 collicular and 26 cortical contralateral neurons (57 in total). Since the great majority of neurons studied were ipsilateral to the cortical electric stimulation, they are simply called collicular or cortical neurons, while the small number of contralateral neurons studied are specified as contralateral collicular or cortical neurons. Of the 469 neurons studied, 193 collicular and 172 cortical neurons showed clear changes in response magnitude, frequency-response curve and BF for cortical electric stimulation. The data obtained from these 365 neurons are shown in the figures.

ES_ar as short as 2 min could evoke BF shifts in the IC and AC. The amount of the BF shifts became larger with the duration of ES_ar and became a maximum for a 30-min-long ES_ar. Figures 1 and 2 show changes in the responses (A and B) and the frequency-response curve (C) evoked by a 30-min-long ES_ar of a collicular and a cortical neuron, respectively. The collicular neuron in Fig. 1 was tuned to 31.5 kHz (C1, ●). When the ES_ar was delivered to cortical neurons tuned to 26.0 kHz, its frequency-response curve and BF shifted from 31.5 to 29.0 kHz at the end of the ES_ar (C2, x), i.e., toward the BF of the electrically stimulated cortical neurons. The shifted frequency-response curve and BF shifted to 30.5 kHz 90 min after the ES_ar (C3, x), and returned to 31.5 kHz 180 min after the ES_ar (C4, ○). The time for full recovery from the maximum shift was ~180 min. Figure 1, A and B, respectively show the changes in the responses to a 31.5 kHz (the BF in the control condition: BF_c) and a 29.0-kHz tone burst (the BF in the maximally shifted condition: BF_s). The ES_ar evoked a decrease in the response at 31.5 kHz (A2) and an increase in the response at 29.0 kHz (B2). In other words, the corticofugal effect on the “unmatched” collicular neuron could be inhibitory or facilitatory depending on the frequency. These corticofugal effects disappeared 180 min after ES_ar.

The cortical neuron in Fig. 2 was tuned to 40.0 kHz (C, ○). When ES_ar was delivered to 35.0-kHz tuned cortical neurons, its frequency-response curve and BF shifted from 40.0 to 38.0 kHz at the end of the ES_ar (C, ●). In C, the arrays of PST cumulative histograms show frequency-response curves. The PST histograms and frequency-response curves were obtained before (1), immediately after (2), 90 min after (3), and 180 min after ES_ar (4). ●, x, and ○, BFs in the control, shifted, and recovery conditions, respectively. (bottom of A and B), 20-ms-long tone bursts.

FIG. 1. Changes in the responses (A and B) and frequency-response curve (C) of a collicular neuron to tone bursts evoked by repetitive focal electrical stimulation of the auditory cortex (ES_c). ES_c was a 6-ms-long train of 4 electric pulses (0.2-ms-long, 100 nA) that was delivered every 100 ms over 30 min. ES_c stimulated cortical neurons tuned to 26.0 kHz (● in C2). In A and B, the poststimulus-time (PST) histograms show changes in the responses at the best frequencies in the control (BF_c: 31.5 kHz) and “shifted” conditions (BF_s: 29.0 kHz). In C, the arrays of PST cumulative histograms show frequency-response curves. The PST histograms and frequency-response curves were obtained before (1), immediately after (2), 90 min after (3), and 180 min after ES_ar (4). ●, x, and ○, BFs in the control, shifted, and recovery conditions, respectively. (bottom of A and B), 20-ms-long tone bursts.

FIG. 2. Changes in the responses (A and B) and frequency-response curve (C) of a cortical neuron to tone bursts evoked by ES_c of cortical neurons tuned to 30.0 kHz. The cortical neuron tuned to 40.0 kHz (C, ○) showed a change in the responses to 40.0 (A2 and B2) and 38.0 kHz (B2) and shifted its frequency-response curve from 40.0 to 38.0 kHz (C, ●). In C, ○, ●, ▲, and - - - represent the frequency-response curves obtained before (1, control), immediately after (2), 90 min after (3), and 180 min after ES_ar (4), respectively. See Fig. 1 for abbreviations and other explanations.
electrically stimulated cortical neurons. The shifted frequency-response curve and BF shifted back to 38.5 kHz 90 min after the ESar (C, – – –), and returned to 40.0 kHz 180 min after the ESar (C, - - -). That is, the time for full recovery was 180 min.

Figure 2, A and B, respectively, shows the changes in the responses to a 40.0-kHz (BFc) and a 38.0-kHz tone burst (BFs). The ESar evoked an overall decrease in the response at 40.0 kHz (A2) and an overall increase in the response at 38.0 kHz (B2). Therefore the effect of ESar on the unmatched cortical neuron could be inhibitory or facilitatory depending on the frequency.

BF shift for a 30- or 60-min-long ESar was studied with 65 collicular neurons. Of the 65, 43 showed BF shifts (Fig. 3A). The directions and amounts of BF shifts of these neurons are represented by the directions and lengths of bars originating from individual symbols, respectively. Regardless of the BFs of electrically stimulated cortical neurons, 30 collicular neurons with a BF within a 10-kHz band immediately above the BF of electrically stimulated cortical neurons (hereafter, cortical BF) showed a downward BF shift without exception. The amount of BF shift differed from neuron to neuron. However, there was no sign that the higher the cortical BF, the larger the collicular BF shift. To substantiate this, BF shifts in Fig. 3A are plotted as a function of cortical BF (Fig. 3B). In Fig. 3B, BF shifts of cortical neurons with a BF 4–6 kHz higher than the cortical BF are shown with filled symbols because it has been demonstrated that these collicular neurons showed the largest BF shifts (Chowdhury and Suga 2000; Yan and Suga 1998). A 40- to 60-kHz band is one octave higher than a 20- to 30-kHz band, and there are several data points in each of these frequency bands. A comparison between the data in these frequency bands indicates that there was no relationship such that collicular BF shifts for 40- to 60-kHz cortical BFs were two times larger than those for 20- to 30-kHz cortical BFs. The mean and SE of BF shifts were 0.52 ± 0.20 kHz (n = 9) for 20–30 cortical BFs and 0.48 ± 0.14 kHz (n = 13) for 40- to 60-kHz cortical BFs. The difference between these two means is statistically insignificant (P = 0.36). Therefore in our present paper, BF shifts are expressed in kilohertz, not in octave.

Figure 4 shows collicular BF shifts evoked by ESar’s with different durations as a function of difference in BF between
recorded collicular and stimulated cortical neurons. The duration of ES<i><sub>ar</sub></i> was 15 (A), 30 (B), or 60 min (C). BF shift became larger with the duration of ES<i><sub>ar</sub></i>. Regardless of the duration, however, the major BF shifts occurred for neurons with BFs within 10 kHz higher than the BF of electrically stimulated cortical neurons, and these were toward the cortical BF. The BF shifts toward the cortical BF may be defined as “centripetal” BF shifts, and those away from the cortical BF may be defined as “centrifugal” BF shifts. Since the major BF shifts occurred only on one side of the cortical BF, these were asymmetrical and centripetal. Minor BF shifts occurred for neurons with BFs ~5 kHz lower or ~13 kHz higher than the cortical BF. These were centripetal and centrifugal, respectively. The largest BF shifts occurred at 4–5 kHz above the cortical BF and were 1.6 kHz for the 15-min-long ES<i><sub>ar</sub></i> and 2.0 kHz for both the 30 and 60 min-long ES<i><sub>ar</sub></i>’s.

About half of the collicular and cortical neurons contralateral to the AC stimulated by ES<i><sub>ar</sub></i> showed no BF shift, but the remaining half did (Fig. 5A). Different from the ipsilateral collicular and cortical BF shifts, which were asymmetrical and centripetal (Fig. 5B), the BF shifts in the contralateral collicular and cortical neurons were somewhat symmetrical and centripetal (Fig. 5A). Centripetal BF shifts occurred for BFs that were within a range between −9 and +12 kHz of the BFs of stimulated cortical neurons. The centripetal BF shifts were sandwiched between centrifugal BF shifts that occurred for BFs that were between −10 and −14 and between +13 and +17 kHz (Fig. 5A). For comparison in BF shift between the contralateral and ipsilateral neurons, Fig. 5B shows the BF shifts of ipsilateral collicular and cortical neurons. It is clear that the BF shifts in the ipsilateral neurons were asymmetrical and that the BF shifts for BFs 5–8 kHz higher than the BF of electrically stimulated cortical neurons were much larger than those in the contralateral neurons.

**Time courses of BF shifts in ipsilateral collicular and cortical neurons**

BF shift was largest for collicular and cortical neurons with a BF ~5 kHz higher than that of electrically stimulated cortical neurons (Fig. 4). Therefore the time courses of BF shifts were measured for collicular and cortical neurons with a BF ~5 kHz higher than the BF of the electrically stimulated cortical neurons.

Since stimulus artifacts interfered with the recording of action potentials, the duration of ES<i><sub>ar</sub></i> was varied from 2 to 90 min and the amounts of collicular and cortical BF shifts were measured immediately after ES<i><sub>ar</sub></i>. The BF shifts of collicular and cortical neurons were 0.6 ± 0.34 and 0.6 ± 0.33 kHz, respectively, for a 2-min-long ES<i><sub>ar</sub></i> (shortest horizontal bar of Fig. 6). Those were 0.68 ± 0.31 and 0.72 ± 0.30 kHz for a 4-min-long ES<i><sub>ar</sub></i> (2nd shortest horizontal bar). There was no significant difference in BF shift between collicular and cortical neurons (<i>P > 0.05</i>). In other words, the BF shifts of the collicular and cortical neurons developed at the same time and to the same amount until 4 min after the onset of ES<i><sub>ar</sub></i>. As the duration of ES<i><sub>ar</sub></i> increased, the BF shifts increased. The amount of increase was larger for the cortical neurons than for the collicular neurons: 1.18 ± 0.20 kHz in the AC and 1.10 ± 0.18 kHz in the IC for a 30-min-long ES<i><sub>ar</sub></i> (5th horizontal bar) and 1.36 ± 0.19 kHz in the AC and 1.19 ± 0.21 kHz in the IC for a 60-min-long ES<i><sub>ar</sub></i> (6th horizontal bar). The difference in BF shift between the cortical and collicular neurons was not significant (<i>P > 0.05</i>) 30 min after the onset of ES<i><sub>ar</sub></i>, but was significant 60 min after that (<i>P < 0.05</i>). The collicular BF shift was 1.10 ± 0.18 and 1.19 ± 0.21 kHz at 30 and 60 min, respectively, after the onset of ES<i><sub>ar</sub></i>. This difference was significant (<i>P < 0.05</i>). Therefore the cortical BF shift reached a plateau value 30 min after the onset of ES<i><sub>ar</sub></i>. The cortical BF shift was 1.18 ± 0.20 and 1.36 ± 0.19 kHz at 30 and 60 min, respectively, after ES<i><sub>ar</sub></i>. This difference was significant (<i>P < 0.05</i>). The cortical BF shifts for a 90-min-long ES<i><sub>ar</sub></i> were the same as those for the 60-min-long ES<i><sub>ar</sub></i>: 1.36 ± 0.19 kHz for 60 min and 1.31 ± 0.26 kHz for 90 min. The cortical BF shift thus reached a plateau value between 30 and 60 min after the onset of ES<i><sub>ar</sub></i>. In other words, the collicular and cortical BF shifts both reached plateaus within 60 min after the onset of the 90-min-long ES<i><sub>ar</sub></i>, and the plateau value was 14% larger for the cortical BF shift than for the cortical BF shift (<i>P < 0.05</i>). The 2-min-long ES<i><sub>ar</sub></i> evoked ~50% of the maximum collicular BF shift and ~44% of the maximum cortical BF shift.

The recovery period differed according to the amount of BF shift. Figure 7, A and B, shows the recovery curves of the BF shifts of collicular and cortical neurons, respectively. The recovery curves of the cortical BF shifts were similar to those of the collicular BF shifts. For example, the collicular and cortical BF shifts evoked by the 2-min-long ES<i><sub>ar</sub></i> recovered 45 min after the ES<i><sub>ar</sub></i> (Fig. 7, ○). Those evoked by the 30-min-
long ESar recovered 180 min after the ESar (Fig. 7, △). The amounts and recovery times of the collicular BF shifts were the same for the 30-, 60-, and 90-min-long ESar’s. However, the amount of cortical BF shift was slightly larger for the 60- and 90-min-long ESar than for the 30-min-long ESar. The recovery time of the cortical BF shift was very similar for the 30-, 60-, and 90-min-long ESar’s (Fig. 7, △, ▲, and □, respectively). There was a noticeable difference in a 50% recovery time between the collicular and cortical BF shifts. For example, the 50% recovery time was 25 min for the collicular neurons, but 45 min for the cortical neurons when ESar was 15 min long (F). It was 60 min for the former but 120 min for the latter when ESar was 30 min long (△). These differences between the collicular and cortical BF shifts were statistically significant (P < 0.005). Therefore cortical neurons tended to maintain BF shift longer than collicular neurons.

Augmentation of collicular and cortical BF shifts by electrical stimulation of the somatosensory cortex

Asymmetrical and centripetal BF shifts of collicular and cortical neurons can be evoked by conditioning the bat with a conditioned acoustic stimulus (a train of tone bursts) followed by an unconditioned electric leg stimulus. The primary somatosensory cortex and the AC are both necessary for these BF shifts (Gao and Suga 1998, 2000). Therefore a train of electric stimuli (ESat) was delivered to the AC, and then a short train of electric stimuli (ESst) was delivered to the somatosensory cortex to evoke the excitation of these cortices, which would be evoked by the conditioning.

ESat delivered at a rate of 2/min over 30 min was less effective in evoking collicular and cortical BF shifts than the 30-min-long ESar in which an electric stimulus was repetitively delivered at a rate of 10/s. Collicular (0.78 ± 0.33 kHz) and cortical BF shifts (0.79 ± 0.34 kHz) were very similar in amount, but different in recovery time: 60 versus 100 min at 50% recovery and 120 versus 150 min at full recovery (Fig. 8, curves a and b). When ESat was followed by ESst, the collicular BF shift became 12% larger and lasted 70 min longer at 50% recovery than that evoked by ESst alone (Fig. 8, curve c). ESst had a larger effect on cortical BF shift than on collicular BF shift. That is, the cortical BF shift became 31% larger and lasted 120 min longer at 50% recovery than that evoked by ESst alone (Fig. 8, curve d). These differences in the changes...
evoked by ES <sub>st</sub> between the collicular and cortical BF shifts were significant (P < 0.05 for amount and P < 0.001 for 50% recovery time).

Figure 9 shows the arrays of PSTC histograms representing frequency-response curves of a single collicular and a single cortical neuron. In Fig. 9A, the collicular neuron was tuned to 36.0 kHz (A1). When ES <sub>at</sub> stimulating 30.5-kHz tuned cortical neurons was followed by ES <sub>st</sub>, the collicular BF shifted from 36.0 to 34.0 kHz at the end of the 30-min-long delivery of ES <sub>at</sub> + ES <sub>st</sub> (A2). The shifted BF recovered to the BF in the control condition 180 min after ES <sub>at</sub> + ES <sub>st</sub> (A4). In Fig. 9B, the cortical neuron was tuned to 41.5 kHz (B1). When ES <sub>at</sub> stimulating cortical neurons tuned to 37.0 kHz was paired with ES <sub>st</sub>, its BF shifted to 39.5 kHz at the end of ES <sub>at</sub> + ES <sub>st</sub> (B2). The shifted BF slowly recovered: 39.5 kHz 180 min after ES <sub>at</sub> + ES <sub>st</sub> (B4) and 41.5 kHz 300 min after ES <sub>at</sub> + ES <sub>st</sub> (B5). ES <sub>st</sub> lengthened the recovery period of cortical BF shift, as unconditioned leg stimulation did (Gao and Suga 1998, 2000).

When an unconditioned leg stimulation is delivered prior to a conditioned acoustic stimulus, collicular and cortical BF shifts are not evoked or not augmented (Gao and Suga 1998). To mimic this backward conditioning, ES <sub>at</sub> was delivered prior to ES <sub>st</sub>. The collicular and cortical BF shifts evoked by ES <sub>at</sub> alone were not augmented at all in magnitude by this ES <sub>at</sub> + ES <sub>st</sub>. However, they showed a tendency toward longer recovery periods, although this was statistically insignificant, P > 0.05 (Fig. 10). In other words, the electrical stimulation of the somatosensory cortex prior to that of the AC was ineffective in evoking the augmentation of BF shifts, the same as backward conditioning.

ES <sub>at</sub> augmented collicular and cortical BF shifts evoked by acoustic stimuli (tone bursts). A 1.0-s-long train of tone bursts...
ASt for the collicular neurons and 60 min after the AS t for the studied. These BF shifts recovered rapidly: 45 min after the kHz; n shift to neither ASt nor AS t by N. The numbers of neurons in the parentheses are those that showed a BF obtained from the number of neurons ranging between 12 and 20, as indicated respectively, evoked by AS t.

Curves c and d represent the time courses of collicular and cortical BF shifts, respectively, evoked by AS t. Curves a and b represent the time courses of collicular and cortical BF shifts, respectively, evoked by AS t + ES ar. Means and SE are based on the data obtained from the number of neurons ranging between 12 and 20, as indicated by N. The numbers of neurons in the parentheses are those that showed a BF shift to neither AS t nor AS t + ES ar.

(10-ms-long, 50 dB SPL, tone burst rate of 33/s; hereafter AS t) delivered at a rate of 2/s for 30 min evoked small collicular (0.38 ± 0.34 kHz; n = 20) and cortical BF shifts (0.42 ± 0.35 kHz; n = 18) in 38 neurons but not in the remaining 41 neurons studied. These BF shifts recovered rapidly: 45 min after the AS t for the collicular neurons and 60 min after the AS t for the cortical neurons (Fig. 11, a and b).

When each AS t was followed by ES ar, the collicular BF shift became 139% larger and lasted 60 min longer at 50% recovery than that evoked by AS t alone (Fig. 11, c). ES ar had a larger effect on the cortical BF shift than on the collicular BF shift. That is, the cortical BF shift became 166% larger and lasted 120 min longer at 50% recovery than that evoked by AS t alone (Fig. 11, d). The full recovery of the BF shifts after AS t + ES ar occurred at 130 min in the IC and 180 min in the AC. These differences between the collicular and cortical BF shifts evoked by ES ar were significant (P < 0.01 for amount and P < 0.005 for 50% recovery time).

**Discussion**

Corticofugal modulation and plasticity of the central auditory system

Electric stimulation of the AC (ES ar) evokes collicular (Yan and Suga 1998; Zhang et al. 1997) and cortical BF shifts (Chowdhury and Suga 2000). As shown in Figs. 6 and 7, these collicular and cortical BF shifts rapidly and simultaneously develop within 2 min after the onset of ES ar and reach a maximum ~30 min after the onset of a 30-min-long ES ar. They recover (return) to the control BF ~180 min after the cessation of the ES ar. For a 90-min-long ES ar, the maximal BF shifts plateau ~60 min after the beginning of ES ar and then recover ~180 min after the cessation of ES ar. The cortical BF shift tends to recover slightly slower than the collicular BF shift. However, both the collicular and cortical changes are short term and similar to each other. The collicular and cortical BF shifts evoked by ES ar do not give us a cue as to whether the cortical BF shift evokes the collicular BF shift or vice versa. Gao and Suga (2000) found that for auditory conditioning, the collicular BF shift develops faster than the cortical BF shift does and that the former can be evoked without the latter. Therefore they hypothesized that the collicular change boosts the cortical change.

The cortical BF shift evoked by a 30-min-long ES ar is similar in amount and time course to that evoked by a 30-min-long conditioning session. Inactivation of the AC during the conditioning evokes no collicular BF shift, which otherwise would be evoked (Gao and Suga 2000). These findings indicate that the collicular change evoked by the conditioning is based on corticofugal modulation.

The cortical and collicular BF shifts evoked by ES ar are similar to each other in amount and time course, as shown in Figs. 5–7. It recovers ~180 min after a 30- or 90-min-long ES ar. However, the cortical and collicular BF shifts evoked by the conditioning are quite different from each other. That is, the cortical BF shift evoked by the conditioning develops slowly, reaches a plateau when the collicular BF shift is almost recovered, and lasts many hours after the conditioning (Gao and Suga 2000). These data indicate that whether the cortical BF shift follows the cortical BF shift depends on nonauditory systems.

Inactivation of the somatosensory cortex during the conditioning abolishes the collicular and cortical BF shifts, otherwise they would be evoked. Therefore Gao and Suga (1998, 2000) hypothesized that the somatosensory cortex and the AC send somatosensory and auditory signals, respectively, to the amygdala through the association cortex and that this pathway is essential for cortical plasticity due to fear conditioning rather than the pathway from the multisensory thalamic nuclei to the amygdala proposed by Weinberger (1990, 1998). Electrical stimulation of the somatosensory cortex immediately after (not before) ES ar or acoustic stimulation augments cortical BF shift in magnitude and, in particular, duration, as shown in Figs. 8, 9, and 11. Therefore our present data strongly favor the hypothesis proposed by Gao and Suga (1998, 2000).

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37°C during the experiments. The bats were not experiencing an abnormally low body temperature. Therefore the first assertion can be dismissed. Asymmetrical and centripetal BF shifts of collicular and cortical neurons are evoked not only by electrical stimulation of the AC (Chowdhury and Suga 2000; Yan and Suga 1998; present paper) but also by auditory conditioning with repetitive acoustic stimuli followed by electric leg stimulation (Gao and Suga 1998, 2000) or by repetitive acoustic stimuli without any electrical stimulation of the AC (Gao and Suga 1998; Yan and Suga 1998). Therefore the second assertion can be dismissed. Asymmetrical and centripetal BF shifts are highly specific to the BF of cortical neurons electrically stimulated and are augmented by electric stimulation of the somatosensory cortex following that of the AC by a 1.0-s gap. A change in brain temperature or blood flow that might be caused by arousal cannot evoke such highly frequency-specific BF shifts, so the third assertion may also be dismissed. Atropine applied to the IC or AC has little effect on auditory responses, frequency-tuning curves and BFs of collicular and cortical neurons. However, it has prominent effects on the BF shifts of these neurons. For example, atropine applied to the IC immediately prior to auditory conditioning selectively abolishes collicular BF shifts that otherwise would be evoked in the same amount as those evoked by cortical electrical stimulation. It does not abolish cortical BF shifts (W. Ji, E. Gao, and N. Suga, unpublished data). These observations indicate that collicular and cortical BF shifts do not depend on possible changes in cochlear hair cells and that the AC and IC have plasticity for reorganization of the cochleotopic map. BF shifts of collicular and cortical neurons (i.e., reorganization of the cochleotopic map) are a fact. However, we don’t yet know the neural mechanisms for the BF shifts. The exploration of the neural mechanisms for BF shifts will be a further step in our research.

Variation in BF shifts

In the big brown bat, ESar evoked asymmetrical and centripetal BF shifts in the ipsilateral IC (Yan and Suga 1998) (Fig. 4) and AC (Chowdhury and Suga 2000) (Fig. 5B) but symmetrical and centripetal BF shifts in the contralateral IC and AC (Fig. 5A). We don’t know of any physiological and anatomical basis for this difference. In the mustached bat, ESar delivered to the DSCF area of the primary auditory cortex evoked symmetrical and centrifugal BF shifts in the ipsilateral IC and MGB (Zhang and Suga 2000). However, ESar delivered to the posterior division of the primary auditory cortex of the mustached bat evoked somewhat symmetrical and centripetal BF shift in the AC (Yan and Suga 2000). The DSCF area is specialized for fine frequency analysis of sound at ~61 kHz and has a radial frequency axis (Suga and Jen 1976; Suga and Manabe 1982). The FM-FM area of the AC of the mustached bat is a specialized area for fine analysis of echo delays (O’Neill and Suga 1979) and has an echo-delay axis (Suga and O’Neill 1979). ESar delivered to the FM-FM area evoked symmetrical and centrifugal best echo-delay shifts in the IC (Yan and Suga 1996). The DSCF and FM-FM areas are very large relative to the nonspecialized (i.e., ordinary) portion of the primary auditory cortex of this species. Therefore Suga et al. (2000) hypothesized that centrifugal BF or best echo-delay shifts are related to increasing contrast in neural representation of echo frequency in the DSCF area or of echo delay in the FM-FM area, respectively, and that centripetal BF shift is related to augmenting neural representation of a signal in the ordinary area such as the posterior division of the AC of the mustached bat and the AC of the big brown bat. This hypothesis is supported by the data obtained by Sakai et al. (2000). They delivered ESar to the AC of the gerbil, Meriones unguiculatus and found that ipsilateral cortical BF shift was asymmetrical and centripetal, the same as in the big brown bat.

As discussed in the preceding text, ESar evoked BF shifts that are significantly different between species and between cortical areas of the same species. We don’t yet know the anatomical and physiological basis for this variation. Yan and Suga (1998) pointed out that symmetrical and asymmetrical BF shifts related to symmetrical and asymmetrical shape, respectively, of tuning curves of neurons and that centrifugal and centripetal BF shifts are related to sharp and broad tuning curves, respectively. Suga et al. (2000) hypothesized that corticofugal positive feedback and negative feedback are respectively related to centripetal and centrifugal BF shifts. Further physiological and anatomical studies of the corticofugal system with different species of animals remain to be performed to explore the neural basis of BF shifts.

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REFERENCES


