Augmentation of Plasticity of the Central Auditory System by the Basal Forebrain and/or Somatosensory Cortex

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Ma, Xiaofeng and Nobuo Suga. Augmentation of plasticity of the central auditory system by the basal forebrain and/or somatosensory cortex. J Neurophysiol 89: 90–103, 2003. 10.1152/jn.00968.2001. Auditory conditioning (associative learning) or focal electric stimulation of the primary auditory cortex (AC) evokes reorganization (plasticity) of the cochleotopic (frequency) map of the inferior colliculus (IC) as well as that of the AC. The reorganization results from shifts in the best frequencies (BFs) and frequency-tuning curves of single neurons. Since the importance of the cholinergic basal forebrain for cortical plasticity and the importance of the somatosensory cortex and the corticofugal auditory system for collicular and cortical plasticity have been demonstrated, Gao and Suga proposed a hypothesis that states that the AC and corticofugal system play an important role in evoking auditory collicular and cortical plasticity and that auditory and somatosensory signals from the cerebral cortex to the basal forebrain play an important role in augmenting collicular and cortical plasticity. To test their hypothesis, we studied whether the amount and the duration of plasticity of both collicular and cortical neurons evoked by electric stimulation of the AC or by acoustic stimulation were increased by electric stimulation of the basal forebrain and/or the somatosensory cortex. In adult big brown bats (Eptesicus fuscus), we made the following major findings. 1) Collicular and cortical plasticity evoked by electric stimulation of the AC is augmented by electric stimulation of the basal forebrain. The amount of augmentation is larger for cortical plasticity than for collicular plasticity. 2) Collicular and cortical plasticity evoked by AC stimulation is augmented by somatosensory cortical stimulation mimicking fear conditioning. The amount of augmentation is larger for cortical plasticity than for collicular plasticity. 3) Collicular and cortical plasticity evoked by both AC and basal forebrain stimulations is further augmented by somatosensory cortical stimulation. 4) A lesion of the basal forebrain tends to reduce collicular and cortical plasticity evoked by AC stimulation. The reduction is small and statistically insignificant for collicular plasticity but significant for cortical plasticity. 5) The lesion of the basal forebrain eliminates the augmentation of collicular and cortical plasticity that otherwise would be evoked by somatosensory cortical stimulation. 6) Collicular and cortical plasticity evoked by repetitive acoustic stimuli is augmented by basal forebrain and/or somatosensory cortical stimulation. However, the lesion of the basal forebrain eliminates the augmentation of collicular and cortical plasticity that otherwise would be evoked by somatosensory cortical stimulation. These findings support the hypothesis proposed by Gao and Suga.

INTRODUCTION

The response properties of cortical frequency-tuned neurons and the cochleotopic (frequency) map of the primary auditory cortex (AC) can be changed by repetitive acoustic stimulation (Chowdury and Suga 2000; Ma and Suga 2001a), auditory fear conditioning (Bakin et al. 1996; Diamond and Weinberger 1986, 1989; Gao and Suga 2000; Ji et al. 2001; Ohl and Scheich 1996; Weinberger et al. 1993), learning of an auditory discrimination task (Edeline and Weinberger 1993; Recanzone et al. 1993), focal electrical stimulation of AC (Chowdury and Suga 2000; Ma and Suga 2001a; Sakai and Suga 2001, 2002), or electric stimulation of the basal forebrain during acoustic stimulation (Bakin and Weinberger 1996; Kilgard and Merzenich 1998a). The response properties of collicular frequency-tuned neurons and the cochleotopic map of the inferior colliculus (IC) can also be changed by repetitive acoustic stimulation (Gao and Suga 1998; Ma and Suga 2001a; Yan and Suga 1998), auditory fear conditioning (Gao and Suga 1998, 2000; Ji et al. 2001), or focal electric stimulation of the AC (Jen et al. 1998; Ma and Suga 2001a; Yan and Ehret 2001; Yan and Suga 1998; Zhang and Suga 2000; Zhou and Jen 2000). Focal cortical electric stimulation also modulates the response properties of collicular neurons tuned to echo delays (Yan and Suga 1996) and sound durations (Ma and Suga 2001a) or sound direction (Jen et al. 1998) and shifts their echo-delay- or duration-tuning curves. Therefore the corticofugal system modulates the functional organization of the IC not only in the frequency domain but also in the time domain. In the big brown bat, Eptesicus fuscus, the cortical and collicular changes (plasticity) both have been found to be greatly due to the corticofugal system (Gao and Suga 2000; Ji et al. 2001). Our present paper deals with both collicular and cortical plasticity in the big brown bat so that papers on collicular and cortical plasticity of the big brown bat are mainly reviewed in the following text.

In the big brown bat, the corticofugal auditory system shifts the best frequencies (BFs) of collicular neurons, together with their frequency-tuning curves, toward the frequency of a repetitively delivered acoustic stimulus (Gao and Suga 1998; Ma and Suga 2001a; Yan and Suga 1998), the frequency of a conditioned sound (Gao and Suga 1998, 2000; Ji et al. 2001), or the BF of electrically stimulated cortical neurons (Ma and Suga 2001a; Yan and Suga 1998). BF shift results in reorganization of the frequency map of the IC. Such “centripetal” BF shifts are basically the same regardless of the means that evoked them. This means that focal electric stimulation of the
AC activates the essential portion of the neural mechanism for plasticity of the central auditory system and that it can be an appropriate method for the exploration of the plasticity (Suga et al. 2000).

Collicular and cortical BF shifts, which otherwise would be evoked by trace conditioning with acoustic stimuli followed by electric leg stimulation, are abolished by inactivation of the somatosensory cortex during conditioning (Gao and Suga 1998, 2000). Electric stimulation of the somatosensory cortex (ES_{ac}) after electric stimulation of the AC (ES_{at}), mimicking trace conditioning, augments the collicular and cortical BF shifts. However, ES_{at} prior to ES_{at}, mimicking backward conditioning, does not augment the BF shifts (Ma and Suga 2001a). Therefore the somatosensory cortex is one of the essential portions for the plasticity caused by conditioning, and the sequence of stimulation of the two cortical areas is important for evoking the plasticity.

Cholinergic nerve fibers originating from the basal forebrain control the acetylcholine level in the cortex and play an important role in cortical plasticity as reviewed by Buonomano and Merzenich (1998), Rasmusson (2000), and Sarter and Bruno (2000). In the AC of the guinea pig, BF shift is caused by a tone burst paired with electric stimulation of the cholinergic basal forebrain but not by the tone burst or the electric stimulation alone. The BF shift is similar to that caused by behavioral learning (Bakin et al. 1996; Bjordahl et al. 1998). In the cat’s AC, massive progressive reorganization of the cochleotopic map is evoked by electric stimulation of the basal forebrain paired with a tone burst (Kilgard and Merzenich 1998a). In the big brown bat, electric stimulation of the basal forebrain augments the collicular and cortical BF shifts evoked by a train of acoustic stimuli or by electric stimulation of the AC (Ma and Suga 2001a).

Gao and Suga (1998, 2000) proposed the following working hypothesis of collicular and cortical plasticity, incorporating their findings with part of the hypothesis proposed by Weinberger and his coworkers (1990). That is, the central auditory system has an intrinsic mechanism for the reorganization of the central auditory system based on the activity of the AC and the corticofugal system. When a behaviorally irrelevant acoustic stimulus is repetitively delivered, the central auditory system shows a small short-term plasticity. However, when it is paired with electric leg stimulation, the auditory and somatosensory sensory signals ascend from the stimulated sensory cells to the auditory and somatosensory cortices, respectively, and then to the amygdala through the association cortex. These signals are probably associated in the amygdala, which is essential for evoking conditioned behavioral response. Then the acoustic stimulus becomes behaviorally relevant to the animal. The amygdala sends the “associated” signal to the cholinergic basal forebrain, which increases the cortical acetylcholine level. Therefore the plasticity in the AC and IC due to the activity of the AC and the corticofugal system is augmented.

Ji et al. (2001) applied acetylcholine or atropine to the AC or IC to examine their effect on the collicular and cortical BF shifts evoked by conditioning and obtained data indicating that acetylcholine augments both the cortical and collicular BF shifts and that the cortical BF shift depends on the cortical neural net, corticofugal system (feedback loops) and cortical acetylcholine (ACh) level. Their data support Gao and Suga’s hypothesis. A 30-min-long conditioning session with acoustic stimulation followed by electric leg stimulation evoked a long-term cortical BF shift (Gao and Suga 2000) and a short-term collicular BF shift (Gao and Suga 1998, 2000). Therefore there is a possibility that the augmentation of the ES_{at}-evoked BF shifts by ES_{ac} is due to the activation of the basal forebrain through the pathway from the somatosensory cortex to the association cortex, then to the amygdala, and finally to the basal forebrain, as hypothesized by Gao and Suga (1998, 2000). The aim of our present research was to further test the validity of their hypothesis. We studied whether electric stimulation of the basal forebrain and/or the somatosensory cortex augments the collicular and cortical BF shifts evoked by ES_{at}. We also studied the effect of a bilateral lesion of the basal forebrain on the collicular and cortical BF shifts evoked by ES_{at} and also on the augmentation of the collicular and cortical BF shifts by ES_{at} paired with ES_{ac}. We have obtained data that support Gao and Suga’s hypothesis.

METHODS

Materials, surgery, recording of neural activity, acoustic stimulation, electric brain stimulation, data acquisition, and data processing were basically the same as those described in Ma and Suga (2001a). Therefore only the essential portion of the methods is summarized in the following text. Eleven adult big brown bats (18-24 g body wt) were used for the present experiments. Under neuroleptanalgesia (Innovar 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 awake 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. 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, IC, primary somatosensory cortex, or dorsal to the basal forebrain. Tungsten-wire electrodes for recording action potentials or for electrically stimulating 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 & Kjael microphone and was expressed in dB SPL. The frequency-tuning curve of a single collicular or cortical neuron was first measured manually. 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. 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 response of a single neuron to the scan was displayed as an array.
of peristimulus time (PST) histograms or PST cumulative (PSTC) histograms.

**Electric stimulation of the primary auditory cortex**

Electric stimulation (ES$_a$) were delivered to the AC 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 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. A 6.2-ms-long train of four monophasic electric pulses (100 nA, 0.2-ms duration, 2.0-ms interval). The train of electric pulses was repetitively delivered at a rate of 10/s for 2–90 min (hereafter, ES$_a$). These stimulus parameters were chosen in the previous studies on corticofugal modulation of bat’s auditory neurons (Chowdhury and Suga 2000; Ma and Suga 2001a,b; Yan and Suga 1998) because the bat emits biosonar pulses at a rate of ~10/s in the search phase of echolocation (Griffin 1962). The electric pulses were estimated to stimulate neurons within a 60-μm radius around the electrode tip (Yan and Suga 1996). Therefore electric stimulation of the AC was quite focal. The bat showed no behavioral response at all to such a weak electric stimulation delivered to the AC.

**Electric stimulation of the primary somatosensory cortex**

To mimic trace conditioning with a train of tone pulses followed by an electric leg stimulation, a train of electrical stimuli delivered to the AC (hereafter ES$_{at}$) was followed by an electric stimulation of the ipsilateral primary somatosensory cortex (hereafter ES$_{st}$) with a 1.0-s gap. The somatosensory cortex was localized by referring to the somatotopic map studied by Krubitzer and Calford (1992) and by recording neural responses to touch stimuli before inserting a pair of electrodes for electric stimulation (Fig. 1A). The ES$_{at}$ was 1.0-s long and consisted of 33 trains. Each train was 6.2-ms long and consisted of four monophasic electric pulses, as in ES$_a$. ES$_{at}$ was delivered twice per minute for 30 min. ES$_{at}$ 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. ES$_{at}$ + ES$_{st}$ was 1.0 s ES$_{at}$ + 1.0 s gap + 50 ms ES$_{st}$. To mimic backward conditioning, ES$_{st}$ was delivered 1.0 s before ES$_{at}$. Because the bat emits biosonar pulses at a rate of 30–40/s at the middle of the approach phase of echolocation and because the mid-approach phase is most likely to be followed by the terminal phase and the contact with an insect (Griffin 1962), the preceding parameters of ES$_{at}$ and ES$_{at}$ + ES$_{st}$ were chosen in our previous studies on the plasticity of the auditory system caused by electric stimulation (Ma and Suga 2001a) mimicking fear conditioning (Gao

![Fig. 1. Dorsolateral view of the brain of the big brown bat (A), frontal sections across the basal forebrain (B), electroencephalograph (EEG, C), and action potentials selected by a time-amplitude window discriminator (D). A: the auditory (AC) and somatosensory (SI) cortices are indicated (- - -), ×, locations where electric stimulation was applied. B: an electrode penetration and an electrolytic lesion at the tip of the stimulating electrodes. CP, caudate putamen; GP, globus pallidus. C: EEG recorded from the auditory cortex is desynchronized by a 30-s-long electric stimulation of the basal forebrain. D: action potentials selected with a time-amplitude window discriminator. Top: an action potential stored at the beginning of data acquisition (template). Bottom 5 traces: action potentials recorded every 1 h. m, an amplitude level and a time delayed window for selecting action potentials.](jnphysiol-89-3-jan03-fig01.png)
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and Suga 1998, 2000). Therefore these parameters were also used in our present studies.

Because ESbr and ESat stimulated small groups of neurons around the tips of the stimulus electrodes, the spatial and temporal pattern of neural activity evoked by these direct electric stimulation might be quite different from that evoked by auditory fear conditioning. However, it was tested whether ESat augmented both the collicular and cortical changes evoked by ESbr.

Electric stimulation and lesion of the basal forebrain

The photomicrographs of frontal sections across the forebrain of the big brown bat were very similar to those of the mouse. A pair of tungsten-wire electrodes fabricated in the same way as those used for ESbr and ESat was inserted dorsoventrally into the basal forebrain ipsilateral to the electrically stimulated AC, referring the atlas of the mouse brain (Slotnick and Leonard 1975), and a 0.2-ms-long, 100-μA electric pulse was repetitively delivered to the basal forebrain at a rate of 100/s over 15 or 30 min through these electrodes. The electrodes were implanted where the electric stimulation evoked the largest augmentation of the collicular and cortical BF shifts evoked by electric stimulation of the AC. Because it has been known that the electric stimulation of the basal forebrain paired with acoustic stimulation evokes plasticity of the AC (Bakin and Weinerberger 1996; Kilgard and Merzenich 1998a,b), this procedure was one of the three criteria to verify that the electrodes were placed in the basal forebrain.

Because it has been known that electric stimulation of the basal forebrain produces desynchronization of an electroencephalogram (EEG) (Bjordahl et al. 1998), we anesthetized the animal with urethane (1.5 g/kg ip) after completing the plasticity experiment, recorded a synchronized EEG (1- to 300-Hz band-pass), and observed desynchronization of the EEG that was induced by 0.2-ms-long, 100-μA electric pulses delivered at a rate of 100/s for 30 s (Fig. 1C). This observation was the second criterion to verify the stimulation site. The third verification of the stimulation site was anatomical. The basal forebrain was lesioned by a 10-s-long 500-μA monophasic electric current. The animal was then killed by pentobarbital sodium (100 mg/kg) and was perfused with a formalin-saline solution. Its brain was frozen-sectioned 50 μm thick and Nissl stained. The electrolytic lesion was ~200 μm in radius and was located at the basal forebrain (Fig. 1B).

Because electrical stimulation of the basal forebrain (ESbr) as well as ESat stimulated a small group of neurons at and around the tips of the stimulating electrodes, the spatial and temporal pattern of neural activity evoked by ESbr might be quite different from that evoked by auditory fear conditioning. However, it was tested whether ESbr augmented both the collicular and cortical changes evoked by ESat.

Data acquisition and processing

The auditory responses of cortical neurons were recorded with a tungsten-wire microelectrode (6–8 μm tip diameter) at depths between 200 and 600 μm. The auditory responses of collicular neurons were recorded with a tungsten-wire microelectrode at depths between 200 and 2,000 μm in the central nucleus of the IC ipsilateral to the electrically stimulated AC. 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 central nucleus of the IC. Therefore BFs of neurons systematically became higher as expected from the tonotopic map (e.g., Yan and Suga 1998).

Action potentials originating from a single neuron were selected from those originating from a few neurons with a time-amplitude-window discriminator (BAK Electric, model DIS–1). The responses of a single neuron to tone bursts in the frequency scan repeated 50 times were recorded before, during, and after electric stimulation of the AC, somatosensory cortex, and/or basal forebrain. The auditory responses of a single neuron to acoustic stimuli were displayed on the computer monitor as arrays of PST or PSTC histograms during the experiments. 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 (Fig. 1D, top). 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 electric and/or acoustic stimulation. Data acquisition was continued as far as action potentials visually matched the template (Fig. 1D, bottom 5 traces). Data were stored on a hard drive of a personal computer and were used for off-line analysis. In a 1-day experiment that lasted ~8 h, a single collicular or cortical neuron was usually studied.

Off-line data processing included plotting the frequency-response curves (the arrays of PST or PSTC histograms displaying the responses of a collicular or a cortical neuron to 50 identical frequency scans) obtained before, during, and after the electric and/or acoustic stimulation (see Ma and Suga 2001a). The BF was determined as the frequency to which the neuron showed the largest response. A recovery time was measured as the time interval between the end of the electric stimulation and the time for a 50 or 100% (full) recovery. A full recovery time was defined as the time when a recovery curve crossed a point 50 Hz below the control BF. For a statistical analysis of the data, means ± SE of maximum BF shifts and recovery times at 50 and/or 100% recovery points were calculated. A t-test was used to test the difference between the BFs obtained before and after the electric and/or acoustic stimulation and to test the difference between the responses of collicular and cortical neurons.

Stimulus parameters were as follows: ASat, train of acoustic stimuli (10-ms-long tone bursts at 50 dB SPL; 33 tone bursts/s for 30 min); ESbr, repetitive electrical stimulation of the auditory cortex (4 0.2-ms-long, 100-nA electric pulses/6.2-ms-long train; 10 train/s for 15, 30, 60 min); ESat, electric stimulation of the auditory cortex (4 0.2-ms-long, 100-nA electric pulses/6.2-ms-long train; 33 train/s for 1 s; 2 ESat/min); ESbr, repetitive electric stimulation of the basal forebrain (0.2-ms-long, 100-μA electric pulses; 100 pulses/s for 15 or 30 min); ESat, train of electrical stimulation of the somatosensory cortex (20 0.2-ms-long, 100-μA electric pulses/50-ms-long train; 2 ESat/min for 30 min); ESbr + ESat, ESat was delivered 1.0 s after ESbr to mimic auditory fear conditioning; ESbr + ESat, ESat was delivered 1.0 s before ESbr to mimic backward conditioning; ESbr + ESat, a 15 (or 30)-min-long session of ESat and 15 (or 30)-min-long session of ESbr were delivered at the same time; and ESbr + ESat + ESat, a 30-min-long ESat + ESat session and a 30-min-long ESbr session were delivered at the same time.

RESULTS

BF shift (plasticity) evoked by electric stimulation of the AC (ESat or ESbr) was studied for 144 collicular and 78 cortical auditory neurons. The effect of ESbr on the BF shift evoked by ESbr or ESat was studied for 133 collicular and 100 cortical neurons. The effects of ESbr alone and ESat alone were studied on 26 collicular and 26 cortical neurons, respectively. ESat or ESbr alone caused no BF shift of the collicular and cortical auditory neurons studied. In some of the preceding neurons, collicular and cortical BF shifts were studied after bilateral lesion of the basal forebrain.

Effect of ESbr on plasticity evoked by ESat

When a 15-min-long ESat was delivered, the BFs of collicular neurons within 10 kHz of the BF of stimulated cortical neurons (hereafter, stimulated cortical BF) mostly shifted to-
ward the stimulated cortical BF (Fig. 2A, ●). The largest negative BF shift was 1.5 kHz, which occurred at 4.5 kHz above the stimulated cortical BF. The largest positive BF shift was 0.5 kHz, which occurred at 15 kHz above and 4 kHz below the stimulated cortical BF. BF shifts were “centripetal” for BF differences between −5 and 13 kHz, but were “centrifugal” at 15 kHz above the stimulated cortical BF. When a 15-min-long ES_{ar} session was delivered at the same time as a 15-min-long ES_{br} session (hereafter, ES_{ar} + ES_{br}), the collicular BF shifts evoked by ES_{ar} were augmented (Fig. 2A, ○). The maximum negative BF shift became 2.0 kHz, which occurred at 4–7 kHz above the stimulated cortical BF. The largest positive BF shift was 0.8 kHz, which occurred at 5 kHz below the stimulated cortical BF. (Note that in ES_{ar} + ES_{br}, the length of a ES_{ar} session was always the same as that of a ES_{br} session and that ES_{ar} consisted of 10 trains of electric pulses/s, whereas ES_{br} consisted of 100 electric pulses/s, so that every 10 electric pulses of ES_{br} coincided with 1 train in ES_{ar}.)

When a 60-min-long ES_{ar} was delivered, collicular BF shifts became larger than those evoked by the 15-min-long ES_{ar} (Fig. 2B, ●). The largest negative and positive BF shifts were, respectively, 2.0 and 0.5 kHz, which occurred, respectively, at 5–6 kHz above the stimulated cortical BF and at ~4 kHz below and 14 kHz above it. For a 60-min-long ES_{ar} + ES_{br}, the BF shifts evoked by ES_{ar} were augmented (Fig. 2B, ○). The largest negative shifts became 2.5 kHz, which occurred at 6–7 kHz above the stimulated cortical BF, and the largest positive BF shifts were 1.0 kHz, which occurred at both 3.0 kHz below and 15 kHz above the stimulated cortical BF. As shown in Fig. 2, A and B, the BF shifts were predominantly centripetal and asymmetrical, i.e., BF shifts toward the stimulated cortical BF were much larger on the high-frequency side of the stimulated cortical BF than on the low-frequency side.

In our data, 58% (23/40) of collicular neurons studied showed BF shifts for the 15-min-long ES_{ar} alone; 89% (32/36) for 15-min-long ES_{ar} + ES_{br}; 69% (24/35) for the 60-min-long ES_{ar} alone; and 73% (24/33) for 60-min ES_{ar} + ES_{br}. A BF shift depends on differences in both BF (Chowdhury and Suga 2000; Ma and Suga 2001a; Sakai and Suga 2001; Yan and Suga 1998; Zhang and Suga 2000) and distance along iso-BF

![Graph](https://via.placeholder.com/150)

**FIG. 2.** Changes in the best frequencies (BFs) of collicular (IC) and cortical neurons (AC) evoked by repetitive electric stimulation of the auditory cortex (ES_{ar}) or ES_{ar} paired with repetitive electric stimulation of the basal forebrain (ES_{br}) for 15 min (A) or 60 min (B–D). In A and B, BF shifts of collicular neurons are plotted as a function of the differences in BF between recorded collicular and stimulated cortical neurons. BF shifts evoked by ES_{ar} (●) were augmented by ES_{br} (○). Because there were many identical data points, they are slightly shifted to be individually identifiable. C and D: the time courses of the development of the BF shifts of collicular and cortical neurons evoked by ES_{ar} (●) or ES_{ar} + ES_{br} (○), respectively. The vertical bars represent SEs. The BFs of stimulated cortical neurons ranged between 16 and 65 kHz (31.6 ± 1.5 kHz), and the BFs of recorded neurons were 14 and 68 kHz (30.3 ± 2.5 kHz). Different symbols indicate different stimulus conditions. N, the number of neurons studied in a given stimulus condition.
lines (Sakai and Suga 2002) between stimulated and recorded neurons. Therefore the preceding percentages of BF-shifted neurons calculated according to the suggestion of the referees of our present paper, ignoring the relationship in BF between recorded and stimulated neurons, were meaningless. As shown in Fig. 2, A and B, a percentage of neurons showing a BF shift varied with BF differences. For 4- to 7-kHz BF differences, 84% of neurons (31/37) showed BF shifts for ES<sub>ar</sub> alone and 92% (26/28) showed BF shifts for ES<sub>ar</sub> + ES<sub>br</sub>. Because BF shifts were largest at and around 5 kHz BF difference as previously reported by Ma and Suga (2001a) and because an increase in BF shift caused by ES<sub>br</sub> was significant at the 4- to 7-kHz BF differences (Table 1), the further measurements of BF shifts in the present studies were performed for BF differences between 3.6 and 6.9 kHz (4.5 ± 0.06 kHz BF difference, \( n = 182 \)).

The collicular BF shift evoked by the 60-min-long ES<sub>ar</sub> developed during the ES<sub>ar</sub> and reached a plateau of 1.01 ± 0.05 kHz (\( n = 15 \)) 30 min after the onset of the ES<sub>ar</sub> (Fig. 2C, ●). For 60-min-long ES<sub>ar</sub> + ES<sub>br</sub>, the BF shift became larger and reached a plateau of 1.50 ± 0.05 kHz (\( n = 16 \)) 60 min after the onset of the ES<sub>ar</sub> + ES<sub>br</sub> (Fig. 2C, ○). The 0.5-kHz difference in plateau was statistically significant (\( P < 0.05 \)). The cortical BF shift also developed during the 60-min-long ES<sub>ar</sub> and reached a plateau of 1.12 ± 0.04 kHz (\( n = 13 \)) 60 min after the ES<sub>ar</sub> (Fig. 2D, ●). For the 60-min-long ES<sub>ar</sub> + ES<sub>br</sub>, it became larger and reached a plateau of 1.70 ± 0.08 kHz (\( n = 15 \)) 60 min after the ES<sub>ar</sub> + ES<sub>br</sub> (Fig. 2D, ○). The 0.6-kHz BF difference in plateau was statistically significant (\( P < 0.05 \)).

The collicular and cortical BF shifts monotonically returned (recovered) to the BF in the control condition (hereafter, control BF) after the 15- or 30-min-long ES<sub>ar</sub> (Fig. 3A, B, C, D, and E), as previously reported by Ma and Suga (2001a). For the 15- or 30-min-long ES<sub>ar</sub> + ES<sub>br</sub>, the BF shifts became larger in magnitude and longer in recovery than those evoked by the ES<sub>ar</sub> alone (Table 2). Figure 3A shows the recovery curves of the collicular BF shifts evoked by the 15- or 30-min ES<sub>ar</sub> with or without ES<sub>ar</sub>. For the 15-min-long ES<sub>ar</sub> alone, the collicular BF shift was 0.82 ± 0.08 kHz (\( n = 15 \)), and the shifted BF recovered to 50% of the control BF 32 ± 3.3 min after the ES<sub>ar</sub> and to the control BF 57 ± 4.6 min after the ES<sub>ar</sub> (Fig. 3A, ○). For the 15-min-long ES<sub>ar</sub> + ES<sub>br</sub>, the collicular BF shift became larger in amount, 1.18 ± 0.07 kHz (\( n = 16 \)), and longer in recovery time: 88 ± 5.6 min for 50% recovery and 153 ± 8.2 min for 100% recovery (Fig. 3A, ●). For the 30-min-long ES<sub>ar</sub> alone, the collicular BF shift was 1.10 ± 0.05 kHz (\( n = 18 \)), the recovery time was 70 ± 4.9 min for 50% and 166 ± 9.6 min for 100% recovery (Fig. 3A, △). For the 30-min-long ES<sub>ar</sub> + ES<sub>br</sub>, the collicular BF shift became larger in magnitude, 1.32 ± 0.06 kHz (\( n = 14 \)) and longer in recovery time: 101 ± 9.6 min for 50% and 176 ± 9.9 min for 100% recovery (Fig. 3A, ▲). All these changes, except the 100% recovery time for the 30-min-long ES<sub>ar</sub> + ES<sub>br</sub>, were statistically significant. The effect of ES<sub>br</sub> was larger on the BF shifts evoked by the 15-min-long ES<sub>ar</sub> than on those evoked by the 30-min-long ES<sub>ar</sub> (Table 2).

Figure 3B shows the recovery curves of the BF shifts of cortical neurons evoked by ES<sub>ar</sub> or ES<sub>ar</sub> + ES<sub>br</sub> that was either 15 or 30 min long. These cortical recovery curves were similar to the collicular ones. However, the cortical BF shifts at the peak tended to last longer than the collicular ones. Accordingly, the 50% recovery time of the cortical BF shift was significantly longer than that of the collicular one: 47 ± 2.9 min for the 15-min-long ES<sub>ar</sub> and 108 ± 8.9 min for the 30-min-long ES<sub>ar</sub>. Lengthening of the duration of ES<sub>ar</sub> from 15

### Table 1. Collicular BF shifts evoked by electric stimulation of the auditory cortex (ES<sub>ar</sub>) and/or basal forebrain (ES<sub>br</sub>)

<table>
<thead>
<tr>
<th>Duration</th>
<th>Electric Stimuli</th>
<th>No. of IC Neurons Shifting/Total</th>
<th>BF Shift, kHz</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>ES&lt;sub&gt;ar&lt;/sub&gt;</td>
<td>15/18 (83)</td>
<td>0.72 ± 0.09</td>
<td>18</td>
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<tr>
<td></td>
<td>ES&lt;sub&gt;ar&lt;/sub&gt; + ES&lt;sub&gt;br&lt;/sub&gt;</td>
<td>13/100 (100)</td>
<td>1.28 ± 0.06*</td>
<td>13</td>
</tr>
<tr>
<td>60 min</td>
<td>ES&lt;sub&gt;ar&lt;/sub&gt;</td>
<td>16/19 (84)</td>
<td>1.16 ± 0.05</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>ES&lt;sub&gt;ar&lt;/sub&gt; + ES&lt;sub&gt;br&lt;/sub&gt;</td>
<td>15/132 (92)</td>
<td>1.66 ± 0.08*</td>
<td>15</td>
</tr>
</tbody>
</table>

The data were pooled for best frequency (BF) differences between 4 and 7 kHz (see Fig. 2, A and B). Values are means ± SE. Parentheses enclose percentages. IC, inferior colliculus. * \( P < 0.05 \) compared with ES<sub>ar</sub> alone (\( t \)-test).
to 30 min had a larger effect on the 50% recovery time of the cortical BF shift than on that of the collicular BF shifts. The difference in 50% recovery time between the collicular and cortical BF shifts for a given duration of ES$_{ar}$ was statistically significant ($P < 0.01$). For the 15- or 30-min-long ES$_{ar}$ + ES$_{br}$, the cortical BF shift became larger: 1.28 ± 0.09 kHz ($n = 14$; $P < 0.05$; Fig. 3B, ○) or 1.41 ± 0.10 kHz ($n = 15$; $P = 0.07$; Fig. 3B, △), respectively. The 50% recovery time significantly increased (Table 2).

Compared with the 15-min-long ES$_{ar}$ + ES$_{br}$ (i.e., simultaneous delivery of the ES$_{ar}$ and ES$_{br}$ sessions), the 15-min-long ES$_{br}$ delivered immediately before or after the 15-min-long ES$_{ar}$ evoked much smaller changes of the collicular and cortical BF shifts in amount and recovery time (Fig. 4). These small changes were statistically insignificant ($P > 0.05$) compared with the BF shifts evoked by the ES$_{ar}$ alone but significant compared with the BF shifts evoked by the ES$_{ar}$ + ES$_{br}$ (Table 3). When the 30-min-long ES$_{br}$ was delivered prior to and during the 15-min-long ES$_{ar}$, the collicular and cortical BF shifts (Fig. 4, A and B, △) tended to be slightly larger and longer-lasting than those evoked by the 15-min-long ES$_{ar}$ + ES$_{br}$ (Fig. 4, A and B, ○). However, the difference in BF shift was small and statistically insignificant in both magnitude and recovery time.

The collicular and cortical BF shifts evoked by the 15-min-long ES$_{ar}$, respectively, recovered by 50% in 32 ± 3.3 and 47 ± 2.9 min and by 100% in 57 ± 4.6 and 71 ± 5.1 min after ES$_{ar}$ (Fig. 3, A and B, ○). When the 15-min-long ES$_{ar}$ was delivered three times with a 1-h time interval, the collicular and cortical BF shifts for the third ES$_{ar}$ were the same in amount as those evoked by the first ES$_{ar}$ but showed a prominent lengthening in recovery time. That is, 50 and 100% recovery times after the third ES$_{ar}$ were, respectively, 85 ± 5.5 and 121 ± 6.2 min for the cortical BF shift (Fig. 5, ○) and 96 ± 5.1 and 150 ± 7.6 min for the cortical BF shift (Fig. 5, △). When the 15-min-long ES$_{ar}$ + ES$_{br}$ was repeated three times, the collicular and cortical BF shifts for the third pair was the same in amount as those evoked by the first pair but showed a recovery time much longer than that for the first pair (Figs. 5, ○ and △, and 3, A and B, ○). That is, for the third ES$_{ar}$ + ES$_{br}$, the collicular BF shift plateaued for ~80 min and then recovered by 50% in ~60 min and by 100% in ~110 min (Fig. 5, ○), whereas the cortical BF shift plateaued for ~160 min and then recovered by 50% in ~90 min and by 100% in ~200 min (Fig. 5, △). The effect of ES$_{br}$ was stronger on the cortical BF shift than on the collicular one.

**Effect of basal forebrain lesion on plasticity evoked by ES$_{ar}$**

When the 15- or 30-min-long ES$_{ar}$ was delivered after a bilateral lesion of the basal forebrain, the cortical BF shift evoked by ES$_{ar}$ tended to become slightly smaller than that evoked by the ES$_{ar}$ without the lesion. However, this decrease

<table>
<thead>
<tr>
<th>Stimulus Duration</th>
<th>Electric Stimuli</th>
<th>BF Shift at Peak, kHz</th>
<th>Recovery Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>50%</td>
</tr>
<tr>
<td>IC 15 min</td>
<td>ES$_{ar}$</td>
<td>0.82 ± 0.08</td>
<td>32 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>ES$<em>{ar}$ + ES$</em>{br}$</td>
<td>1.18 ± 0.07*</td>
<td>88 ± 5.6**</td>
</tr>
<tr>
<td>30 min</td>
<td>ES$_{ar}$</td>
<td>1.10 ± 0.05</td>
<td>70 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>ES$<em>{ar}$ + ES$</em>{br}$</td>
<td>1.32 ± 0.06*</td>
<td>101 ± 9.6*</td>
</tr>
<tr>
<td>AC 15 min</td>
<td>ES$_{ar}$</td>
<td>0.91 ± 0.08</td>
<td>47 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>ES$<em>{ar}$ + ES$</em>{br}$</td>
<td>1.28 ± 0.09*</td>
<td>121 ± 8.8**</td>
</tr>
<tr>
<td>30 min</td>
<td>ES$_{ar}$</td>
<td>1.18 ± 0.07</td>
<td>108 ± 8.9</td>
</tr>
<tr>
<td></td>
<td>ES$<em>{ar}$ + ES$</em>{br}$</td>
<td>1.41 ± 0.10*</td>
<td>146 ± 9.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE. AC, auditory cortex. See Fig. 3. * $P < 0.05$, ** $P < 0.01$ compared with ES$_{ar}$ alone (t-test).
TABLE 3. Collicular and cortical BF shifts evoked by electric stimulation of the auditory cortex (ES\textsubscript{ar}) and/or basal forebrain (ES\textsubscript{br})

<table>
<thead>
<tr>
<th>Electric Stimuli</th>
<th>BF Shift at Peak, kHz</th>
<th>Recovery Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC</td>
<td>ES\textsubscript{ar}</td>
<td>0.82 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>ES\textsubscript{ar} before ES\textsubscript{ar}</td>
<td>0.92 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>ES\textsubscript{ar} after ES\textsubscript{ar}</td>
<td>0.90 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>ES\textsubscript{ar} together with ES\textsubscript{ar}</td>
<td>1.18 ± 0.07*</td>
</tr>
<tr>
<td>AC</td>
<td>ES\textsubscript{ar}</td>
<td>0.91 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>ES\textsubscript{ar} before ES\textsubscript{ar}</td>
<td>1.12 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>ES\textsubscript{ar} after ES\textsubscript{ar}</td>
<td>1.08 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>ES\textsubscript{ar} together with ES\textsubscript{ar}</td>
<td>1.28 ± 0.09*</td>
</tr>
</tbody>
</table>

Values are means ± SE. See Fig. 4. *P < 0.05, **P < 0.01 compared with ES\textsubscript{ar} alone (t-test).

was statistically insignificant, P > 0.05 (Fig. 6A, O vs. ●; △ vs. ●; also see Table 4). On the other hand, the cortical BF shift evoked by the 15- or 30-min-long ES\textsubscript{ar} after the basal forebrain lesion became smaller and shorter-lasting (Fig. 6B, O vs. ● and △ vs. ●). The decrease in the amount of the BF shift was statistically significant (P > 0.05). However, the decrease in the 50 or 100% recovery time of the BF shift was statistically significant for the 30-min-long ES\textsubscript{ar} (P < 0.05) but not for the 15-min-long ES\textsubscript{ar} (Fig. 6B and Table 4). The preceding data obtained through electrical stimulation of the AC with a basal forebrain lesion indicate that the cortical BF shift evoked by ES\textsubscript{ar} was slightly augmented by the basal forebrain, but the cortical BF shift was not.

Effect of ES\textsubscript{ar} on plasticity evoked by ES\textsubscript{ar} or ES\textsubscript{ar} + ES\textsubscript{br}

To mimic fear conditioning by Gao and Suga (1998, 2000), the AC, somatosensory cortex and basal forebrain were electrically stimulated for 30 min. A short train of electric stimulation of the somatosensory cortex (ES\textsubscript{st}) following ES\textsubscript{ar} with an 1.0-s delay significantly augmented the collicular and cortical BF shifts evoked by ES\textsubscript{ar} (Fig. 7, ○ vs. ● in A and △ vs. ● in B), as previously reported by Ma and Suga (2001a). The recovery curve of the collicular BF shift for the ES\textsubscript{ar} + ES\textsubscript{st} (Fig. 7A, ●) was very similar to that for a 30-min-long conditioning session obtained by Gao and Suga (2000) (Fig. 7A, - - -). However, the recovery curve of the cortical BF shift for the ES\textsubscript{ar} + ES\textsubscript{st} (Fig. 7B, ●) was quite different from that for the conditioning obtained by Gao and Suga (2000) (Fig. 7B, - - -). When a 30-min-long ES\textsubscript{ar} session was delivered together with a 30-min-long session of ES\textsubscript{st} + ES\textsubscript{st}, the collicular and cortical BF shifts evoked by the ES\textsubscript{st} + ES\textsubscript{st} were further augmented in magnitude and lengthened in recovery time. The magnitude of the collicular BF shift was 0.78 ± 0.11 kHz (n = 15) for ES\textsubscript{st} alone, 1.08 ± 0.08 kHz (n = 15) for ES\textsubscript{st} + ES\textsubscript{st}, and 1.32 ± 0.08 kHz (n = 12) for ES\textsubscript{st} + ES\textsubscript{st} + ES\textsubscript{br} (Fig. 7A). The recovery curve of the cortical BF shift for ES\textsubscript{st} + ES\textsubscript{st} + ES\textsubscript{br} showed a plateau lasting ~90 min and a gradual recovery after the plateau (Fig. 7A, ●). The magnitude of the cortical BF shift was 0.79 ± 0.12 kHz (n = 12) for ES\textsubscript{st} alone, 1.27 ± 0.07 kHz (n = 12) for ES\textsubscript{st} + ES\textsubscript{st}, and 1.52 ± 0.09 kHz (n = 11) for ES\textsubscript{st} + ES\textsubscript{st} + ES\textsubscript{br} (Fig. 7B). The recovery of the cortical BF shift for ES\textsubscript{st} + ES\textsubscript{st} + ES\textsubscript{br} was much longer than that of the collicular BF shift. Namely, the cortical BF shift showed a plateau lasting ~210 min and then started to recover (Fig. 7B, ●). This cortical recovery curve was quite different from that for the conditioning which showed a plateau lasting >360 min (Fig. 7B, - - -) (Gao and Suga 2000).

As described in the preceding text, ES\textsubscript{ar} augmented the collicular and cortical BF shifts evoked by ES\textsubscript{st} (Figs. 7 and 8, ● in A and △ in B). ES\textsubscript{ar} augmented the BF shifts evoked by ES\textsubscript{st} (Fig. 7, ● in A and B). When the 30-min-long ES\textsubscript{st} + ES\textsubscript{br} was delivered to the animal after the basal forebrain lesion, the cortical BF shift evoked by the ES\textsubscript{st} + ES\textsubscript{br} tended to be smaller and shorter-lasting than that without the lesion (Fig. 8A, ● vs. ●). It became the same in magnitude and recovery as that evoked by ES\textsubscript{ar} alone. The 50% recovery time was 108 ± 6.5, 133 ± 8.8, and 148 ± 9.8 min for the ES\textsubscript{ar}

FIG. 5. Collicular (○ and ●) and cortical BF shifts (△ and ●) evoked by a 15-min-long ES\textsubscript{ar} (○ and ●) or ES\textsubscript{ar} + ES\textsubscript{br} (△ and ●) delivered 3 times with a 60-min interval. The stimulated cortical BFs ranged between 16 and 64 kHz (32.3 ± 3.5 kHz, n = 29). The BF difference between the stimulated cortical neurons and the recorded collicular or cortical neurons was 4.5 ± 0.05 or 6.8 ± 0.11 kHz, respectively. See Fig. 3 for abbreviations and other explanations.
FIG. 6. Effect of a bilateral lesion of the basal nucleus of the forebrain (BN) on the collicular (A) and cortical BF shifts (B) to be evoked by the 15 (○ and ●) or 30-min-long ES<sub>st</sub> (● and ▲). The lesion of the BN had little effect on the collicular BF shifts (P > 0.05) but a significant effect on the cortical BF shifts (P < 0.05). "No BN" means the lesion of the BN. The stimulated cortical BF ranged between 20 and 60 kHz (34.5 ± 1.8 kHz, n = 46) for collicular studies and between 20 and 70 kHz (38.8 ± 1.5 kHz, n = 42) for cortical studies. The BF difference between the stimulated cortical neurons and the recorded collicular or cortical neurons was 4.0 ± 0.09 or 4.8 ± 0.16 kHz, respectively. See Fig. 3 for abbreviations and other explanations.

alone, ES<sub>st</sub> + ES<sub>br</sub> with the lesion, and ES<sub>st</sub> + ES<sub>br</sub> without the lesion, respectively. The difference in 50% recovery time was statistically insignificant (P > 0.05). On the other hand, the cortical BF shift evoked by the ES<sub>st</sub> + ES<sub>br</sub> after the lesion of the basal forebrain was significantly smaller in magnitude (1.02 ± 0.02 vs. 1.25 ± 0.03 kHz; P < 0.05) and shorter in recovery than that evoked by the ES<sub>st</sub> + ES<sub>br</sub> without the lesion (123 ± 8.9 vs. 210 ± 11.2 min for 50% and 230 ± 10.6 vs. 325 ± 11.2 min for 100% recovery; P < 0.05) (Fig. 8B, filled triangles vs. filled squares). These data indicate that the augmentation of the cortical BF shift evoked by the ES<sub>st</sub> was not due to subcortical interaction between the auditory and somatosensory systems but the basal forebrain activated by ES<sub>st</sub> + ES<sub>br</sub>.

One may consider that the augmentation of the BF shifts by ES<sub>st</sub> and/or ES<sub>br</sub> was not related to the BF shifts evoked by a train of acoustic stimuli (AS<sub>t</sub>) because ES<sub>st</sub> was unnatural. Therefore the effects of ES<sub>st</sub> or ES<sub>br</sub> on the collicular and cortical BF shifts evoked by AS<sub>t</sub> were studied. For a 30-min-long AS<sub>t</sub>, 20 of the 23 collicular neurons studied and 18 of the 24 cortical neurons studied showed small and short-lasting BF shifts (Fig. 9A, ○ and ▲, and B, - - -; Table 5). When AS<sub>t</sub> was paired with a 30-min-long ES<sub>st</sub>, the collicular and cortical BF shifts became larger and longer-lasting (Fig. 9A, ● and ▲; Table 5). These changes are statistically significant (P < 0.05). The effect of ES<sub>st</sub> was larger on the cortical neurons than on the collicular neurons (P < 0.05).

When AS<sub>t</sub> was paired with ES<sub>st</sub> mimicking trace conditioning, the collicular and cortical BF shifts also became larger and

<table>
<thead>
<tr>
<th>Stimulus Duration</th>
<th>Electric Stimuli</th>
<th>BF Shift at Peak, kHz</th>
<th>Recovery Time, min</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50%</td>
<td>Full</td>
<td></td>
</tr>
<tr>
<td>IC</td>
<td>ES&lt;sub&gt;st&lt;/sub&gt;</td>
<td>0.82 ± 0.08</td>
<td>32 ± 3.3</td>
<td>57 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>ES&lt;sub&gt;st&lt;/sub&gt; (no BN)</td>
<td>0.79 ± 0.07</td>
<td>22 ± 4.8</td>
<td>41 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>ES&lt;sub&gt;br&lt;/sub&gt;</td>
<td>1.10 ± 0.05</td>
<td>70 ± 4.9</td>
<td>166 ± 9.6</td>
</tr>
<tr>
<td></td>
<td>ES&lt;sub&gt;br&lt;/sub&gt; (no BN)</td>
<td>1.05 ± 0.08</td>
<td>65 ± 5.3</td>
<td>118 ± 5.9*</td>
</tr>
<tr>
<td>AC</td>
<td>ES&lt;sub&gt;st&lt;/sub&gt;</td>
<td>0.91 ± 0.08</td>
<td>47 ± 2.9</td>
<td>71 ± 5.1</td>
</tr>
<tr>
<td></td>
<td>ES&lt;sub&gt;st&lt;/sub&gt; (no BN)</td>
<td>0.88 ± 0.09</td>
<td>35 ± 4.6</td>
<td>51 ± 3.2*</td>
</tr>
<tr>
<td></td>
<td>ES&lt;sub&gt;br&lt;/sub&gt;</td>
<td>1.18 ± 0.07</td>
<td>108 ± 8.9</td>
<td>192 ± 8.9</td>
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<tr>
<td></td>
<td>ES&lt;sub&gt;br&lt;/sub&gt; (no BN)</td>
<td>1.12 ± 0.12</td>
<td>88 ± 6.3</td>
<td>135 ± 5.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE (see Fig. 6). * P < 0.05 compared with ES<sub>st</sub> alone (t-test).

FIG. 7. Collicular (A) and cortical BF shifts (B) evoked by the trains of electric stimulation of the auditory cortex (ES<sub>st</sub>, ○ and ▲) were augmented by the trains of electric stimulation of the somatosensory cortex (ES<sub>br</sub>, ● and ▲). They were further augmented by electric stimulation of the basal forebrain (ES<sub>br</sub>). The ○, ●, ▲, and ▲, respectively, represent ES<sub>st</sub>, ES<sub>br</sub>, and ES<sub>st</sub> + ES<sub>br</sub> - - - in A and B, respectively: the recovery curves of the collicular and cortical BF shifts evoked by auditory conditioning (Gao and Suga 2000). The stimulated cortical BF ranged between 20 and 60 kHz (35.6 ± 2.1 kHz, n = 22) for collicular studies and between 20 and 71 kHz (36.8 ± 2.4 kHz, n = 21) for cortical studies. The BF difference between the stimulated cortical neurons and the recorded collicular or cortical neurons was 3.9 ± 0.05 or 4.5 ± 0.08 kHz, respectively. See Fig. 3 for abbreviations and other explanations.
longer-lasting (Fig. 9B, ● and ▲; Table 5). When the AS + ES st was delivered to the animal after the basal forebrain lesion, the augmentation of the collicular and cortical BF shifts that otherwise would be evoked by the AS + ES st was hardly evoked (Fig. 9B, □ and ■; Table 5). ES br and ES st augmented the collicular and cortical BF shifts evoked by AS as well as those evoked by ES ar, and the augmentation by ES st was evoked via the basal forebrain.

**DISCUSSION**

**Electric stimulation (ES ar, ES st and/or ES br) versus auditory fear conditioning**

ES ar alone evokes collicular and cortical BF shifts as previously reported (Chowdhury and Suga 2000; Ma and Suga 2001a; Sakai and Suga 2001, 2002; Yan and Suga 1998; Zhang and Suga 2000). A lesion of the basal forebrain had no effect on the ES ar-evoked collicular BF shift, but a small effect on the ES ar-evoked cortical BF shift. These observations indicate that the auditory system has an intrinsic mechanism to evoke BF shifts and that the cortical BF shift is augmented by the basal forebrain. It has been found that the cortical ACh level is increased by acoustic stimuli that perhaps activate the basal forebrain. The horizontal dotted bar represents AS. See Figs. 3 and 7 for abbreviations and other explanations.

**TABLE 5. Collicular and cortical BF shifts evoked by acoustic stimulation (AS) and/or electric stimulation of the basal forebrain (ES br) or the somatosensory cortex (ES st)***

<table>
<thead>
<tr>
<th>Stimuli (30 min)</th>
<th>BF Shift at Peak, kHz</th>
<th>Recovery Time, min</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50%</td>
<td>Full</td>
</tr>
<tr>
<td>IC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AS</td>
<td>0.40 ± 0.02</td>
<td>28 ± 1.9</td>
<td>50 ± 3.6</td>
</tr>
<tr>
<td>AS + ES ar</td>
<td>0.88 ± 0.08*</td>
<td>88 ± 3.3**</td>
<td>106 ± 4.2**</td>
</tr>
<tr>
<td>AS + ES st</td>
<td>0.95 ± 0.07*</td>
<td>100 ± 6.8**</td>
<td>130 ± 5.0**</td>
</tr>
<tr>
<td>AS + ES st (no BN)</td>
<td>0.56 ± 0.04</td>
<td>47 ± 3.9</td>
<td>62 ± 6.2</td>
</tr>
<tr>
<td>AC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AS</td>
<td>0.42 ± 0.03</td>
<td>48 ± 2.8</td>
<td>58 ± 4.1</td>
</tr>
<tr>
<td>AS + ES ar</td>
<td>1.05 ± 0.07*</td>
<td>108 ± 6.8**</td>
<td>140 ± 4.0**</td>
</tr>
<tr>
<td>AS + ES st</td>
<td>1.12 ± 0.06*</td>
<td>132 ± 7.6**</td>
<td>170 ± 6.0**</td>
</tr>
<tr>
<td>AS + ES st (no BN)</td>
<td>0.61 ± 0.04</td>
<td>59 ± 3.8</td>
<td>75 ± 3.512</td>
</tr>
</tbody>
</table>

Values are means ± SE. No BN: bilateral lesion of the basal forebrain. * P < 0.05, ** P < 0.01 compared with AS alone.

*Fig. 8. Effect of a bilateral lesion of the basal forebrain on the augmentation of the collicular (A) and cortical BF shifts (B) evoked by ES ar + ES ar. The BF shifts evoked by ES ar (○ or □) were augmented by ES st (● or ▲). However, the augmentation to be evoked was abolished when the basal forebrain (BN) was bilaterally lesioned (■). The stimulated cortical BF’s ranged between 20 and 60 kHz (35.6 ± 3.5 kHz, n = 38) for collicular studies and between 20 and 71 kHz (36.8 ± 2.9 kHz, n = 31) for cortical studies. The BF difference between the stimulated cortical neurons and the recorded collicular or cortical neurons was 4.5 ± 0.05 or 4.1 ± 0.07 kHz, respectively. See Fig. 3 for abbreviations and other explanations.

*Fig. 9. Collicular (○) and cortical BF shifts (▲) evoked by the trains of acoustic stimuli (AS) were augmented by ES br (● and ▲ in A) or ES st (● and ▲ in B). In B, the augmentation of the collicular and cortical BF shifts to be evoked by ES st (● and ▲) was abolished by a bilateral lesion of BN (□ and ■). The horizontal dotted bar represents AS. See Figs. 3 and 7 for abbreviations and other explanations.
forebrain (Hemsworth and Mitchell 1969; Neal et al. 1968). This finding matches with our observation that the ES_{at} evoked cortical BF shift partially depended on the activity of the basal forebrain.

As described in METHODS, the spatial and temporal pattern of neural activity evoked by electric stimulation of the AC, somatosensory cortex, and basal forebrain might be quite different from that evoked by conditioning. However, the collicular and cortical BF shifts evoked by ES_{at} were both augmented by ES_{st} and/or ES_{br}, as expected by Gao and Suga’s hypothesis (2000). A lesion of the basal forebrain prevented the augmentation of the ES_{at}-evoked collicular and cortical BF shifts that would otherwise be evoked by ES_{at}. These observations indicate that the somatosensory system does not directly evoke the augmentation but through the basal forebrain.

Our present data support Gao and Suga’s hypothesis (1998, 2000), as discussed later in detail. However, there were differences between the BF shift evoked by the electric stimulation and conditioning. The collicular BF shifts evoked by the 30-min-long ES_{at} + ES_{st} or ES_{at} + ES_{br} was very similar in amount and recovery time to that caused by a 30-min-long conditioning session, whereas the collicular BF shift evoked by the 30-min-long ES_{at} + ES_{st} + ES_{br} was slightly larger in amount and longer in recovery time than that caused by the conditioning. The cortical BF shift evoked by electric stimulation was quite different in time course from that evoked by the conditioning, although the amount of the BF shift was similar to one another. Namely, cortical BF shift slowly develops, reaches a plateau ~180 min after the conditioning and shows no sign of recovery even 360 min after the conditioning, whereas the cortical BF shift evoked by electric stimulation was largest at the end of the stimulation and stayed large for ~30 min for the 30-min-long ES_{at} + ES_{st} or ES_{at} + ES_{br} and for ~180 min for the 30-min-long ES_{at} + ES_{st} + ES_{br}. It always showed a recovery after the plateau. The cortical BF shift was always longer in recovery time than the collicular BF shift in identical stimulus conditions. However, the 30-min-long ES_{at} + ES_{st} + ES_{br} did not evoke a long-lasting cortical BF shift as the 30-min-long auditory fear conditioning did (Gao and Suga 2000; Ji and Suga 2001).

The time course of the cortical BF shift caused by the conditioning is presumably due to a slow increase in a cortical ACh level and maintenance of the increased ACh level. As reported in our present paper, ES_{at} augmented the collicular and cortical BF shifts evoked by ES_{at}, the development of this augmentation was prevented by a lesion of the basal forebrain, and ES_{br} augmented the BF shifts evoked by ES_{at}. Ji et al. (2001) demonstrated that an ACh application to the AC makes the cortical BF shift long-term but not the collicular BF shift. Therefore it is clear that ACh plays an important role in determining the time course of the cortical BF shift. Direct electric stimulation of the somatosensory cortex and/or the basal forebrain together with the electric stimulation of the AC perhaps evoked a rapid increase in a cortical ACh level but did not maintain the increased ACh level to produce a long-lasting cortical BF shift. It may be predicted that a long-lasting ES_{br} produces a long-lasting cortical BF shift.

It has been suggested that the lateral amygdala is the place of the plasticity directly related to memory storage of fear conditioning and that short- and long-term memories caused by fear conditioning are, respectively, related to early and late phases of long-term potentiation (a review by Schafe et al. 2001). It has been known that auditory fear conditioning causes the long-term BF shifts of cortical auditory neurons (Bakin et al. 1996; Diamond and Weinberger 1986, 1989; Gao and Suga 2000; Ji et al. 2001; Ohl and Scheich 1996; Weinberger et al. 1993) and that electric stimulation of the cholinergic basal forebrain paired with acoustic stimuli evokes the long-lasting BF shifts of cortical auditory neurons (Bakin and Weinberger 1996; Kilgard and Merzenich 1998a). ACh apparently plays an important role in causing long-term BF shifts. However, it has not yet been known in what way ACh receptors are involved in producing long-term potentiation, although it has been suggested that CGMP may act as a second messenger for producing ACh-evoked depolarization (Woody et al. 1978). It is most likely that this depolarization lasts long and causes the cellular and molecular changes well studied by Kandel and his coworkers (review by Kandel 2001). In our present experiments, the basal forebrain was presumably not activated long enough by electric stimulation to produce the long-term BF shift of cortical neurons.

Our present data support Gao and Suga’s hypothesis

Gao and Suga (1998, 2000) proposed a working hypothesis of the neural pathways for the plasticity of the IC and AC. Their hypothesis contains the following four key statements, which are supported by our current data.

STATEMENT 1. The central auditory system has an intrinsic mechanism for BF shift. The collicular BF shift is evoked by the corticofugal system working together with the cortical neural net.

Focal electric stimulation of the AC or repetitive acoustic stimulation evokes collicular and cortical BF shifts, as previously reported by Yan and Suga (1998), Gao and Suga (1998), and Chowdhury and Suga (2000). The collicular BF shift that would be evoked by conditioning is abolished by inactivation of the AC with muscimol (Gao and Suga 1998). Atropine applied to the AC abolishes the cortical BF shift that otherwise would be evoked by conditioning. However, it does not abolish but slightly reduces the collicular BF shift evoked by conditioning (Ji et al. 2001). These observations indicate that the corticofugal system plays an essential role in evoking the collicular BF shift. Atropine applied to the IC abolishes the collicular BF that otherwise would be evoked by conditioning (Ji et al. 2001). Therefore there was a possibility that electric stimulation of the AC or repetitive acoustic stimulation excited the basal forebrain, which in turn evoked not only cortical, but also collicular BF shifts. A lesion of the basal forebrain tends to reduce these BF shifts but does not abolish them (Fig. 6). Therefore the central auditory system has an intrinsic mechanism for BF shift.

STATEMENT 2. The cortical BF shift evoked by acoustic stimulation is augmented by the excitation of the somatosensory cortex following the excitation of the AC. This augmentation is presumably due to the excitation of the cholinergic basal forebrain that is evoked through the pathway from the somatosensory cortex to the association cortex, then to the amygdala and to the basal forebrain. The augmentation does not depend on the integration of auditory and somatosensory signals in the subcortical auditory nuclei.
Electric stimulation of the somatosensory cortex immediately after electric stimulation of the AC or acoustic stimulation, mimicking the cortical excitation evoked by fear conditioning, augments the collicular and cortical BF shifts evoked by the stimulation of the AC or by acoustic stimulation (Figs. 7 and 8). The somatosensory cortex apparently plays an important role in augmenting the collicular and cortical BF shifts during the conditioning. However, this augmentation does not occur when the basal forebrain is bilaterally lesioned prior to the conditioning (Figs. 8 and 9B). Therefore the somatosensory cortex does not directly augment the collicular and cortical BF shifts but does so through the pathway involving the basal forebrain. The importance of the basal forebrain for auditory cortical plasticity was hypothesized (Weinberger et al. 1990) and has been proved to be correct (Bakin et al. 1996; Kilgard and Merzenich 1998a). Atropine applied to the AC or the IC respectively abolishes the cortical or collicular BF shift (Ji et al. 2001). ACh apparently plays an essential role in cortical and collicular plasticity.

**STATEMENT 3.** The cortical BF shift augmented by the cholinergic basal forebrain in turn augments the collicular BF shift through the corticofugal system.

Electric stimulation of the basal forebrain augments not only the cortical but also the collicular BF shifts evoked by electric stimulation of the AC (Figs. 2–5 and 7). As described in the preceding text, the collicular BF shift is evoked by the corticofugal system. Therefore the augmentation of the collicular BF shift evoked by electric stimulation of the basal forebrain is evoked through the corticofugal system.

**STATEMENT 4.** The cortical BF shift depends on both the subcortical BF shift and an increase in cortical ACh level.

The 30-min-long auditory conditioning evokes “short-term” collicular and “long-term” cortical BF shifts (Gao and Suga 2000). The 30-min-long electric stimulation of the AC evokes short-term collicular and cortical BF shifts (Chowdhury and Suga 2000; Ma and Suga 2001a; Yan and Suga 1998). ACh applied to the AC changes the short-term cortical BF shift to a long-term shift but does not change the short-term collicular BF shift to a long-term shift. Atropine applied to the IC abolishes the collicular BF shift and reduces the cortical BF shift and changes it from long to short term (Ji et al. 2001). Electric stimulation of the AC, somatosensory cortex and basal forebrain for 30 min makes both collicular and cortical BF shifts longer lasting, in particular the cortical BF shift (Fig. 7). However, this long-lasting cortical BF shift was still shorter than the cortical BF shift evoked by the 30-min-long conditioning. In spite of the difference in the time course, our present experiment supports the statement that the cortical ACh level and the collicular BF shift both play an important role in the cortical long-term BF shift.

**Cortical versus thalamic pathways for plasticity of the central auditory system**

Weinberger and his coworkers (1990, 1998) hypothesized that the pathway from the IC to the AC through the multisensory thalamic nuclei is essential for cortical plasticity. However, the importance of this pathway is doubtful because of the following data obtained from the big brown bat. 1) Inactivation of the somatosensory cortex abolishes the cortical and collicular BF shifts that otherwise would be caused by fear conditioning (Gao and Suga 1998, 2000). 2) Electric stimulation of the somatosensory cortex augments the cortical and collicular BF shifts evoked by electric stimulation of the AC. Therefore the somatosensory cortex as well as the AC plays an essential role in cortical and collicular plasticity caused by the conditioning. 3) The collicular BF shift evoked by the conditioning develops faster than the cortical BF shift (Gao and Suga 2000). 4) Inactivation of the AC abolishes the collicular BF shift that otherwise would be evoked by the conditioning (Gao and Suga 1998). 5) Elimination of the collicular BF shift with atropine applied to the IC reduces and shortens the long-term cortical BF shift that otherwise would be evoked by the conditioning (Ji et al. 2001). 6) Fear conditioning evokes plasticity of the IC, which is the nucleus one step below the thalamus (Gao and Suga 1998, 2000; Ji et al. 2001). Therefore the short-term collicular BF shift evoked by the corticofugal system plays an important role for the cortical BF shift, but the pathway through the multisensory thalamic nuclei does not.

According to Gao and Suga’s working hypothesis, the short-term subcortical changes evoked by the corticofugal system and an increased ACh level in the AC are both essential for the production of the long-term cortical BF shift caused by auditory conditioning (associative learning). The long-term cortical BF shift probably represents memory for improved auditory signal processing in the frequency domain. Weinberger (1998) stated that “physiological memory is enduring neuronal change sufficiently specific to represent learned information” and hypothesized that “physiological memory in the auditory cortex is not procedural memory, i.e., is not tied to any behavioral conditioned responses but can be used flexibly.” We completely agree with his definition and hypothesis of physiological memory.

**Gao and Suga’s hypothesis to be elaborated**

The dopaminergic ventral tegmentum plays an important role in reorganization of the AC (Bao et al. 2001) as does the cholinergic basal forebrain (Bakin and Weinberger 1996; Ji et al. 2001; Kilgard and Merzenich 1998a,b; Mercado et al. 2001). The medial or ventromedial prefrontal cortex has been considered to play a role in extinction of conditioned behavioral response (Brenner et al. 1999; Herry et al. 1999; Morgan et al. 1995; Morrow et al. 1999; Quirk et al. 2000). Gao and Suga’s working hypothesis deals with only part of the neural pathways for reorganization of the central auditory system. Their hypothesis remains to be elaborated to incorporate at least the dopaminergic tegmentum.

**Nonplastic neurons in the IC**

Under tranquilization and analgesia, Zhou and Jen (2000) repetitively stimulated the AC of the big brown bat with a 0.1-ms-long, 5- to 50-μA electric pulse for 30 min and studied its effect on the response (latency and number of impulses per stimulus) and minimum threshold of collicular neurons of the big brown bat. They found two groups of collicular neurons: neurons (51%) corticofugally modulated (inhibited or facilitated) for only ≤10 s (brief-term modulation) and those (49%) corticofugally modulated ≤35 min (short-term modulation). They concluded that half of the collicular neurons was not plastic and did not participate in reorganization of the auditory system. In our present studies, repetitive stimulation of the AC
with a 0.2-ms-long, 100-nA electric pulse for 15 min evoked short-term BF shifts in 58% of collicular neuron and no BF shifts in the remaining 42%. When the AC and the basal forebrain both were electrically stimulated, however, the number of neurons showing no BF shift decreased to 11%. As already pointed out in our present paper, BF shifts depend on the relation in BF (Gao and Suga 1998; Ma and Suga 2001a; Sakai and Suga 2001; Yan and Suga 1998) and location along an iso-BF line (Sakai and Suga 2002) between stimulated and recorded neurons. When BF differences in recorded and stimulated neurons were 4–7 kHz, 92% of collicular neurons showed BF shifts for electric stimulation of both the AC and the basal forebrain and the remaining 8% did not. Therefore there may be no “nonplastic” neurons in the IC if the location of cortical neurons electrically stimulated is appropriately chosen relative to that of recorded collicular neurons.

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