Tone-Specific and Nonspecific Plasticity of the Auditory Cortex Elicited by Pseudoconditioning: Role of Acetylcholine Receptors and the Somatosensory Cortex

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Submitted 7 March 2008; accepted in final form 1 July 2008

Ji W, Suga N. Tone-specific and nonspecific plasticity of the auditory cortex elicited by pseudoconditioning: role of acetylcholine receptors and the somatosensory cortex. J Neurophysiol 100: 1384–1396, 2008. First published July 2, 2008; doi:10.1152/jn.90340.2008. Experience-dependent plastic changes in the central sensory systems are due to activation of both the sensory and neuromodulatory systems. Nonspecific changes of cortical auditory neurons elicited by pseudoconditioning are quite different from tone-specific changes of the neurons elicited by auditory fear conditioning. Therefore the neural circuit evoking the nonspecific changes must also be different from that evoking the tone-specific changes. We first examined changes in the response properties of cortical auditory neurons of the big brown bat elicited by pseudoconditioning with unpaired tonal (CS_u) and electric leg (US_u) stimuli and found that it elicited nonspecific changes to CS_o (a heart-rate decrease, an auditory response increase, a broadening of frequency tuning, and a decrease in threshold) and, in addition, a small tone-specific change to CS_o (a small short-lasting best-frequency shift) only when CS_o frequency was 5 kHz lower than the best frequency of a recorded neuron. We then examined the effects of drugs on the cortical changes elicited by pseudoconditioning. The development of the nonspecific changes was scarcely affected by atropine (a muscarinic cholinergic receptor antagonist) and mecamylamine (a nicotinic cholinergic receptor antagonist) applied to the auditory cortex and by muscimol (a GABA_A-receptor agonist) applied to the somatosensory cortex. However, these drugs abolished the small short-lasting tone-specific change as they abolished the large long-lasting tone-specific changes elicited by auditory fear conditioning. Our current results indicate that, different from the tone-specific change, the nonspecific changes depend on neither the cholinergic neuromodulator nor the somatosensory cortex.

INTRODUCTION

A conditioning tonal stimulus (CS) paired with an unconditioned foot stimulus (US), i.e., auditory fear conditioning, evokes tone-specific plastic changes represented by a best-frequency (BF) shift in the primary auditory cortex (AI), whereas the CS unpaired with the US, i.e., pseudoconditioning, evokes nonspecific plastic changes in AI that have been called sensitization (for review see Bakin et al. 1992; Weinberger 2007) or nonspecific augmentation (Suga 2008). It makes a huge difference in cortical plasticity whether the CS is paired or unpaired with the US. The cortical plasticity evoked by the conditioning and pseudoconditioning depends on the activation of the sensory and neuromodulatory systems. It is neurophysiologically very interesting and important to explore the neural circuits for producing the tone-specific and nonspecific changes. Therefore Gao and Suga (1998) proposed a model for tone-specific plasticity elicited by fear conditioning and Suga (2008) proposed a model for nonspecific plasticity elicited by pseudoconditioning.

The model proposed by Gao and Suga (1998), elaborated on by Suga et al. (2000, 2002), states that a subthreshold short-term cortical BF shift is evoked by the CS exciting the neural net in AI and the corticofugal feedback system via the ventral division of the medial geniculate body (MGBv), that this BF shift is changed into a large long-term BF shift by acetylcholine (ACh) released by the cholinergic basal forebrain (the nucleus basalis [NB]), and that the NB is activated by AI and the somatosensory cortex (SI) through the amygdala where the CS–US association occurs. For the measurement of a BF shift, the frequency of a tone burst is varied by, e.g., 0.5-kHz steps. Then, the BF shift <0.5 kHz is defined as a subthreshold BF shift. The presence of the subthreshold BF shift is evident because it is easily changed into a large BF shift by acetylcholine or N-methyl-D-aspartate applied to AI (Ji et al. 2001, 2005) or by electric stimulation of the NB (Bakin and Weinberger 1996; Bjordahl et al. 1998; Kilgard and Merzenich 1998; Ma and Suga 2003; Yan and Zhang 2005; Zhang et al. 2005). The subthreshold BF shift elicited by a short train of tone bursts such as a CS (Gao and Suga 1998; Ji et al. 2001) can also be changed into the small BF shift by lengthening the train duration (Chowdhury and Suga 2000; Gao and Suga 1998, 2000; Ma and Suga 2001, 2003; Yan and Suga 1998).

The Gao–Suga model is different from the model proposed by Weinberger (1998, 2004), which states that the small short-term cortical BF shift is evoked by the medial division of the MGB (MGBm) and the posterior interlaminar nucleus (PIN) only after the CS–US association occurs in these nuclei, and that this BF shift is changed into the large long-term shift by the NB activated by the MGBm/PIN through the amygdala. However, the Weinberger model has not been supported by the following findings: 1) the cortical BF shift can be evoked without the CS–US association in the MGBm/PIN (Bakin and Weinberger 1996; Bjordahl et al. 1998; Chowdhury and Suga 2000; Gao and Suga 1998, 2000; Kilgard and Merzenich 1998; Ma and Suga 2001, 2003; Yan and Zhang 2005; Zhang et al. 2005); and 2) corticofugal feedback and SI play an important role in evoking the cortical BF shift (Gao and Suga 1998; Ji et al. 2001; Ma and Suga 2003, 2005; Yan and Suga 1998). Our current experiments further demonstrate that muscimol
applied to SI abolish the BF shift elicited by pseudoconditioning without affecting auditory responses and frequency tuning of cortical auditory neurons.

The MGBm and PIN project layer I of the auditory cortex (Winer and Morest 1983). They respond to CS and US and show BF shifts (Weinberger 2004, 2007). However, there have been no data indicating that the MGBm/PIN evokes the cortical BF shift, so that it is important to explore what kind of cortical plasticity is evoked by them. Suga (2008) speculated that the MGBm and PIN are involved in evoking the cortical nonspecific plasticity due to pseudoconditioning and that the nonspecific plasticity is mostly augmented by noncholinergic neurotransmitters. Both the Gao–Suga model for the BF shifts and the Suga model for the nonspecific augmentation are working models to be further tested and refined.

Our current studies were designed to examine the following two aspects involved in these models: 1) According to the Gao–Suga model, the CS would evoke the subthreshold short-term cortical BF shift regardless of whether it is paired or unpaired with the US, and this BF shift is augmented by ACh released by the NB which is indirectly activated not only by SI, but also by AI. According to the Weinberger model, however, the CS unpaired with the US would not evoke the cortical BF shift and SI inactivation would have no effect on the cortical BF shift, even it was evoked. Therefore we examined whether the pseudoconditioning evoked the small BF shift and whether this BF shift was abolished by bilateral inactivation of SI with an agonist of γ-aminobutyric acid type A receptor (GABA\textsubscript{A}R), muscimol, applied to SI. 2) The Suga model speculates that, unlike the BF shift, the nonspecific plasticity elicited by pseudoconditioning is not augmented by ACh. If the nonspecific plasticity was increased by ACh, the small short-term BF shift elicited by the pseudoconditioning would also be increased and would change into the large long-term BF shift. Therefore we examined whether the antagonists of ACh receptors, atropine and mecamylamine, abolish the small BF shift but not the nonspecific plasticity. Our current data support part of the Suga model as well as the Gao–Suga model. Here, we first report the cortical tone-specific (BF shift) and nonspecific (augmentation) plasticity in the bat’s AI evoked by the pseudoconditioning. We then describe our findings that the ACh receptor antagonists applied to AI and the GABA\textsubscript{A}R agonist applied to SI abolished the cortical tone-specific plasticity, but not the nonspecific plasticity.

**METHODS**

**Experimental subjects**

Twenty-four adult big brown bats (Eptesicus fuscus, 18–24 g body weight) were used for the experiments. All experimental procedures were approved by the Animal Studies Committee of Washington University in St. Louis and were the same as those previously described (Gao and Suga 2000; Ji et al. 2001).

**Surgical and recording procedures**

Under neuroleptanalgesia (innovar 4.08 mg/kg of body weight, 0.4 ml fentanyl citrate in 20 mg/ml droperidol), a 1.5-cm-long metal post was glued to the dorsal surface of the bat’s skull. Experiments began 3–4 days after the surgery. An awake bat was placed in a polyethylene-foam body mold suspended at the center of a soundproof room maintained at 31°C. The bat’s head was immobilized by fixing the metal post glued on the skull onto a metal rod with set screws to ensure a uniform and stable position of the head, which directly faced a loudspeaker located 80 cm away. For single-unit recording, a tungsten-wire microelectrode with a tip diameter of about 7 μm was inserted orthogonally into AI at a depth between 200 and 700 μm. Only one neuron was studied in a 1-day experiment, and the same animal was used with a 3-day interval to minimize the cumulative effect of the pseudoconditioning. Local anesthetic (5% lidocaine; E. Fougera) and antibiotic (0.2% nitrofurazon; RXV Products) ointments were applied to the surgical wound. The recording session lasted about 7 h. Water was provided with a dropper and lidocaine was reapplied every 2 h. The bat was neither anesthetized nor tranquilized during the experiments. If the bat continued to show signs of discomfort, recordings were terminated and the bat was returned to its cage.

**Acoustic stimulation**

Acoustic stimuli were 20-ms tone bursts, including a 0.5-ms rise-decay time, delivered to the bat at a rate of 4/s from a leaf tweeter with Real Time processors 2.1 (Tucker-Davis Technologies, Alachua, FL). The frequency and amplitude of the tone bursts were manually varied or computer-controlled to measure the BF and the minimum threshold (MT) of a given neuron. Frequency-tuning curves and iso-spike-count contour lines were obtained from the response of a single neuron to a frequency–amplitude scan that was repeated 10 times. The scan consisted of 21 time blocks of 250 ms for a frequency scan and 13 time blocks of 250 ms for an amplitude scan. The frequency and amplitude of the tone burst were randomly varied by 1.0-kHz steps and 5-dB steps, respectively. The receptive field of a neuron is the excitatory response area derived from the response to the frequency–amplitude scan. To measure a frequency–response curve and a BF shift, the amplitude of the tone burst was set at 10 dB above the MT of a given neuron. Then, the frequency of the tone burst was randomly varied in 0.5- or 1.0-kHz steps and was delivered every 250 ms. This frequency scan consisted of 21 time blocks of 250 ms and was repeated 50 times. These scans were delivered by the stimulus control and recording software (BrainWare v. 8.0). The amplitude of the tone bursts delivered from the leaf tweeter was calibrated with a microphon (Bruel & Kjaer Instruments, Naerum, Denmark). It was flat within ±5 dB from 10.0 to 70.0 kHz. The amplitude of the tone bursts was expressed in decibels sound pressure level (dB SPL) referred to 20-μPa root mean square.

**Pseudoconditioning**

In the big brown bat, auditory fear conditioning elicits the largest shifts of the cortical (Gao and Suga 2000) and collicular (Gao and Suga 1998) neurons with a BF 6.0–7.0 kHz higher than the frequency of the conditioning stimulus (CS), whereas it does not elicit the BF shifts of the cortical and collicular neurons with a BF >10 kHz lower or >15 kHz higher than the CS frequency. Furthermore, it has been known that a 30-min-long repetitive tone burst stimulation evokes the small BF shift of a neuron with a BF approximately 5 kHz higher than the tone burst frequency (Gao and Suga 1998). Therefore to demonstrate tone-specific and nonspecific plasticity evoked by pseudoconditioning, the frequency of the CS was either the same as, 10 kHz higher, 5.0 kHz lower, or 15 kHz lower than the BF of a given neuron. We particularly examined whether the pseudoconditioning with the CS at 5.0 kHz lower than the BF of a given neuron elicited a BF shift in addition to the nonspecific augmentation. In the pseudoconditioning, the CS was unpaired with the US, so that this unpaired CS is hereafter abbreviated as the “CS\textsubscript{u}.” Accordingly, the unpaired US is abbreviated as the “US\textsubscript{u}.”

Since the CS in our auditory fear conditioning was a train of tone bursts that were 50 dB SPL, 10 ms long, 33/s over 1.0 s and this CS was delivered every 30 s over 30 min (Gao and Suga 1998, 2000; Ji and Suga 2003; Ji et al. 2001), all the parameters characterizing the
CSu for pseudoconditioning were exactly the same as those of the CS for the auditory fear conditioning and this CSu was delivered every 30 s for 30 min. As in our auditory fear conditioning, the unconditional stimulus for pseudoconditioning (USu) was a 50-ms, 0.1- to 0.4-mA monophasic electric pulse. However, the USu was randomly delivered to the bat in a time interval between 5 and 25 s after the CSu, making sure to avoid delivering the USu in a period between 5.0 s before and 5.0 s after the CSu. The mean interval of the USu was 14.8 s. There were 60 CSu-USu in total per session.

A stimulus design to elicit behavioral changes is different between sensitization and pseudoconditioning, although the behavioral changes evoked by them might be almost the same (Erickson and Walters 1988). Therefore we hereafter call the changes in neural responses evoked by pseudoconditioning “nonspecific augmentation” instead of sensitization. The ascending reticular activating system evokes “general augmentation” for cortical arousal, so we avoid using this term.

Heart-rate recording

To record electrocardiograms of the bat during and after the pseudoconditioning, tab electrodes (PSS Select, Physician Sale and Services) were attached to the chest and wing of a bat. The electrocardiograms were amplified by an RP 2.1 enhanced real-time processor (TDT) and stored by BrainWare v 8.0 software. The heart-rate change evoked by the pseudoconditioning was plotted as a function of time.

Drug applications

In the big brown bat, auditory responses can be recorded within the 4.52-mm² cortical area that is mostly AI (Dear et al. 1993). The approximate center of AI is dorsoventrally crossed by a 30-kHz iso-BF line. The approximate midpoint of this iso-BF line was first electrophysiologically located by recording auditory responses at five to six cortical loci. Then, a hole, about 1.0 mm in diameter, was made on the cortex. The approximate center of AI is dorsoventrally crossed by a 30-kHz iso-BF line. The approximate midpoint of this iso-BF line was first electrophysiologically located by recording auditory responses at five to six cortical loci. Then, a hole, about 1.0 mm in diameter, was made there for single-unit recording and drug applications. The drug applied to the AI surface was 0.2 µl of 0.4 M atropine or 10 µM mecamylamine (Sigma Chemical, St. Louis, MO) dissolved in a 0.9% saline solution. SI was localized by recording neural responses to touch stimuli (Krubizer and Calford 1992). Muscimol, 0.2 µl of 8.8 mM (1 µg/µl), was bilaterally applied to the SI surface, the same way as did Gao and Suga (1998). The drug applications were made 2–5 min prior to the pseudoconditioning with a 1.0-µl Hamilton syringe. The dura mater was not removed, but punctured by the penetrations of the recording electrode, so that the drug applied to the cortex was diffused into it through these puncture holes.

Data acquisition

Action potentials of a single cortical neuron tuned to a specific frequency were amplified (Medusa base station; TDT) and selected with a time–amplitude window-discriminator (BrainWare v 8.0). During and after the pseudoconditioning and/or a drug application, the action potentials discharged by the neuron were continuously compared with the template stored and displayed on the monitor screen at the beginning of the study of the neuron. An array of poststimulus time (PST) histograms displaying the auditory responses to the frequency scan repeated 50 times or the frequency–amplitude scan repeated 10 times was acquired every 15 or 30 min, up to 240 min after the onset of the pseudoconditioning, as long as action potentials visually matched the template. The response latency of a neuron was determined to be the time between the onset of a tone burst at the bat’s ears and the onset of the response of the neuron, displayed by a PST cumulative histogram.

Off-line data processing

To obtain the frequency–response curve of a neuron with the frequency scan, the magnitude of the auditory response of the neuron was expressed by the number of spikes per 50 identical stimuli as a function of the frequency of the tone burst. The BF of the neuron was defined as the frequency at which the frequency–response curve peaked. To study the development of the nonspecific augmentation of auditory responses, the magnitude of the response at the BF was plotted as a function of time. Because an identical frequency scan was delivered 50 times, there were 50 samples of BFs that could be used to compute a mean ± SE of the BFs and to perform statistical analysis. To determine whether there were differences in response magnitude between a BF and the adjacent frequencies, or between the BFs obtained before and after the pseudoconditioning or a drug application, a two-tailed unpaired t-test was used for a significant difference of P < 0.01 or P < 0.05.

For processing the data obtained with the frequency–amplitude scan, the magnitude of the response of a neuron was expressed by the number of spikes per 10 identical stimuli and was plotted in the frequency–amplitude coordinates and iso-spike-count contour lines were drawn by using SigmaPlot 8.0 software. The recovery of an auditory response was defined as the changed response at the BF recovered to that of the control response within ±10% in the neurons studied.

RESULTS

Non-specific heart-rate change elicited by pseudoconditioning

When a train of 30.0-kHz tone bursts (CSu) unpaired with electric leg-stimulation (USu) was delivered to the bats, their heart rates decreased 21.0 ± 5.5% on the average (n = 4; Fig. 1, 1). After this pseudoconditioning, the bats showed a heart-rate decrease not only to the CSu delivered alone, but also to non-CSu tone bursts at 15.0 or 60.0 kHz (Fig. 1, 2–4). The average decrease of the heart rate for these three sounds was

![Fig. 1. Heart-rate change evoked by pseudoconditioning. 1: pseudoconditioning; an unpaired conditioning stimulus, CSu (1.0-s train of 10-ms, 50-dB SPL, 30-kHz tone bursts at 33/s) was delivered every 30 s for 30 min, whereas an unconditioned stimulus, USu (electric leg stimulus, 0.1–0.4 mA) was randomly delivered. 2–4: a 10-ms, 50-dB SPL tone burst at 15 kHz (2), 60 kHz (3) or 30 kHz (4) was delivered at a rate of 4/s for 3 min without the USu after the pseudoconditioning. The symbols and bars represent means and SEs in the percentage of heart-rate change. The data were obtained from 4 animals.]
20.0 ± 4.1, 17.4 ± 6.5, and 18.5 ± 5.0%, respectively. There were no significant differences between them (P > 0.05). Therefore these nonspecific autonomic responses (the decrease in heart rate) were quite different from a conditioned autonomic response specific to the CS paired with the US (Ji and Suga 2007).

**BFs of cortical neurons and the frequencies of CS_u**

AI of the big brown bat represents the frequencies of sounds from 10 to 80 kHz along its caudorostral axis (Dear et al. 1993; Shen et al. 1997). Without a drug application, the effects of pseudoconditioning were examined on 28 cortical neurons with BFs ranging between 20 and 34 kHz (mean ± SE: 29.5 ± 1.3 kHz). Their BFs were either the same as (6 neurons), 5.0 kHz higher (10 neurons), 15.0 kHz higher (6 neurons), or 10.0 kHz lower (6 neurons) than the frequencies of CS_u’s (Table 1, first row).

The effects of drugs were studied on the changes in 45 cortical neurons elicited by pseudoconditioning: 14 neurons for the effect of atropine applied to AI, 15 neurons for the effect of mecamylamine applied to AI, and 16 neurons for the effect of muscimol applied to SI (Table 1, second to fourth rows). All the drugs were applied 2–5 min prior to the pseudoconditioning. The BFs of these cortical neurons ranged between 23 and 58 kHz (mean ± SE: 32.8 ± 1.7 kHz). For the studies of the drug effects on nonspecific and tone-specific cortical changes elicited by the pseudoconditioning, the CS_u frequencies were always 5.0 kHz lower than the BFs of the neurons studied because in the big brown bat, auditory fear conditioning elicits the largest centripetal BF shift when the BF of a given neuron is about 5.0 kHz higher than the CS paired with the US (Gao and Suga 1998, 2000).

**Nonspecific and tone-specific plasticity of cortical auditory neurons elicited by pseudoconditioning**

Pseudoconditioning increased the auditory responses, broadened the frequency-tuning curves, lowered the thresholds, and/or increased the background discharges (Table 1, first row). The increase in the auditory responses was always associated with the shortening of the response latencies. All those changes were nonspecific to the CS_u. In addition, the pseudoconditioning elicited a small BF shift that was specific to the unpaired CS_u. Those cortical changes are illustrated in Figs. 2–5.

In Fig. 2, the top row displays the receptive fields of four cortical auditory neurons in which the iso-spike-count contours are color-coded (Fig. 2, A–D). The outer edge of the faint blue zone represents the excitatory frequency-tuning curve of a given neuron. When the pseudoconditioning was delivered to the bat with the CS_u at the frequency being the same as (A), 5.0 kHz lower (B), 15.0 kHz lower (C), or 10.0 kHz higher (D) than the BF of a given neuron, the responses of these four neurons were augmented, as shown by the large red areas (Fig. 2, middle row). This augmentation gradually reduced and disappeared 180–240 min after the onset of the pseudoconditioning (Fig. 2, bottom row). For example, the neuron in Fig. 2A was tuned to 29.0 kHz. Its maximum response was 18 spikes/10 stimuli, which was evoked only by a tone burst at 26 kHz and 90 dB SPL. After the pseudoconditioning with the CS_u at 29 kHz, its responses to tone bursts were augmented so that 21 spikes/stimulus occurred at many different frequency–amplitude combinations within the large red area. The neuron in Fig. 2C was tuned to 26.0 kHz. Its responses to tone bursts were also augmented by the pseudoconditioning with the CS_u at 11.0 kHz. The neuron in Fig. 2D was tuned to 26.0 kHz. Its responses to tone bursts were also augmented by the pseudoconditioning with the CS_u at 36.0 kHz. The lowest and highest BFs of the neurons studied were 9.0 and 80.0 kHz, respectively. The responses of the 9.0-kHz-tuned neuron to tone bursts were augmented by the pseudoconditioning with the CS_u at 24.0 kHz, and those of the 80.0-kHz-tuned neuron were augmented by the pseudoconditioning with the CS_u at 70.0 kHz. Therefore the augmentation was nonspecific to the CS_u frequencies. On average, the nonspecific augmentation of the response to the tone burst at the BF and 10 dB above the MT was 56.7 ± 3.4% (n = 23). In Fig. 2, it is clear that the nonspecific augmentation of the response was accompanied with a broadening of a frequency-tuning curve, a decrease in threshold, and an increase in background discharge. The

<table>
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<tr>
<th>Group</th>
<th>Response Increase at MT + 10 dB, %</th>
<th>BW Increase at MT + 10 dB, kHz</th>
<th>Threshold Decrease at BF, dB</th>
<th>Background Discharge Increase, %</th>
<th>BF Shift at +5 kHz, threshold, h</th>
<th>Recovery Time of Response, h</th>
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<tr>
<td>P-cond with saline to AI (BF: 0, +5, +15, or −10 kHz from CS_u)</td>
<td>56.7 ± 3.4**</td>
<td>3.8 ± 0.4**</td>
<td>6.1 ± 0.8*</td>
<td>14 ± 4.1*</td>
<td>−1.0±0.1**</td>
<td>4.0 ± 0.8**</td>
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<td>P-cond with Atropine to AI (BF: +5 kHz from CS_u)</td>
<td>48.6 ± 5.1*</td>
<td>3.2 ± 0.3</td>
<td>5.4 ± 0.7</td>
<td>10 ± 9.6</td>
<td>0</td>
<td>2.8 ± 0.3**</td>
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<tr>
<td>P-cond with Mecamylamine to AI (BF: +5 kHz from CS_u)</td>
<td>50.4 ± 5.2</td>
<td>3.0 ± 0.4</td>
<td>5.0 ± 1.0</td>
<td>10 ± 4.5</td>
<td>0</td>
<td>3.4 ± 0.6*</td>
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<td>P-cond with Muscimol to SI (BF: +5 kHz from CS_u)</td>
<td>58.2 ± 4.6</td>
<td>3.7 ± 0.2</td>
<td>5.0 ± 1.0</td>
<td>16 ± 9.8</td>
<td>0</td>
<td>3.1 ± 0.5*</td>
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Values are means ± SE. Nonspecific augmentation and tone-specific BF shifts of cortical auditory neurons elicited by pseudoconditioning (unpaired CS–US) and the effects of drugs applied to the primary auditory cortex (AI) or somatosensory cortex (SI) on the changes elicited by pseudoconditioning (p-cond). The data were obtained from the cortical neurons whose best frequencies (BFs) were either 0, 5, 15, or −10 kHz different from the frequency of an unpaired conditioning tone stimulus (CS_u). Atropine (an antagonist of muscarinic cholinergic receptors); BW, bandwidth; Mec, mecamylamine (an antagonist of nicotinic cholinergic receptors); MT, minimum threshold; Mus, muscimol (an agonist of GABA_A receptors). In the fractions, the denominators and nominators, respectively, indicate the total number of neurons studied and the number of neurons that showed a given change. *P < 0.05, **P < 0.01, t-test; the effects of p-cond are compared with pre-p-cond (i.e., control). *P < 0.05, **P < 0.01, t-test; the drug effects are compared with p-cond alone.
amounts of all these changes were calculated and are listed in the first row of Table 1. All these changes disappeared (i.e., the neurons recovered) 4.0 ± 0.75 h after the onset of the pseudoconditioning.

When the unpaired CSu was 5.0 kHz lower than the BF, we found that the pseudoconditioning elicited a small short-lasting BF shift in addition to the nonspecific augmentation. Figure 2B2 shows both the nonspecific augmentation and the BF shift of the receptive field of the neuron tuned to 24.0 kHz. The BF shift toward the unpaired CSu at 19.0 kHz was small (1.0 kHz), but it was clear because it was accompanied with the overall shift of the receptive field.

FIG. 2. Nonspecific augmentation of 4 cortical neurons elicited by pseudoconditioning (p-cond). The best frequencies (BFs) of the 4 single neurons (A–D) were either 29.0, 24.0, 26.0, or 26.0 kHz (vertical dotted lines). The arrows along the frequency axis indicate the frequencies of the CSu, which were either 0 (A), 5 (B), 10 (D), or 15 (C) kHz different from the BFs of the recorded neurons. In each column, 1–3 show the receptive fields obtained before (control) and 30 and 210 min after the onset of p-cond, respectively. Note the augmentation of the responses, broadening of the receptive field, increase in background discharges, and decrease in threshold. The scale bars from dark blue to dark red show low to high spike counts per 10 stimuli. Note a small BF shift toward 19 kHz (i.e., tone-specific change) in B2.

FIG. 3. The frequency–response curves of 4 cortical neurons (A–D) showing nonspecific augmentation and a BF shift (B) elicited by p-cond. The BFs of the neurons in A–D were 20.0, 32.0, 31.0, and 24.0 kHz, respectively (open circles on vertical dashed lines). Note the overall augmentation of the responses 30 min after the onset of p-cond and, in addition, the 1.0-kHz BF shift in B. The key of the curves is shown in the inset. All the curves were obtained with tone bursts at 10 dB above the minimum threshold of a given neuron. The arrows along the frequency axis indicate the frequencies of CSu’s. The difference between the BF and CSu frequency is shown at the top of each graph.
The cortical nonspecific augmentation and BF shift are further demonstrated in Fig. 3, which shows the frequency–response curves of four cortical neurons obtained at 10 dB above their MTs (open circles). Their BFs were either the same as (A), 5.0 kHz higher (B), 15.0 kHz higher (C), or 10.0 kHz lower (D) than the CSu frequencies. The responses of all four neurons to tone bursts were augmented by the pseudoconditioning regardless of whether the BFs of the recorded neurons were the same as or different from the CSu frequencies (Fig. 3, filled circles or triangles). The amount of augmentation was 110% in A, 60% in B, 118% in C, and 67% in D.

When the BF of a cortical neuron was 5.0 kHz higher than the CSu frequency, the neuron showed a BF shift in addition to the nonspecific augmentation. In Fig. 3B, the neuron showed a 1.0-kHz BF shift for the pseudoconditioning. This BF shift disappeared within 50 min after the onset of the pseudoconditioning, but the nonspecific augmentation lasted much longer than that. The BF shift was small (1.0 ± 0.1 kHz, n = 5) and short-lasting (45.0 ± 15.1 min, n = 5). The BF shift observed always occurred toward the frequency of the CSu and it was statistically significant in 5 of the 10 neurons studied (P < 0.01). The arrays of PST histograms in Figs. 7A and 8B, which are subsequently explained, show additional examples of the BF shifts.

The pseudoconditioning induced nonassociative learning (Fig. 1; Bakin et al. 1992), so that the USu alone should evoke nonspecific augmentation. As a matter of fact, the USu alone evoked it. The amount of the augmentation measured at the BF and 10 dB above the MT of a given neuron ranged from 43 to 67%. The mean and SE of the augmentation were 51 ± 1.6% (n = 10). Since the mean ± SE of the augmentation evoked by the CSu and USu was 57 ± 3.4% (n = 23), as shown in Table 1, the nonspecific augmentation evoked by the USu alone was not statistically different from that evoked by the CSu and USu (two-tailed unpaired t-test, P = 0.36). The CSu (20-ms, 50 dB SPL) delivered 50 times. The tone burst was 29.0 kHz, 55 dB SPL, and 20 ms (horizontal bars at the bottom). The minimum threshold of the neuron was 45 dB SPL. 1–3: control and 60 and 180 min after the onset of the p-cond. The gap between 2 vertical dashed lines indicates a 5.2-ms shift in response latency. The arrows in A indicate the onsets of the responses. The inset lists the response latencies of 12 neurons measured before and after the p-cond.

The cortical nonspecific augmentation and BF shift are subsequently explained, show additional examples of the pseudoconditioning. This BF shift disappeared within 50 min after the onset of the pseudoconditioning, but the nonspecific augmentation lasted much longer than that. The BF shift was small (1.0 ± 0.1 kHz, n = 5) and short-lasting (45.0 ± 15.1 min, n = 5). The BF shift observed always occurred toward the frequency of the CSu and it was statistically significant in 5 of the 10 neurons studied (P < 0.01). The arrays of PST histograms in Figs. 7A and 8B, which are subsequently explained, show additional examples of the BF shifts.

The cortical nonspecific augmentation and BF shift are further demonstrated in Fig. 3, which shows the frequency–response curves of four cortical neurons obtained at 10 dB above their MTs (open circles). Their BFs were either the same as (A), 5.0 kHz higher (B), 15.0 kHz higher (C), or 10.0 kHz lower (D) than the CSu frequencies. The responses of all four neurons to tone bursts were augmented by the pseudoconditioning regardless of whether the BFs of the recorded neurons were the same as or different from the CSu frequencies (Fig. 3, filled circles or triangles). The amount of augmentation was 110% in A, 60% in B, 118% in C, and 67% in D.

When the BF of a cortical neuron was 5.0 kHz higher than the CSu frequency, the neuron showed a BF shift in addition to the nonspecific augmentation. In Fig. 3B, the neuron showed a 1.0-kHz BF shift for the pseudoconditioning. This BF shift disappeared within 50 min after the onset of the pseudoconditioning, but the nonspecific augmentation lasted much longer than that. The BF shift was small (1.0 ± 0.1 kHz, n = 5) and short-lasting (45.0 ± 15.1 min, n = 5). The BF shift observed always occurred toward the frequency of the CSu and it was statistically significant in 5 of the 10 neurons studied (P < 0.01). The arrays of PST histograms in Figs. 7A and 8B, which are subsequently explained, show additional examples of the BF shifts.

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SPL tone bursts) was apparently unnecessary for eliciting the nonspecific augmentation. Needless to say, the USₙ alone did not evoke BF shifts.

Shortening of the response latencies of cortical neurons elicited by pseudoconditioning

The response latency of a cortical neuron was measured as the time interval between the onset of a tone burst at the BF and at 10 dB above the MT of a given neuron and the first spike of the response of the neuron to the tone burst. The PST histograms in Fig. 4A display the responses of a cortical neuron to 50 identical tone bursts at 29.0 kHz, which was the BF of the neuron. The response of the neuron increased by about 110% and its response latency shortened from 19.2 to 14.0 ms after the pseudoconditioning (Fig. 4A2). The response magnitude and latency reverted back to the control values about 180 min after the onset of the pseudoconditioning (Fig. 4A3). Since the pseudoconditioning augmented not only auditory responses, but also background discharges, the measurement of the response latency with a PST histogram was not necessarily accurate. Therefore a PST cumulative histogram was plotted instead of a PST histogram and its deflection point (arrows in Fig. 4B) was used for the latency measurement. Figure 4B shows a 5.2-ms decrease in latency, a 110% increase in response, and a 24% increase in background discharges elicited by the pseudoconditioning. In 12 cortical neurons studied, the latencies of the responses were measured in the same way as described earlier. On average, the latency was shortened from 22.4 ± 0.4 to 18.3 ± 0.5 ms (P < 0.01) by the pseudoconditioning (Fig. 4B, inset).

Time course of the nonspecific augmentation elicited by pseudoconditioning

The cortical nonspecific augmentation gradually developed to a peak over 60–90 min after the onset of the pseudoconditioning, after which it slowly decreased and disappeared about 240 min after that (Fig. 5A, curve 1). The array of the PST histograms in Fig. 5B1 shows the time course of such a nonspecific augmentation of a cortical neuron tuned to 26.0 kHz. It has been demonstrated that atropine applied to the surface of AI, without the pseudoconditioning, reduces cortical auditory responses with the maximum reduction at about 30 min after its application, and that the reduction gradually disappears over 90 min after that (Fig. 5A, curve 3; Ji et al. 2001). Therefore it was expected that atropine applied to AI 2–5 min prior to the pseudoconditioning would initially reduce the auditory responses of cortical neurons. In fact, atropine first reduced the auditory responses of cortical neurons and then the responses increased to the peak value at about 60 min after the onset of the pseudoconditioning (Fig. 5A, curve 2a). This initial decrease in the responses was the same as the drug effect of atropine application alone (curve 3), so this direct atropine effect was subtracted from curve 2a to obtain the time course of the augmentation due to the expression of the pseudoconditioning (curve 2b). This curve clearly indicated that the development of the nonspecific augmentation was scarcely affected by atropine. The augmentation at the peak tended to be slightly smaller than that elicited by the pseudoconditioning without atropine, and the recovery of the responses to the control was faster than that for the pseudoconditioning without atropine (curve 2b; Table 1, second row; P < 0.05). A saline solution applied to AI had no effect at all on the auditory responses (Fig. 5A, curve 4). The array of the PST histograms in Fig. 5B2 shows the change evoked by atropine and the pseudoconditioning in the response of a cortical neuron tuned to 29.0 kHz. These histograms clearly show an initial decrease in response and delayed augmentation. Of the 14 cortical neurons studied with atropine applied to AI prior to the pseudoconditioning, 12 showed an initial decrease in the response followed by the nonspecific augmentation, whereas the remaining 2 neurons showed nonspecific augmentation without the initial decrease in response.

Effects of ACh-receptor antagonists applied to AI on the BF shift and nonspecific augmentation elicited by pseudoconditioning

As reported by Bakin et al. (1992), nonspecific augmentation was frequency independent; that is, it occurred equally at different frequencies. Therefore drug effects were studied on the nonspecific augmentation at the CSₙ frequency 5.0 kHz lower than the BF, instead of at different CSₙ frequencies. Since the pseudoconditioning with the CSₙ 5.0 kHz lower than the BF of a given neuron evoked both the small BF shifts and the prominent nonspecific augmentation of cortical neurons (Figs. 2B and 3B) and since the cortical BF shift evoked by auditory fear conditioning is abolished by atropine applied to AI (Ji et al. 2001), the effect of atropine or mecamylamine applied to AI was studied on the BF shifts and nonspecific augmentation of 29 cortical neurons elicited by the pseudoconditioning. All of the 29 neurons studied showed no BF shifts, but they did show the nonspecific augmentation. The amount of the augmentation tended to be smaller than that elicited without a drug application (Table 1, second and third rows).

Figure 6 shows the receptive fields of three cortical neurons (Fig. 6, A–C) to tone bursts with the color-coded iso-spike-count contours. In Fig. 6A1, the neuron was tuned to 27.0 kHz. When atropine was applied to AI prior to the pseudoconditioning with a 22.0-kHz CSₙ, the auditory response of the neuron was initially reduced (Fig. 6A2), but the nonspecific augmentation was fully developed about 60 min after the onset of the pseudoconditioning (Fig. 6A3). The BF shift was not elicited at all. In Fig. 6B1, the neuron was tuned to 34.0 kHz. Mecamylamine applied to AI prior to the pseudoconditioning with a 29.0-kHz CSₙ did not reduce the auditory response of the neuron, so that the nonspecific augmentation of the neuron rapidly developed as shown in Fig. 6B2. The neuron showed no BF shift.

In Fig. 7, the effects of atropine on the BF shift and nonspecific augmentation were further demonstrated with the PST histograms and the frequency–response curves of a cortical neuron tuned to 26.0 kHz. First, the pseudoconditioning with a saline application to AI was delivered to the animal. Then, the 1.0-kHz BF shift and nonspecific augmentation were elicited 30 min after the onset of the pseudoconditioning (Fig. 7A2). However, 90 min after that, the BF shift disappeared and the nonspecific augmentation developed further (Fig. 7A3). The augmentation disappeared about 210 min after the pseudoconditioning (Fig. 7A4). After the recovery from this pseudoconditioning, atropine was applied to AI immediately prior to the second pseudoconditioning and the same neuron was restudied (Fig. 7B). Then, the responses of the
neuron to tone bursts first became smaller and no BF shift was evoked by the pseudoconditioning (Fig. 7B2); however, the nonspecific augmentation developed after that. It became prominent about 90 min after the onset of the pseudoconditioning (Fig. 7B3). The changes in the response described earlier are also shown by the frequency–response curves in Fig. 7, C and D. The effect of atropine applied to AI on the BF shift was clearly different from its effect on the nonspecific augmentation. This finding indicates that the neuromodulator for the nonspecific augmentation is different from that for the BF shift.

**Effects of a GABA<sub>A</sub>R agonist bilaterally applied to SI on the BF shift and nonspecific augmentation elicited by pseudoconditioning**

Bilateral inactivation of SI by muscimol abolishes the cortical and collicular BF shifts elicited by auditory fear conditioning without affecting their auditory responses (Gao and Suga 1998, 2000). Therefore the effects of muscimol bilaterally applied to the surface of SI were studied on the BF shifts and nonspecific augmentation of 16 cortical neurons elicited by pseudoconditioning. With the muscimol application, all of these 16 neurons showed no BF shifts, but they did show nonspecific augmentation (Table 1, fourth row). There was no sign that cortical auditory neurons were influenced directly by muscimol applied to SI.

Figure 6C shows the receptive field of a cortical neuron tuned to 44.0 kHz (Fig. 6C1). When muscimol was bilaterally applied to SI prior to the pseudoconditioning, the neuron showed no BF shift, but it did show nonspecific augmentation (Fig. 6C2). The nonspecific augmentation was strong about 60 min after the onset of the pseudoconditioning (Fig. 6C3), but disappeared about 180 min after that (Fig. 6C4).

The effects of muscimol bilaterally applied to SI on the BF shift and nonspecific augmentation are further demonstrated with the PST histograms and frequency–response curves of a cortical neuron tuned to 23.0 kHz in Fig. 8. First, pseudoconditioning with a bilateral saline application to SI was delivered...
to the animal. Then, 30 min after that, both the 1.0-kHz BF shift and nonspecific augmentation were elicited (Fig. 8A2). By 90 min after the pseudoconditioning, the BF shift disappeared and the nonspecific augmentation became larger (Fig. 8A3). The augmentation disappeared about 210 min after the pseudoconditioning (Fig. 8A4). After recovery from this pseudoconditioning, muscimol was bilaterally applied to SI prior to the second pseudoconditioning and the same neuron was restudied. Its responses to tone bursts were augmented 30 min after the pseudoconditioning, but no BF shift was elicited because of the bilateral inactivation of SI (Fig. 8B2). Figure 8, C and D shows the changes in the response described earlier by the frequency–response curves. It was clear that muscimol applied to SI abolished the development of the BF shift, as did that elicited by auditory fear conditioning (Gao and Suga 2000), but did not abolish the nonspecific augmentation.

DISCUSSION

BF shift elicited by the pseudoconditioning

In our pseudoconditioning experiments, the USa was not delivered to the animal in the time period between 5 s prior to and after the CSa so as to avoid CSa–USa association. However, one may consider that weak CSa–USa association might occur and cause the small BF shift. This simple and straightforward explanation may make sense, but may not necessarily be correct because it has been repeatedly demonstrated that the small short-term BF shift is evoked without the CS–US association, as elucidated in the following text. 1) A long repetitive tone burst stimulation lasting 30 min evokes a small short-term BF shift (Gao and Suga 1998; Yan and Suga 1998). 2) A short repetitive tone burst stimulation lasting 1.0 s delivered every 30 s over 30 min (i.e., CS) evokes the subthreshold BF shift (Gao and Suga 1998), which is augmented by the NB stimulation. 3) Acoustic stimulation paired with electric stimulation of the NB evokes the BF shift (Bakin and Weinberger 1998; Kilgard and Merzenich 1998; Ma and Suga 2003; Puckett et al. 2007; Yan and Zhang 2005). 4) Electric stimulation of AI evokes the BF shift, which is augmented by electric stimulation of SI. This augmentation is blocked by a lesion of the NB (Ma and Suga 2001, 2003). 5) Electric stimulation of the ventral division of the medial geniculate body (Jafari et al. 2007; Wu and Yan 2007) or the central nucleus of the inferior colliculus (Zhang and Suga 2005) evokes the BF shift. Furthermore, it
has been known that a sensory stimulus excites a corresponding sensory cortex and, without CS–US association, increases the cortical ACh level through the prefrontal cortex and NB (Golmayo et al. 2003; Rasmusson et al. 2007; Zaborszky et al. 1999). That is, the US, USu, CS, or CSu alone can activate the NB through the prefrontal cortex in addition to other brain structures. Therefore a subthreshold BF shift evoked by the CSu can be changed into a small BF shift by the USu.

Interpretation of the effect of muscimol applied to SI on the BF shift

Inactivation of SI by muscimol blocks the development of the cortical and collicular BF shifts elicited by auditory fear conditioning without affecting their auditory responses and frequency tunings (Gao and Suga 2000). Electric stimulation of SI after, not before, electric stimulation of AI augments the small BF shift evoked by the AI stimulation (Ma and Suga 2001, 2003). Furthermore, our current experiment demonstrated that the pseudoconditioning elicits not only the nonspecific augmentation, but also the small short-lasting cortical BF shift and that bilateral SI inactivation abolishes the development of this BF shift without affecting the auditory responses and nonspecific augmentation of cortical auditory neurons.

These inactivation and activation experiments clearly indicate that SI plays an important role in evoking the conditioning-elicited and pseudoconditioning-elicited BF shifts. Weinberger (2004, 2007) stated that muscimol applied to SI abolished the conditioning-dependent cortical BF shift because of its spread from SI to AI. The data shown in Figs. 6C and 8, as well as the data presented by Gao and Suga (2000), clearly indicate that muscimol applied to SI did not directly affect cortical auditory neurons by spreading from SI to AI and selectively abolished the development of the BF shift.

Interpretation of the effect of atropine or mecamylamine applied to AI on the BF shift and nonspecific augmentation

Atropine applied to AI reduces the cortical response to a tone burst. Thus in the normal condition the response is slightly augmented by ACh (Ji et al. 2001) spontaneously released into AI by the NB (Rasmusson et al. 2007). Atropine or mecamylamine applied to AI immediately prior to the pseudoconditioning blocked the development of the cortical BF shift, but did not block the development of the cortical nonspecific augmentation. How can this observation be interpreted? A comparison of these current findings with our previous findings of the conditioning-elicited BF shift (Ji et al. 2001) gives us an important...
insight into the mechanism for nonspecific augmentation as described in the following text.

The cortical BF shift elicited by fear conditioning and the cortical nonspecific augmentation elicited by pseudoconditioning, respectively, develop to the plateau and the peak in the same time course (Fig. 9, curves 1 and 3). The BF shift maintains the plateau for many hours because of a cortical ACh level increased by the NB (Ji and Suga 2003; Ma and Suga 2005), whereas the nonspecific augmentation reduces without showing a plateau and disappears 150 min after the peak. The same time course in the development of these two types of cortical changes may suggest the same neuromodulator, ACh, for them. Then, why did the nonspecific augmentation not last a long time as does the cortical BF shift? Since the pseudoconditioning-elicited BF shift was small, the short-lasting nonspecific augmentation may arise from a much smaller increase in a cortical ACh level for the pseudoconditioning than for the conditioning. However, this interpretation does not hold because atropine applied to AI completely blocks the BF shift, but not the nonspecific augmentation (Fig. 9, curves 2b and 4). This dramatic difference in the atropine effect indicates that the nonspecific augmentation depends on noncholinergic neuromodulators.

However, we may consider alternative interpretations for precaution because ACh applied to AI increases the auditory responses of AI neurons (Ji et al. 2001) and because it has presumably been considered that a cortical ACh level increased by the ascending reticular activating system (ARAS) evokes the cortical nonspecific augmentation.

**Cortical nonspecific augmentation and the ARAS**

The ARAS consists of the dorsal and ventral ascending pathways starting from the pontomesencephalic reticular formation. In its dorsal pathway, the glutamatergic intralaminar and midline nuclei of the thalamus diffusely project to the cerebral cortex. In the ventral pathway, the histaminergic hypothalamus and the cholinergic NB broadly project to the cerebral cortex (Siegel 2002). The ARAS would be activated by CS1 and US1, and would evoke cortical general arousal, which depends on an increase in the cortical ACh level (Jones 2002). The cortical large BF shift also depends on an increase in the cortical ACh level (Bakin and Weinberger 1998; Chen and Yan 2007; Kilgard and Merzenich 1998; Ma and Suga 2003). The increase in the cortical ACh level by the pseudoconditioning is perhaps much smaller for the general arousal than for the conditioning-elicited large BF shift; otherwise, the small BF shift evoked by the pseudoconditioning would be the large long-term BF shift. In other words, if the nonspecific augmentation were the enhanced general arousal, the pseudoconditioning would elicit the large BF shift in addition to the nonspecific augmentation. Apparently, this is not the case. Therefore an increased ACh level by the ARAS (Magoun 1963) is likely not involved in evoking the nonspecific augmentation.

The auditory fear conditioning or tone-burst stimulation paired with electric stimulation of the NB increases the cortical ACh level. However, it does not elicit the nonspecific augmentation in addition to the large long-term BF shift. This lack of nonspecific augmentation may be interpreted in two different ways: 1) ACh is not involved in evoking the nonspecific augmentation and 2) ACh is involved in evoking the nonspecific augmentation, but it requires activation of the cortical projection from the multisensory thalamic nuclei. The first interpretation is correct because atropine did not block the nonspecific augmentation. It has been known that the NB contains more GABAergic neurons than cholinergic ones (Freud and Meskenaite 1992). The GABAergic NB neurons project to cortical GABAergic interneurons and could evoke widespread disinhibition (Sarter and Bruno 2002), so they are suited for evoking the nonspecific augmentation. However, their functional role in evoking cortical plasticity has not yet been explored.

The ARAS and the Suga model both include the multisensory thalamic nuclei, so that involvement of the ARAS in evoking the nonspecific augmentation cannot be completely excluded. The ARAS controls not only the cortical ACh level, but also the cortical histamine level (Jones 2005; Seigel 2002). It is unknown whether histamine is involved in evoking the nonspecific augmentation. The Suga model, assuming the involvement of noncholinergic neuromodulators, is thus far partially supported only by our current data. Further studies are clearly needed to test this model.

**Our current data and the Gao–Suga and Suga models**

The conditioning induces associative learning and the large long-lasting cortical BF shift. In the conditioning, both the CS and US are necessary for sensory association (Gao and Suga 1998, 2000; Weinberger 1998). On the other hand, the pseudoconditioning induces nonassociative learning and large nonspecific augmentation (Bakin et al. 1992; this study). In the pseudoconditioning, the randomized US1 is essential because of no sensory association.

As described in the introduction, our experiments were performed to test part of the Gao–Suga model (1998) proposed for the conditioning-elicited BF shift and the Suga model (2008) proposed for the pseudoconditioning-elicited nonspecific augmentation.

The Gao–Suga model states that the MGBv is involved in evoking a small or subthreshold cortical BF shift in AI, that this BF shift is augmented by the cholinergic neuromodulator, and that the SI, as well as AI plays a role in indirectly activating the cholinergic neuromodulator. Our current data
clearly indicate that both SI and the cholinergic neuromodulator are involved in evoking the BF shift regardless of whether it is elicited by the pseudoconditioning or conditioning. The Suga model states that the MGBm and PIN are involved in evoking a small cortical nonspecific augmentation, that this augmentation is further augmented by noncholinergic neuromodulators, and that SI is not involved in evoking the nonspecific augmentation. Our current data clearly indicate that SI is not involved in evoking the nonspecific augmentation and that noncholinergic neuromodulators are involved in evoking it. Electric stimulation of the NB paired with tone-burst stimulation elicits the large cortical BF shift, but not the cortical nonspecific augmentation (Bakin and Weinberger 1996; Kilgard and Merzenich 1998; Ma and Suga 2003; Puckett et al. 2007). This well-known finding also indicates that the cholinergic neuromodulator is not involved in evoking the nonspecific augmentation.

The important components in the Gao–Suga and Suga models are the thalamic nuclei, MGBv and MGBm. The MGBv shows the conditioning-elicited short-lasting BF shift (Edeline and Weinberger 1991), and electric stimulation of the MGBv evokes the cortical BF shift (Jafarei et al. 2007). Electric stimulation of the central nucleus of the inferior colliculus evokes the collicular BF shift via AI (Zhang and Suga 2005). Therefore it is certain that the MGBv is involved in evoking the cortical BF shift. The MGBm also shows the conditioning-elicited short-lasting BF shift (Edeline and Weinberger 1992) and has been speculated to be involved in evoking the cortical BF shift (Weinberger 2004, 2007). However, it has not yet been demonstrated whether electric stimulation of the MGBm evokes the cortical BF shift. In addition, there have been many studies indicating that the cortical BF shift is elicited without CS–US association in the MGBm (for a review see Suga and Ma 2003). Therefore it is not clear what kind of cortical plastic changes are evoked by the MGBm. It is unlikely that the MGBm evokes the cortical BF shift because of the following three reasons. 1) MGBm neurons project widely over cortical auditory areas, including AI, have a broad or multipeaked frequency-tuning curve, and rapidly habituate, different from MGBv neurons, which project specifically to AI, are sharply tuned in frequency, and do not habituate (Aitkin 1973; Bordi and Ledoux 1994a, b; Calford 1983). 2) Electric stimulation of AI facilitates MGBv neurons, but inhibits MGBm neurons (Yu et al. 2004). 3) The cholinergic brain stem nuclei project to the MGB (Levey et al. 1987). ACh depolarizes MGBv neurons involved in evoking the BF shift, whereas it hyperpolarizes MGBm neurons (Mooney et al. 2004).

The parameters characterizing the CSs and USs for the pseudoconditioning were exactly the same as those characterizing the CS and US for the auditory fear conditioning. The CSs and USs produced the cortical changes remarkably different from the CS–US, as shown herein. The only difference between them is that, unlike the US, the USs was randomly delivered to the animal. The CS–US elicits CS–US association and evokes an increase in a cortical ACh level (Weinberger et al. 2006). On the other hand, the CSs–USs excites the multisensory thalamic nuclei without CSs–USs association and perhaps evokes an increase in the cortical non-ACh level. Therefore the important key question is whether the brain has the multisensory thalamic nuclei that are more strongly excited by randomly delivered stimuli rather than regularly delivered ones and that project broadly to the cortex. The MGBm and PIN are the multisensory nuclei, broadly project to the cortex (Winer and Moster 1983), and habituate rapidly to acoustic stimuli (Aitkin 1973; Bordi and LeDoux 1994a,b; Calford 1983).

Suga (2008) hypothesized the following four neurophysiological events: 1) when the electric leg stimulation is randomized in time, MGBm/PIN neurons do not habituate and strongly augment cortical activity; 2) when the cortical BF shift is elicited by conditioning, MGBm neurons projecting to the auditory cortex are suppressed by descending signals from AI through the thalamic reticular nucleus; 3) when the nonspecific augmentation is augmented by noncholinergic neuromodulators, the BF shift is not augmented; and 4) when the BF shift is augmented by the cholinergic neuromodulator, the nonspecific augmentation is not augmented. An important mechanism included in the Suga model is activation of multisensory thalamic nuclei by randomized stimulation. Then, the cortical nonspecific augmentation must be elicited by randomized leg stimulation (USs) alone. This was apparently the case. The tone-specific (BF shift) and nonspecific (augmentation) plastic changes are mutually exclusive, so that when one is elicited, the other should not be elicited. The Suga model proposes a possible mechanism for eliciting either the tone-specific or nonspecific cortical plasticity.

ACKNOWLEDGMENTS
We thank S. E. Miller for editing the current manuscript.

GRANTS
This work was supported by National Institute on Deafness and Other Communication Disorders Grant DC-000175.

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