Multiparametric Changes in the Receptive Field of Cortical Auditory Neurons Induced by Thalamic Activation in the Mouse

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The functional organization of the sensory cortex is constructed to process sensory information based on experience and learning. Importantly, it is plastic so that it can quickly adapt to environmental changes. Because the thalamus gates all ascending information, it is critical to understand how the thalamocortical system contributes to the plasticity of the sensory cortex. We show here that the neuronal receptive field (RF) in the auditory cortex faithfully tends toward the RF of the electrically stimulated auditory thalamic neurons. We characterized the RF of auditory neurons by measuring the best frequency, minimum threshold, bandwidth, RF area, and averaged response magnitude. All these parameters of the cortical RF showed robust changes toward the values of the parameters of the stimulated thalamic neuron following focal thalamic stimulation. Our data suggest that the thalamocortical system possesses intrinsic mechanisms that underlie the input specificity of learning-induced or experience-dependent cortical plasticity.

Keywords: auditory cortex, auditory plasticity, auditory thalamus, mouse, receptive field

Introduction

The construction of the functional organization of the sensory cortex is primarily guided by information relayed by thalamocortical inputs (Read and others 2002; Hirsch 2003; Yan 2003). This is clearly illustrated by cross-modal plasticity; the redirection of visual inputs to the auditory thalamus in neonatal animals makes auditory cortical neurons respond to visual stimuli (Sharma and others 2000).

The functional contributions of thalamocortical projections, specifically to the response properties and receptive fields (RFs) of cortical neurons, have extensively been investigated in vivo and in vitro studies. (Kyrizzi and Simons 1993; Bazhenov and others 1998; Steriade and others 1998; Miller, Escabi, Read, and others 2001; Miller, Escabi, and Schreiner 2001; Suder and others 2002; Metherate and others 2005). We are now aware that the activation of thalamocortical inputs primarily produces excitatory postsynaptic potentiation that underlies thalamocortical excitation of cortical neurons. On the other hand, thalamocortical activation also produces inhibitory postsynaptic potentiation that may impact cortical integration (Gil and Amitai 1996; Bazhenov and others 1998; Cruikshank and others 2002; Nicolelis 2002; Hirsch 2003; Fuentealba and others 2004). These in vivo and in vitro studies have focused upon some fundamental issues related to thalamocortical synaptic transmission.

The functional organization and neuronal RFs of the sensory cortex are also characterized by a continuous adaptation to environmental changes or plasticity that is highly specific to sensory inputs or to newly acquired information (Weinberger 1998; Jones 2000; Kilgard and others 2002; Pantev and others 2003; Suga and Ma 2003). Studies of ocular dominance plasticity of the visual cortical neurons demonstrate that the RFs of cortical neurons are shaped through thalamocortical synaptic competition (Wiesel and Hubel 1963, 1965; Schmidt and others 1999). Similar findings are also observed in the auditory cortex where neonatal deprivation of 1 ear leads to enhanced auditory responses and reduced response thresholds of cortical neurons to the intact ear (Reale and others 1987). The rule of thalamocortical synaptic competition, however, seems inapplicable in this case because such changes in cortical responses are already seen in the auditory midbrain, a subthalamic nucleus in the central auditory system (Moore and others 1993). To date, it remains unclear how the thalamocortical system contributes to the input-specific plasticity. How is the cortical RF precisely shaped or altered by the thalamocortical system?

The central lemniscal auditory system provides a unique model to study the thalamocortical impact on cortical plasticity. Its tonotopic organizations, preserved from the cochlea, precisely represent a single stimulus parameter, that is, sound frequency. The major goal of this study was to explore the thalamocortical alteration of cortical RFs. The alteration of cortical RFs was analyzed under duress of the relationship between the thalamic and cortical RFs. We found that focal thalamic stimulation significantly changed the cortical RFs. These changes tended toward the RFs of the electrically stimulated thalamic neurons, which were quantified by a number of parameters.

Materials and Methods

Female C57 mice, 5–7 weeks old, with a body weight ranging from 19.1 to 21.6 g were used in this study. All experimental procedures were in accordance with the Animal Care Committee of the University of Calgary and the Canadian Council on Animal Care. Animals were anesthetized with a mixture of ketamine (110 mg/kg, intraperitoneal) and xylazine (20 mg/kg, intraperitoneal) during surgery and throughout the experiments. The anesthetic status was examined approximately every 30 min by pinching the animal’s tail with forceps. If the animal exhibited any response to tail pinching, additional dosages of ketamine (20 mg/kg) and xylazine (3.5 mg/kg) were administered to maintain anesthesia. During anesthesia, the mouse’s head was fixed in a custom-made head holder by rigidly clamping between the palate and nasal/frontal bones. The head holder was adjustable and designed to align bregma and lambda points of the skull in 1 horizontal plane. Once the mouse’s head was positioned, the scalp was incised at the midline to expose the left skull. A dental drill was used to make 2 openings in the skull. The openings were prepared for the placement of electrodes to the primary auditory cortex and the ventral division of the medial geniculate body of the thalamus (MGB). After surgery, the animal was placed in a soundproof chamber for electrophysiological experiments. The animal’s body temperature was maintained at ~37 °C by a feedback-controlled heating pad throughout surgery and electrophysiological experiments.
Acoustic Stimulation

To evoke neural responses of auditory neurons, a 60-ms-long pure tone with 5-ms rise/decay times was used as acoustic stimuli. Tone bursts were digitally synthesized by SigGen software and played back by BrainWare software (Tucker-Davis Tech., Gainesville, FL). Digital sinusoidal waves were converted into 20-V peak-to-peak analog signals through a digital-analog converter (DA3) and an anti-aliasing filter (FT6). The signals were then fed to a tweeter via a digital attenuator (PA5) and power amplifier. The tweeter was placed 45 degrees to the right of and 90 cm away from the mouse’s right ear. During calibration, the tweeter was driven by sinusoidal bursts of peak-to-peak 20 V without attenuation. The output amplitude of the tone burst ranged from 1 to 70 kHz and was expressed as decibel sound pressure level (SPL) with 1 decimal accuracy (re. 20 μPa). It was calibrated at the position of the animal’s right ear with a Larson-Davis condenser microphone (Model 2520) and a microphone preamplifier (Model 2200C). Frequencies and intensities of tone bursts were varied either manually or automatically. The excitatory responses of auditory neurons to acoustic stimuli were measured with computer-controlled frequency/amplitude scanning (frequency scan plus amplitude scan, FA-scan). The excitatory areas of RFs and frequency/threshold tuning curves were derived from the FA-scan data. An FA-scan varied from 3 to 40 kHz in frequency with increments of 1 kHz and from -20 to -80 dB with increments of 5 dB. Therefore, 1 FA-scan consisted of 495 frequency/amplitude blocks. The last block without tone presentation was designed for spontaneous spike counting. The interval between stimuli was 500 ms. The tone frequency and amplitude in an FA-scan were randomly arranged by the BrainWare software. The identical stimulus was repeated five times for each frequency/amplitude set.

Recording and Electrical Stimulation in the MGB

The stereotaxic coordinates of the MGB are 3.1 mm posterior to the bregma, 1.8 mm lateral (left) to the midline, and -3 mm below the brain surface (Fig. 1A; Franklin and Paxinos 1996). A tungsten electrode of ~2-MΩ impedance was perpendicularly placed to the MGB with the use of the above stereotaxic coordinates. The electrode was initially connected to the preamplifier of the data acquisition system (see details below). A pure tone with manual alteration of frequencies and amplitudes was continuously delivered once per second during electrode penetration. This process facilitated the search for neurons that responded to tone stimulus. Sharply tuned neurons were frequently located at about 3 mm below the brain surface. Once a neuron was found to respond to tone stimulus, the neuronal responses to the FA-scan were recorded. If the neuron was sharply tuned to a particular frequency in addition to demonstrating an overt excitatory response area, the electrode tip was most likely in the ventral division of the MGB. The position of the electrode was maintained for the remainder of the experiment. The electrode was later disconnected from the recording system and reconnected to the output of a constant current isolator in the electrical stimulation system. This procedure ensured that the locations of the recording and stimulating sites were identical.

Electrical stimulation consisted of single electric pulses (0.2 ms-long, monophasic, and constant current square wave) or a train of electric pulses (0.2 ms-long, monophasic, and constant current square wave, 120 Hz, and 200-ms train duration). Electrical pulses were generated by a Grass S88 stimulator (Astro-Medical, Inc., West Warwick, RI) and an A360 constant-current isolator (WPI Inc., Sarasota, FL). The output current of the A360 was manually set within a range of 100-1000 nA.

Cortical Recording and Data Acquisition

Neuronal activities of the primary auditory cortex were recorded with 2 tungsten electrodes of ~2-MΩ impedance inserted ~300 μm below the surface of the cortical epithelium. Bioelectrical signals obtained from the 2 electrodes were led to a 4-channel DB4/HS4 amplifier with a bandpass of 0.3–10 kHz (Tucker-Davis Tech.). After a 10 000-time amplification, the bioelectrical signals from 2 channels were fed to an oscilloscope for observation and to the BrainWare data acquisition system for data collection. Single-unit action potentials from both channels were isolated based on 8 parameters of the action potential waveform, including peak, valley, spike height, spike width, peak time, valley time, and 2 selected voltage values (Yan and others 2002, 2003, 2005).

Anatomic Localization of the Stimulus Site

Upon completion of the physiological experiments, a 30-s-long and 1-mA electrical current was applied to the site of electrical stimulation in the MGB. The animal was then given a cardiac perfusion with 10% formalin under deep anesthesia. The brain was sectioned, and the electrolytic lesion point was examined under microscope in all cases (Fig. 1A).

Data Processing

Single-unit responses to the FA-scans were eventually displayed by dot-rasters, peristimulus time (PST) histograms, or PST cumulative

Figure 1. (A) Microscopic photograph of the coronal section of the left brain at 3.1 mm posterior to the bregma with a superimposed schematic drawing of the coronal section of the mouse brain at 3.16 mm posterior to the bregma. A lesion site can be seen at the MGBv as indicated by a black arrow. ml, medial lemniscus; Hipp, hippocampus; SN, substantia nigra. (B) The percentage of the shifts in cortical BFs evoked by the ESMGB as the function of the current level for the ESMGB. A remarkable shift in cortical BFs was observed when the current was 700 nA or larger.

When the action potential recordings were stable, the neuronal responses to the FA-scans were recorded and saved as control responses. The negative current of signal or train electrical pulses was then delivered to the MGB for the microelectrical stimulation of the site where the neuronal RF was known (1/s for 6 min). After 6 min of stimulation, the responses of cortical neurons to the FA-scan were again immediately recorded and at 30-min intervals until a recovery rate of at least 50% in the best frequency (BF), minimum threshold (MT), and/or auditory response was obtained.
histograms with a bin width of 1 ms. The RF, that is, the excitatory frequency-tuning curve, was derived from response thresholds across frequencies based on the FA-scan data. The response threshold to each frequency was the boundary between 2 neighboring amplitudes in which one showed more than 1 spike and the other showed no response per 5 stimuli. If spontaneous activity was seen to have interfered with spike counting, the threshold was determined by at least 1 spike difference between neighboring amplitudes, depending on the spontaneous rate (Suga and others 1997; Yan and Zhang 2003; Yan and others 2005). A comprehensive evaluation of the RF was developed by examining the following 5 parameters obtained from the FA-scan data.

1. BF: the frequency to which a neuron showed the lowest response threshold to tone stimuli.
2. MT: the lowest response threshold to tone stimuli across all frequencies.
3. Bandwidths of 10 and 30 dB above the MT (BW10 and BW30, respectively): the frequency (kHz) ranges at 10 and 30 dB above the MT that showed excitatory responses to tone stimuli.
4. RF area: 1) The entire RF area represented the number of frequency/amplitude sets below 50-dB SPL to which a neuron showed excitatory responses. 2) The overlayered RF area indicated the cortical RF area that overlapped the thalamic RF area. 3) The nonoverlayered RF area represented the cortical RF area that did not overlap the thalamic RF area. The size of the RF areas was defined in units of kilohertz × 5 dB.
5. Average spike number (ASN) including ASNs of entire, overlapped, and nonoverlapped RF areas: ASNs were the quotient of the sum of spike numbers at the frequency/amplitude sets in entire, overlapped, or nonoverlapped RF areas divided by the size of entire, overlapped, or nonoverlayered RF areas. Spike numbers of each frequency/amplitude set were counted based on the spikes within a poststimulus time window of 0-60 ms that were evoked by 5 identical stimuli.

The values of these parameters of the cortical RF were compared before and after electrical stimulation of the MGB. The BF shifts have been expressed in a linear kilohertz scale because the amount of a BF shift does not increase with the BF of stimulated neurons in an octave relationship but rather it is constant over a wide range of the stimulated BFs (Sakai and Suga 2001; Yan and Ehret 2002; Zhang and Suga 2005). The changes in the values of RF parameters of single cortical neurons were analyzed according to the differences in these parameters between isolated single units of the recorded cortical neurons and unisolated multiple units of the stimulated thalamic neurons.

Data were generally expressed by a mean ± standard deviation. A 2-test was used to compare the differences between 2 groups of data. In all statistical evaluations, \(P < 0.05\) was considered as the criterion for statistical significance.

Results

To determine the exact contribution of thalamocortical inputs to the cortical RF, it is essential to minimize the electrical stimulation of thalamic neurons (ESMGB). We first determined the level of current that could effectively induce changes in cortical BFs. We intentionally selected cortical neurons with BFs that were 3–6 kHz different from the BFs of thalamic neurons. Ten neurons were examined for each current level. ESMGB-evoked cortical BF shifts of individual samples could vary from 17% to 100%. Figure 1A shows the average percentage changes in cortical BFs as the function of the electrical current used for ESMGB. The changes in BFs were <10% when the electrical current was smaller than 600 nA. A steep slope was found between 600 and 700 nA when a single electrical pulse was delivered at a rate of 1/s for 6 min. When the current varied from 700 to 1000 nA, the increase in BF shifts became limited. However, 500 nA could be effective if a train of pulses were delivered. This suggested that a 700-nA single electrical pulse stimulation or a 500-nA train stimulation was appropriate stimulation for inducing cortical plasticity with minimal activation of thalamic neurons. In order to make the electrical stimulation comparable with that used in our previous studies, a 700-nA single electrical pulse was selected for this study.

In order to study the thalamic contribution to the cortical RF plasticity, we first recorded the thalamic RF using a FA-scan with an ~2-MΩ tungsten electrode. Once the response data of the thalamic RF were saved on computer, we maintained the electrode’s position but changed its role to that of a stimulating electrode. This provided very exacting RF data on the stimulated neurons. The effects of the ESMGB on cortical RFs were studied in 33 cortical neurons from 15 mice. Cortical and thalamic neurons showed variation in their RF shapes but typically had sharp tuning and displayed clear BFs and MTs. Their BFs ranged from 10 to 23 kHz (17.6 ± 3.5 kHz), and their MTs ranged from 9.6–to 34.3-dB SPL (22.5 ± 6.11–dB SPL); these values fell within the central range of mouse hearing (Zhang and others 2005). The cortical and thalamic RFs always overlapped each other in varying degrees. ESMGB altered the shape of cortical RFs in all cases. As illustrated in Figure 2, the multiunit RF of the thalamic neurons at the stimulated site was smaller in area. These neurons had a BF of 17 kHz with an MT of 23.8–dB SPL (Fig. 2A). A single-unit RF of the recorded cortical neuron was larger in area, and this neuron tuned to 22 kHz with an MT of 11.0–dB SPL (Fig. 2B). After ESMGB with 700 nA at a rate of 1/s for 6 min, the RF area of this cortical neuron was largely reduced; its BF was shifted to 17 kHz, and its MT was elevated by 12 dB (Fig. 2C). This neuron exemplified the effectiveness of thalamic stimulation in inducing RF plasticity of cortical neurons.

For a comprehensive evaluation of the changes in cortical RFs and the correlation of these changes to thalamocortical inputs, we characterized the RFs with BF, MT, bandwidth, RF area, and ASN. We then used the differences in these parameters between cortical and thalamic RFs as references to analyze the change in cortical RF.

Centripetal Shift in the Cortical BF After ESMGB

The BF shift-difference functions obtained in bats (Chowdhury and Suga 2000), gerbils (Sakai and Suga 2002), and mice (Yan and Ehret 2001, 2002; Yan and others 2005) indicate that the cortical BF does not change if the difference in BFs between the recorded collicular or cortical and stimulated cortical neurons is large and beyond a certain range. In the mouse, cortical electrical stimulation evokes collicular plasticity when the BF difference between them is <10 kHz (Yan and Ehret 2002). To demonstrate that thalamic stimulation evokes cortical plastic changes, we mainly focused on the cortical neurons whose BFs were within 10 kHz of those of stimulated thalamic neurons.

ESMGB either increased or decreased cortical BFs. The changes in the cortical BFs were highly specific to the BFs of stimulated thalamic neurons. As illustrated in Figure 3, following the ESMGB with a BF of 17 kHz, a 13-kHz-tuned cortical neuron shifted its BF up to 16 kHz (Fig. 3Aa). On the other hand, following the ESMGB with a BF of 20 kHz, a 25-kHz-tuned cortical neuron shifted its BF down to 20 kHz (Fig. 3Ba). The shifts in cortical BFs appeared to result from the differential changes in response thresholds; the response thresholds increased at the control BF, and the response threshold decreased at the shifted
BF (Fig. 3Aa). Such frequency-dependent changes in response thresholds could also be reflected by the increase and decrease in response magnitude. As illustrated by the changes in tone-evoked unit firing at 10 dB above the MT, the response magnitude was decreased at the control BF and increased at the shifted BF (Fig. 3Ab, c and Bb, c). However, the response thresholds at both control and shifted BFs could be increased (Fig. 2Bc) or decreased depending on the thalamic MTs as discussed below. In these cases, the BF shifts should theoretically be dependent on the differential degrees of threshold changes at the control and shifted BFs.

Analysis of all samples indicated that the cortical BF changes were systematically associated to the difference in the BFs between the stimulated thalamic neurons and the recorded cortical neurons. When thalamic BFs were higher than cortical BFs, ES_MGB shifted cortical BFs upward (Aa) or downward (Ba) depending on the BFs of the stimulated thalamic neurons. Accordingly, the auditory responses were decreased at the control BFs (Ab and Bb) and increased at the shifted BFs (Ac and Bc) following the ES_MGB. The shift in cortical BF was linearly correlated to the difference in BFs between thalamic and cortical neurons (filled circles in C). ES_MGB rarely shifted the cortical BFs when the difference between cortical and thalamic BFs was >10 kHz (open circles in C). In Ab, c and Bb, c, “1” represents control, “2” represents post-ES_MGB, and “3” represents recovery. The arrows in (Aa) and (Ba) indicate the BFs of the stimulated thalamic neurons.

Figure 2. The multiunit RF of the stimulated thalamic neurons (A) and the single-unit RFs of the recorded cortical neuron before (B) and after (C) ES_MGB. The thalamic RF shows a lower BF, higher MT, shorter bandwidth, smaller size, and lower response magnitude when compared with the pre-ES_MGB cortical RF. The ES_MGB changed the cortical RF toward the thalamic RF. The contour line represents 2-spike increments.

Figure 3. Effects of the ES_MGB on the BFs of cortical neurons. ES_MGB shifted the BFs of cortical neurons upward (Aa) or downward (Ba) depending on the BFs of the stimulated thalamic neurons. Accordingly, the auditory responses were decreased at the control BFs (Ab and Bb) and increased at the shifted BFs (Ac and Bc) following the ES_MGB. The shift in cortical BF was linearly correlated to the difference in BFs between thalamic and cortical neurons (filled circles in C). ES_MGB rarely shifted the cortical BFs when the difference between cortical and thalamic BFs was >10 kHz (open circles in C). In Ab, c and Bb, c, “1” represents control, “2” represents post-ES_MGB, and “3” represents recovery. The arrows in (Aa) and (Ba) indicate the BFs of the stimulated thalamic neurons.
When the difference in BFs between cortical and thalamic neurons was larger than 10 kHz, cortical BFs showed a little change after the ESMGB (open circles in Fig. 3C).

**Centripetal Change in the Cortical MT After ESMGB**

The cortical MT was $22.70 \pm 5.86$-dB SPL before the ESMGB and $22.96 \pm 6.36$-dB SPL after the ESMGB. There was no statistical significance between them ($P > 0.05$). However, the ESMGB could markedly increase or decrease the cortical MTs with respect to each individual neuron when the difference between cortical and thalamic BFs were <10 kHz (Fig. 2B,C, filled circles in Fig. 3B). The increase or decrease in the cortical MTs appeared to be independent of the relationship of the frequency tunings between cortical and thalamic neurons. No matter whether the thalamic BFs were higher or lower than cortical BFs, ESMGB could result in either an increase or a decrease in the cortical MT (Fig. 4A). The changes in cortical MTs were only correlated to the MT difference between thalamic and cortical neurons ($r = 0.62, P < 0.001$). However, for those neurons in which BFs were larger than 10 kHz from the BFs of thalamic neurons, the MT changes evoked by ESMGB were different (open circles in Fig. 4B).

**Centripetal Change in the Cortical Bandwidth After ESMGB**

Because changes in both the BFs and MTs potentially affected the level-dependent frequency selectivity of auditory neurons, we analyzed the changes in bandwidths at 10 and 30 dB above the MT after the ESMGB. The BW10 and BW30 could be narrowed, broadened, or unchanged following the ESMGB; the changes ranged from $-6$ to 5 kHz for the BW10 and from $-5$ to 9 kHz for the BW30. Because the changes occurred in both directions, the BW10 ($11.12 \pm 2.90$ kHz) and the BW30 ($11.36 \pm 1.77$ kHz) before the ESMGB were not significantly different from the BW10 ($15.30 \pm 3.49$ kHz) and the BW30 ($16.36 \pm 2.68$ kHz) after the ESMGB (both $P > 0.05$). The significance of the changes in cortical BW10 and BW30 is described in Figure 5. When the thalamic BW10 was wider than the cortical BW10, the ESMGB broadened the cortical BW10. On the other hand, when the thalamic BW10 was narrower than the cortical BW10, the ESMGB narrowed the cortical BW10. The larger the difference in the BW10 between thalamic and cortical neurons, the larger the changes in the cortical BW10 (filled circles in Fig. 5A). The change in the cortical BW10 was linearly correlated to the difference in the BW10 between thalamic and cortical neurons ($r = 0.84, P < 0.001$). The same linear correlation was also found for the BW30 (filled circles in Fig. 5B, $r = 0.81, P < 0.001$). When the BFs of cortical neurons were 10 kHz or greater than the BFs of thalamic neurons, the cortical BW10 and BW30 were little affected by ESMGB (open circles in Fig. 5).

Figure 4. The changes in cortical MTs after the ESMGB were plotted as the function of the difference in BFs (A) and MTs (B) between thalamic and cortical neurons. The changes in MTs were only correlated to the MT difference between thalamic and cortical neurons (filled circles). Such correlation did not apply to the changes in MTs of those cortical neurons that differed by >10-kHz BF from that of the thalamic neurons (open circles).

Figure 5. Changes of cortical bandwidth following the ESMGB. The BW10s (A)/BW30s (B) were systematically correlated to the difference in BW10s/BW30s between thalamic and cortical neurons (filled circles). For the cortical neurons with >10-kHz difference from thalamic neurons (open circles), the ESMGB did not significantly change their bandwidths.
Centripetal Change in the Cortical RF Size After ESMGB

The ESMGB could lead to an increase or decrease in the size of the entire cortical RF area as well as have no impact upon it at all. In general, the size of the entire cortical RF area was slightly increased by the ESMGB, but the increase was statistically insignificant ($P > 0.05$). We also measured the size of the cortical RF area that overlapped the thalamic RF as well as the area that did not overlap the thalamic RF. On average, an insignificant increase was seen in the overlapped RF size ($P > 0.05$) but not in the nonoverlapped RF size. It appeared that the increase in the cortical RF size most likely resulted from the increase in the overlapped RF size following the ESMGB (Fig. 6A). Because the changes in the sizes of entire, overlapped, and nonoverlapped RF areas were similar following ESMGB, we have limited ourselves to an analysis of the entire RF area in the discussion below.

Because the RF size is directly related to the BF, MT, and bandwidth, we examined the relationship between the changes in cortical RF size and the difference in these parameters between thalamic and cortical neurons. The regression analysis showed that the changes in the cortical RF size was not correlated to the difference in BF ($r = 0.23$, $P > 0.05$) or MT ($r = 0.05$, $P > 0.05$) between thalamic and cortical neurons (filled circles in Fig. 6B,C), whereas it was significantly correlated to the difference in BW $10$ ($r = 0.38$, $P < 0.05$) or BW $30$ ($r = 0.46$, $P < 0.005$) between thalamic and cortical neurons (filled circles in Fig. 6D,E). Importantly, the changes in cortical RF size showed a high correlation to the difference in the RF size between thalamic and cortical neurons ($r = 0.66$, $P < 0.001$). When the thalamic RF size was larger than the cortical RF size, ESMGB enlarged the cortical RF size; otherwise, it reduced the cortical RF size. However, when the thalamic and cortical RF sizes were similar, the ESMGB did not clearly change the cortical RF size (filled circles in Fig. 6F). This correlation was not seen in the cortical neurons whose BFs were $> 10$ kHz from the BFs of thalamic neurons (open circles in Fig. 6B–F).

Centripetal Change in the ASN within the Cortical RF Area After ESMGB

The neuronal response magnitude was expressed by the ASN. The change in the cortical ASN calculated from the entire RF area following the ESMGB was similar to the changes in other RF parameters as described above. When the thalamic ASN was smaller than the cortical ASN, ESMGB decreased the cortical ASN, and when the thalamic ASN was larger than the cortical ASN, ESMGB increased the cortical ASN. There was statistical significance between them (Fig. 7A). The larger the difference in the ASN between thalamic and cortical neurons, the larger the changes in the cortical ASN (Fig. 7B). The changes in the cortical ASN were linearly correlated to the difference in the ASN between thalamic and cortical neurons ($r = 0.84$, $P < 0.001$). We further found that the changes in the ASN calculated from the overlapped and nonoverlapped RF areas showed similar correlations between the ASN changes and the RF difference between thalamic and cortical neurons (Fig. 7C). Namely, the changes in ASNs of both overlapped and nonoverlapped RF areas were dependent on the relationship of the ASNs between thalamic and cortical neurons. The ASNs of both overlapped and nonoverlapped RF areas increased when the thalamic ASN was higher; otherwise, they decreased. There were significant correlations between them ($r = 0.55$, $P < 0.001$ for the ASN of the overlapped area and $r = 0.51$, $P < 0.001$ for the ASN of the nonoverlapped area). Because the changes in the ASNs of both overlapped and nonoverlapped RF areas were similar to the changes in the ASN of entire RF area, we have limited ourselves to an analysis of the ASN of the entire RF area in the discussion below. Again, there was no correlation between the changes in cortical ASNs and the difference of cortical and thalamic ASNs in neurons in which BFs deviated from the thalamic BFs by $> 10$ kHz.

Discussion

Determination of an Appropriate Level of Current for Electrical Stimulation

Electrical stimulation is often used for exploring the functions of the central nervous system. Determining an appropriate level
Current levels for cortical stimulation may also vary at times. In the big brown bat, electrical stimulation of cortical neurons with either a 100-nA current (Yan and Suga 1998) or a 5- to 50-μA current (Jen and Zhou 2003) can induce frequency-dependent changes in frequency tuning and the response magnitude of auditory midbrain neurons. However, the practice of using increasingly larger currents may mask some changes because neurons tuned to neighboring frequencies may also be stimulated. For instance, when the auditory cortex in the cat is stimulated with a current from 100 to 500 μA, the frequency-dependent changes in the response magnitude of thalamic neurons are reported but the frequency-dependent changes in thalamic frequency tunings are not (He 1997).

In our experiment involving the ESMGB, we first measured the shift in cortical BF s as the function of a current level that ranged from 100 to 1 μA. We showed that the ESMGB with a current ≤600 nA was ineffective. A current level higher than 600 nA was required. We demonstrated that 700 nA was the smallest but most effective current for analyzing the thalamocortical contribution to cortical plasticity (Fig. 1B). The effectiveness with 700-nA stimulation should be associated with both the degree of neuronal activation and the number of activated ventrally geniculate body of the thalamus (MGBv) neurons. For any type of stimulation, the degree of neuronal activation and the number of activated neurons should be synchronously increased or decreased as the function of stimulus intensity in a certain range. According to the review by Ranck (1975), the affecting area of a 700-nA current may be <50 μm. Major neurons in the MGBv have a body size of 6–10 μm. The density of MGBv tufted neurons that send projections to the auditory cortex is about 2–3 neurons/100 μm. The interneuron are very rare, probably about 1 neuron/100 μm (Winer and others 1999). If the mouse MGBv is presumed to be comparable with the rat MGBv, the total number of neurons that would be stimulated with the 700-nA pulse should be ~20 and the number of thalamocortical neurons should be <17 within a radius of <50 μm. However, the exact number of thalamic neurons directly stimulated by 700-nA electric pulses remains to be measured.

We occasionally isolated up to 5 units with a cluster cutting method and found that their BFs were almost the same within 1 kHz. The measurement of the RF of stimulated thalamic neurons was based upon the largest action potentials in multunit recordings, so that these neurons were perhaps most strongly stimulated by 700-nA electric pulses. We examined the changes in the RF of a single cortical neuron referring to the RF of these thalamic neurons. The thalamic RF measured in our study was much sharper than that of local evoked potential (Norena and Eggermont 2002).

The impact of anesthesia on neuronal activity and plasticity is frequently addressed. Auditory cortical plasticity can be readily evoked, even in the anesthetized animal. As we know, the electrical stimulation of the cholinergic basal forebrain paired with a tone shifts cortical frequency tuning toward even to the frequency of the paired tone. It has been documented that the tuning shifts of cortical neurons evoked by basal forebrain stimulation paired with a tone were similar in both anesthetized and awake animals (Bakin and Weinberger 1996; Bjordahl and others 1998). In the auditory cortex of the rat, the characteristics of cortical frequency tuning and cortical plasticity evoked by intracortical microstimulation under ketamine anesthesia are similar to those without anesthesia (Maldonado and Gerstein 1996; Talwar and Gerstein 2001). These data suggest that the
frequency-tuning shifts observed in the present study were insignificantly affected by ketamine.

**Multiparametric Specificity of Thalamocortical Adjustment**

Unlike most previous studies that examine cortical plasticity with 1 or 2 RF parameters, we examined a number of RF parameters that are essential for characterizing RF. Our data show that the focal stimulation of the auditory thalamus leads to centripetal changes in the cortical RF. In summary, the focal thalamic activation shifted the cortical BFs toward the thalamic BFs (Fig. 3C) and shifted the cortical MTs toward the thalamic MTs (Fig. 4B). It shortened or lengthened bandwidths of cortical frequency tuning toward the bandwidths of thalamic frequency tuning (Fig. 5), decreased or increased the size of the cortical RF area toward the size of the thalamic RF area (Fig. 6F), and, finally, increased or decreased the average response magnitude of cortical neurons toward that of thalamic neurons (Fig. 7B). These data strongly suggest that the cortical RF can faithfully adapt to the emphasized thalamic RF. However, the thalamocortical modulation appears to be limited to a 10-kHz difference between the stimulated thalamic neurons and the recorded cortical neurons (open circles in Figs 3-6). This 10-kHz range in thalamocortical modulation shares some similarity with our previous findings in corticofugal modulation (Jones 1985; Steriade and Llinas 1993; Castro-Alamancos 2004). Our data demonstrated that focal thalamic stimulation did not specifically alter the overlapped RF size and ASN in the overlapped RF area (Figs 6A and 7). This suggests that the plastic changes evoked by thalamic stimulation cannot be simply due to direct thalamocortical projections to the recorded cortical neurons. In other words, the neural mechanisms underlying cortical plasticity is perhaps not as simple as once thought. Our previous study suggests that the electrical stimulation of the cholinergic basal forebrain paired with a tone leads to 2 types of changes in cortical RF: one is a frequency-specific threshold decrease and the other is a frequency-specific tuning shift (Yan and Zhang 2005). We propose that the direct thalamocortical connection is responsible for the frequency-specific threshold decrease, whereas the intracortical interaction and corticofugal modulation must somehow influence the frequency-tuning shift. Considering the nature of the tonotopic organization of thalamocortical projections (Redies and others 1998), focal stimulation of particular thalamic neurons must more strongly facilitate, in addition to the recorded cortical neurons, the cortical neurons that tune to the same frequency of stimulated thalamic neurons. These neurons may further alter the RF of the recorded cortical neurons through intracortical interaction and corticothalamic modulation (Steriade and others 1998; Zhang and Suga 2000; Sakai and Suga 2001; Fox and others 2002; Suga and Ma 2003; Yan 2003; Metherate and others 2005; Winer and others 2005; Yan and Zhang 2005). Therefore, the neural mechanisms for the plasticity of cortical RFs evoked by thalamic activation appears to involve a delicate collaboration: the modifications of thalamocortical synapses to the target cortical neurons, the thalamocortical synapses to neighboring cortical neurons incorporated with intracortical lateral excitatory/inhibitory projections, and the thalamocortical synapses to neighboring cortical neurons incorporated with corticothalamic projections. In other words, the centripetal plasticity of cortical RF is the result of a delicate balance in thalamocortical, intracortical, and corticofugal circuitry rather than simple thalamocortical competition.

Our current data indicate that the thalamocortical projection can decrease or increase cortical MTs. Our previous data also indicate that focal cortical stimulation can decrease or increase the collicular MTs depending on the relationship of MTs between cortical and collicular neurons (Yan and Ehret 2002). However, electrical stimulation of the basal forebrain paired with a tone can decrease cortical threshold when tone amplitude is lower than cortical threshold, whereas it does not occur randomly but is guided by sensory inputs (Singer 1995; Ehret 1997; Buonomano and Merzenich 1998; Fox and others 2000; Kilgard and others 2002; Rauschecker and others 2002; Schnupp and Kacelnik 2002; Suga and others 2002; Yan 2003; Polley and others 2004). In other words, the central sensory system modifies its functional organization by adapting to emphasized information in the environment. For example, a young rat’s exposure to a particular sound augments the cortical representation of that sound (Zhang and others 2001). Auditory learning or training can enhance the cortical coding of the frequency and amplitude of the learned or experienced sound (Bakin and Weinberger 1990; Recanzone and others 1993; Gao and Suga 2000; Polley and others 2004). The frequency tuning of a cortical neuron can even be shifted toward a sound frequency that is simply repetitively presented (Yan and Suga 1998). Conversely, cortical tuning shifts away from weakened or eliminated peripheral inputs (Wiesel and Hubel 1963, 1965; Reale and others 1987; Harrison and others 1991; Moore and others 1993). Therefore, neural plasticity in the cerebral cortex appears to be dependent on the occurrence frequency and the biological significance of specific environmental information as well as on the health of the peripheral sensory system. Because the thalamus gates all bottom-up information to the cortex (Jones 1985; Steriade and Llinas 1988; Steriade 1993; Castro-Alamancos 2004), our results present new insights into the neural mechanisms that underlie input-specific cortical plasticity induced by learning or experience.
not increase cortical threshold when tone amplitude is higher than cortical threshold (Yan and Zhang 2005). It would be very interesting to explore how thalamocortical function is implemented in entire cortical and corticosubcortical circuitry during learning or experience.

**Thalamocortical Contribution to the Cortical RF**

Another important information derived from our data is that the cortical RF may be constructed from a large range of thalamocortical inputs. We observed that focal stimulation of thalamic neurons with 700 nA always induced detectable changes in values of cortical RF parameters that adapted to the values of thalamic RF parameters. This occurred as long as the values of the parameters between thalamic and cortical neurons were different. This implies that the cortical RF is constructed by the convergent integration of a large range of thalamocortical inputs. This implication is supported by other studies that involved different approaches (Miller, Escabi, and Schreiner 2001; Miller, Escabi, and Shapley 2002; Fuentealba and others 2004) and by the anatomical findings that single neurons in the auditory cortex could receive inputs from a large number of thalamic neurons (Andersen and others 1980; de Venecia and McMullen 1994; Huang and Winer 2000). Using the premise that the RF of individual cortical neuron is the product of thalamocortical competition (Wiesel and Hubel 1963, 1965; Singer 1995), our data also suggest that the cortical RF is in a state of dynamic stability. If any factor disturbs or alters this state, the cortical RF changes and adjusts to a new state; the cortical RF tends toward the thalamocortical inputs that become relatively stronger.

Excitation evoked by thalamic stimulation would spread beyond the primary auditory cortex via synapses. Anatomical and physiological studies strongly suggest that the cortical lateral projections and corticofugal projections are crucial for auditory information processing and plasticity (Suga and Ma 2003; Metherate and others 2005; Winer and others 2005; Yan and Zhang 2005). Therefore, the cortical plasticity reported in this paper may be elicited not only by thalamocortical neurons but also by corticofugal and corticocortical feedback. Zhang and Suga (2005) recently found that electrical stimulation of the inferior colliculus evokes changes in the RF of nearby collicular neurons, and these changes are not evoked when the auditory cortex is inactivated. In other words, the corticocollicular feedback plays an essential role in evoking the collicular plasticity. Therefore, it is quite possible that thalamic stimulation in our experiments evoked not only cortical but also thalamic and collicular RF changes through the corticofugal system that, in turn, augmented cortical plasticity.

**Notes**

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**References**


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