Physiological Correspondence Dictates Cortical Long-Term Potentiation and Depression by Thalamic Induction

Xiuping Liu, Carol Wang, Christopher Pan and Jun Yan

Department of Physiology and Pharmacology, Hotchkiss Brain Institute, University of Calgary Faculty of Medicine, Calgary, Alberta, Canada

Address correspondence to Jun Yan, Email: juyan@ucalgary.ca

The auditory cortex exhibits frequency-specific plasticity over a life cycle. Although thalamocortical long-term potentiation (LTP) and depression (LTD) are components of a widely held model underlying the receptive field (RF) plasticity of cortical neurons, the model lacks direct supporting evidence. We show here that conventional high-frequency tetanic stimulation (TS) of the auditory thalamus induced long-term changes in cortical field excitatory postsynaptic potentials, including both LTP and LTD, in mice. Thalamic TS induced LTP when the stimulated thalamic and recorded cortical neurons were tuned to the same frequency and induced LTD when they were tuned to different frequencies. The thalamocortical LTP was N-methyl-D-aspartate-dependent, but the LTD also involved cortical γ-aminobutyric acidergic inhibition. Notably, the frequency-specificity of cortical LTP/LTD was in accordance with the frequency-specific plasticity of spike-based RFs of cortical neurons. Our results suggest that cortical LTP and LTD induced by thalamic induction can be a consequence of identical stimuli, occur in an input-specific manner, and account for frequency-specific remodeling of RFs of auditory cortical neurons.

Introduction

Receptive fields (RFs) of sensory cortical neurons are primarily assembled on thalamocortical information (Kyriazi and Simons 1993; Miller et al. 2001; Lomber and Eggermont 2002; Hirsch 2003; Metherate et al. 2005; Barkat et al. 2011; Hackett et al. 2011), they are refined during early development and continuously remodeled in adulthood (Zhang et al. 2001; Dorm et al. 2010; Shideler and Yan 2010; Sun et al. 2010; Blundon et al. 2011). Since the discovery of the thalamocortical role in cortical plasticity and the long-term remodeling of excitatory synapses >40 years ago (Wiesel and Hubel 1963; Lømo 1966; Bliss and Lømo 1973), thalamocortical long-term potentiation (LTP) and depression (LTD) have been a prevalent model for the interpretation of learning-induced and experience-dependent RF plasticity of the sensory cortex that is highly specific to the configuration of inputting sensory information (Buonomano and Merzenich 1998; Nelson and Turrigiano 2008; Feldman 2009).

This model, however, faces challenges that stem from properties, some even fundamental, of long-term synaptic plasticity. First, LTP and LTD are typically induced by different protocols, including those using high-/low-frequency tetanic stimulation (TS) or the various timing of pre- and postsynaptic firing (Dan and Poo 2006; Dahmen et al. 2008; Nelson and Turrigiano 2008; Habib and Dringenberg 2010). Secondly, the development of thalamocortical LTP and LTD by sensory deprivation has various time courses. For example, in ocular dominance plasticity of the visual cortex, the LTD to the deprived eye occurs more rapidly than the LTP to the nondeprived eye (Feldman 2009; Smith et al. 2009). Finally, in vivo LTD is rarely induced in the adult brain (Hölscher 1997; Banerjee et al. 2009). These properties of long-term synaptic plasticity are not easily reconciled with our current understanding of frequency-specific RF plasticity exhibited by auditory cortical neurons in response to learning and experience (Liu et al. 2011).

In adult animal and human subjects, auditory learning and experience enhance the neural representation of the frequency of the learned sound in the primary auditory cortex (AI). The enhanced frequency representation results from the RF shift of cortical neurons toward the frequency of the learned sound (Suga et al. 2000; Ji et al. 2001; Zhang et al. 2001; Ji and Suga 2003; Pantev et al. 2003; Polley et al. 2006; Weinberger 2007; Krishnan et al. 2012; Fritz et al. 2013; Guo et al. 2013). Of note, the RF shift results from the facilitation of neuronal responses to the frequency of the learned sound together with simultaneous suppression of the responses to other frequencies (Bakin and Weinberger 1990, 1996; Gao and Suga 2000; Suga et al. 2000; Xiao and Suga 2002; Yan and Zhang 2005). Thus, cortical LTP and LTD may not only occur in the adult brain, but their actions must be induced in a frequency-specific manner by stimulation of the auditory thalamus with an identical protocol. Synaptic inputs to the cortical neurons tuned to the relevant frequency must be strengthened or enhanced (LTP). Conversely, inputs to the cortical neurons tuned to irrelevant frequencies must be weakened or reduced (LTD). If these scenarios are correct, LTP and LTD of synaptic inputs to cortical neurons must exhibit a direct correspondence to RF plasticity of cortical neurons. To date, supporting evidence for these connections is lacking.

Materials and Methods

Fifty-six female C57 mice 4–5 weeks old and weighing 15–20.5 g were used in our study. Animal use was in accordance with the Canadian Council on Animal Care, and the protocol (M10029) was approved by the Animal Care Committee at the University of Calgary.

Animal Preparation

Mice were anesthetized with an intraperitoneal injection of ketamine and xylazine (Supplementary Materials A). The initial dose of ketamine and xylazine was 85 and 15 mg/kg, respectively. An additional dose of ketamine (17 mg/kg, intraperitoneally, i.p.) and xylazine (3 mg/kg, i.p.) was injected approximately every 40 min to maintain anesthesia, as assessed by the animal’s response to tail pinching. Surgery and physiological experiments were then performed in a sound-proof room. The mouse was mounted on a custom-made head holder and its head was immobilized by clamping the palate and nasal bone. The skull was exposed by making a midline incision and removing connective tissue and muscle. The position of bregma and lambda was aligned at the same horizontal plane. For electrode placement, 2 openings approximately 2 mm in diameter were then drilled into the skull to expose the...
brain surface above the ventral division of the medial geniculate body of the thalamus (MGBv; 3.1 mm posterior to the bregma, 1.8 mm left to the midline) and the auditory cortex. The opening to the auditory cortex was enlarged for physiological identification of the AI. The body temperature of the mouse was maintained at 37 °C using a feedback-controlled heating pad.

**Acoustic Stimulation**
A sixty-millisecond long pure tone with 5 ms rising/decay time was used for acoustic stimulation. A digital sinusoidal wave burst was generated and converted into a 20-V peak-to-peak analog wave burst using a real-time processor (RP2, Tucker-Davis Tech., Inc., Gainesville, FL, USA). The analog signal was then sent to an electrostatic speaker driver (ED1) via a digitally controlled attenuator (PAS). The frequency and amplitude of tone bursts, delivered by the Brainware software (Tucker-Davis Tech., Inc.), were either manually or digitally varied during experiments. Tone bursts were delivered from an electrostatic loudspeaker positioned 15 cm away from and 45° right of the mouse right ear. The loudspeaker output was calibrated offline with a Larson-Davis condenser microphone (Model 2520) and a microphone preamplifier (Model 2200C), and the tone intensity was expressed as dB SPL (re. 20 µPa). Tone bursts were delivered to the mouse at a rate of 1 Hz when neuronal responses to identical tones were examined, or at a rate of 4 Hz when neuronal responses to a series of tones were examined.

**Recording of Single-Unit Firing, RF, and Field Excitatory Potentials in the Primary Auditory Cortex**
Tungsten electrodes or multibarrelled glass electrodes with a carbon fiber in the central tubing were used for recording neural activity in the left auditory cortex. The impedance of the recording electrodes was approximately 2 MΩ. The multibarrelled electrode used for both recording and drug injection is described below. Electrodes were connected to a 16-channel preamplifier (PA16) and amplifier (RA16). Bioelectrical signals were amplified and digitized. Two sets of frequencies were used to separate the recorded electrical signals; one filter was set at 300 Hz to 10 kHz for sampling unit firing and the other, at 1–100 Hz for sampling the cortical local field potential. The electrode was perpendicularly oriented toward the cortical surface for penetration. Optimal neuronal responses to tone stimulation were often recorded at a depth of about 300–600 µm below the brain surface (layers III–IV). At this depth, the local field potential displayed a negative waveform followed by a positive waveform (Supplementary Materials B). It is generally thought that the negative wave of cortical local field potential represents summated excitatory postsynaptic potentials (fEPSP) in the vicinity of the electrode tip (Mitzdorf 1985; Berens et al. 2008). We typically made 5–8 electrode penetrations in each animal to confirm the location of the AI. The RF was sampled based on unit firing in response to a series of tones. The minimum threshold of cortical neurons was determined audiovisually. A frequency scan (F-scan) was used for sampling RF data (the responses of AI neurons to a series of tones). In an F-scan, tone amplitude was set at 10 dB above the minimum threshold and tone frequencies varied from 5 to 35 kHz in increments of 1 kHz. Identical tone stimuli were repeated 15 times. Auditory responses to the F-scan were used to construct the auditory response curve, that is, RF. The best frequency (BF) of neurons was measured using the auditory response curve. The frequency at which a neuron showed its greatest response (spike number) was denoted the BF. These data were recorded and stored using the Windows-based Brainware data acquisition software (Tucker-Davis Tech., Inc.).

The cortical local field potential induced by electrical stimulation of the MGBv (ESMGBv) initially displayed a negative waveform followed by a positive waveform. The negative deflection reflected a summation of ESMGBv-induced fEPSPs. All fEPSPs used in this study were averaged waveforms in response to 30 identical MGBv stimuli.

**Recording and Electrical Stimulation in the MGBv**
A tungsten electrode with tip impedance of approximately 2 MΩ was vertically advanced into the brain through the opening above the left MGBv. The electrode was initially connected to the RA-16 preamplifier of the recording system. Electrical signals were filtered with a bandwidth of 0.3–10 kHz. Tone bursts were repetitively delivered at a rate of 1 Hz over the period of electrode penetration, and tone-evoked responses were usually observed when the electrode was about 3 mm below the brain surface. When tone-evoked responses were discernible, the electrode was advanced. Once the auditory response disappeared, the positioning of the electrode tip at the ventral border of the MGBv was confirmed. The electrode was withdrawn 50–100 µm. The RF and BF were measured in the manner described above. If the neurons displayed sharp frequency tuning, positioning of the electrode in the MGBv was confirmed (Jafari et al. 2007; Supplementary Materials C). The electrode was then disconnected from the recording system and connected to a constant-current isolator in the stimulating system.

Electrical pulses were generated by a Grass S88 stimulator (Astro-Medical, Inc., West Warwick, RI, USA) and an A360 constant-current isolator (WPI, Inc., Sarasota, FL, USA). The electrical pulse for inducing cortical fEPSP was monophasic with a current-constant square wave of 0.1 ms in duration. For ESMGBv, an electrical pulse was delivered to the MGBv at a rate of 1 Hz. The input–output (IO) function of ESMGBv-induced cortical fEPSP (i.e., the amplitude of fEPSP as a function of the stimulus current) was first measured. The stimulus current for subsequent electrical stimulation and TS was defined as the current that induced 50% of maximal fEPSP amplitude.

**Induction of LTP/LTD of Thalamocortical Synaptic Transmission**
High-frequency TS of the medial geniculate body was used for inducing long-term changes of thalamocortical synaptic transmission. A negative current was delivered to the tungsten electrode placed in the MGBv. The TS consisted of a 100-ms long train of 100-Hz electrical pulses. The duration of the electrical pulse was 0.1 ms. The train was delivered to the MGBv 5 times with a train interval of 500 ms. LTP and LTD were expressed as the percentage changes in fEPSP amplitudes and changes were required to exceed 20 min in duration.

**Micronotophoretic Injection of Saline, APV, and BMI**
The multibarrelled glass electrode had a tip diameter of 15–20 µm and consisted of 6 glass tubes. Five glass tubes surrounded a central tube that contained a carbon fiber connected to the PA16 preamplifier of a recording system (see above). Three of the surrounding tubes were filled with isotonic saline solution (0.9% NaCl, pH 7.0); one for grounding, one for balancing, and one for control injection. The remaining 2 barrels were filled with DL-2-amino-5-phosphonopentanoic acid (APV, 50 mM, pH 8.0) and bicuculline methiodide (BMI, 10 mM, pH 3.0). All the surrounding barrels were connected to the Neuro Phore System (BH-2, Harvard Apparatus, Inc., USA) for microiontophoresis injection of APV and/or BMI or saline. The injection current was −50 nA for APV and +50 nA for BMI. The saline injection was 50 nA. A retention current of +10 nA for APV and −10 nA for BMI was administered to each drug-filled barrel during noninjection periods. Drug injections began 10 min prior to TS and continued throughout the recording period.

**Experimental Protocols**
Each mouse was subjected to 1 of the 3 protocols described below. TSMGBv was applied once per animal.

Protocol 1 examined TSMGBv-induced changes in cortical fEPSP in the absence of any drug application to the recorded site. The responses of AI and MGBv neurons to tones with various frequencies and amplitudes were measured to determine their BFs and minimum thresholds. The results were used to construct the F-scan. The MGBv electrode was connected to a stimulation system. The IO function was measured to help determine the intensity of electrical current for ES and TS. ESMGBv-evoked cortical fEPSPs were measured every 2 min for 10 min. The responses of AI neurons to the F-scan were measured at 4 min prior to the next procedure. TSMGBv was delivered and ESMGBv-evoked fEPSPs were immediately sampled every 2 min for 60 min. At this time, the responses of AI neurons to the F-scan were also sampled at 4 and 50 min after TSMGBv or when the fEPSP returned to the pre-TSMGBv level.
Protocol 2 measured TSMGBV-induced changes in cortical fEPSPs under microiontophoresis of APV to the recorded site. The experimental procedures were identical to Protocol 1 with one additional procedure. Prior to TSMGBV, APV was microiontophoretically injected throughout the electrophysiological experiment. During the APV injection, ESMGBV-evoked cortical fEPSPs were sampled every 2 min until they stabilized at a new level. The responses of AI neurons to the F-scan were sampled 4 min prior to TSMGBV.

Protocol 3 measured TSMGBV-induced changes in cortical fEPSP under microiontophoresis of APV and BMI to the recorded site. The procedures were identical to those in Protocol 2.

Data Processing and Statistical Analysis

The auditory responses of AI neurons to tone were illustrated using peristimulus time histograms (PST) or cumulative PST (PSTC). The response magnitude was represented by spike number measured at the control BF of AI neurons before and after TSMGBV. The time window for counting spike number was 60 ms from tone burst onset. The RFs of AI neurons were plotted using spike numbers or PSTC as the function of tone frequencies, that is, F-scan. The neuronal BF was the tone frequency to which neurons demonstrated the greatest response or largest spike number. The change in BF was the difference in BFs before and after manipulation including drug application and TSMGBV.

All data were expressed as a mean ± standard deviation. A paired t-test was used to compare the data differences before and after TSMGBV, and/or drug application. A P-value of <0.05 was considered statistically significant.

Results

Cortical fEPSPs and action potentials were sampled using the same electrode, whether a tungsten or a multibarreled electrode in design (Fig. 1A). Tone or ESMGBV-induced a negative wave followed by a positive wave at the middle layers of the AI, which represented the compound thalamocortical fEPSP (Kaur et al. 2004, 2005, Fig. 1B and Supplementary Materials B).

Once the RFs of AI and MGBV neurons were determined, the IO function of the cortical fEPSPs was measured. As the 2 examples in Figure 1B show, the amplitude and slope of fEPSP increased following an increase in stimulus intensity. The threshold and IO function illustrate clear differences in the ESMGBV-evoked fEPSP between matched and unmatched neurons. The threshold of matched neurons (1.18 ± 0.59 µA, n = 22) was significantly lower than that of unmatched neurons (3.15 ± 1.54 µA, n = 41, P < 0.001). In contrast, the IO function (fEPSP amplitude/stimulus current that induced 50% of maximal fEPSP amplitude) of matched neurons (58.46 ± 22.19 µV/µA) was significantly higher than that of unmatched neurons (26.41 ± 12.39 µV/µA, P < 0.001). More importantly, the threshold (Fig. 1C) and IO function (Fig. 1D) of cortical fEPSP systematically changed as the function of the deviation of AI BF from the MGBV BF. These data clearly indicate that the ESMGBV was more influential in inducing the fEPSP of matched neurons. In other words, ESMGBV-evoked AI fEPSPs appeared most efficient when the AI and MGBV BFs were similar; this showed a positive correlation to the difference in BFs between the recorded AI neurons and the stimulated MGBV neurons. Once we determined the IO function, subsequent data sampling was based on a stimulus current that induced 50% of maximal fEPSP amplitude. The stimulus current ranged from 0.3 to 13.0 µA, 4.93 ± 3.19 µA. At the given stimulus current, the amplitude and latency of ESMGBV-induced fEPSP were 310.31 ± 69.64 µV and 15.29 ± 4.23 ms, respectively, for matched neurons (n = 22) and 270.46 ± 89.19 µV and 17.55 ± 4.29 ms, respectively, for unmatched neurons (n = 41). The results were not statistically significant (P > 0.05). Latencies >15 ms suggest that the recorded cortical events resulted from electrical stimulation of thalamocortical neurons rather than antidromic stimulation of corticothalamic nerve terminals.

High-frequency TSMGBV-induced long-term changes in thalamocortical fEPSP. A unique finding was that TSMGBV induced
not only an increase, but also a decrease in cortical fEPSPs (Fig. 2A). Since these changes lasted >30 min, we used LTP to represent the long-term increase. Of note, we used the term LTD-analog (LTDa) to represent the long-term decrease in cortical fEPSP in contrast to the term LTD associated with low-frequency TS. The analysis of the BFs of the recorded AI neurons and the stimulated MGBv neurons indicated that the increase or decrease in cortical fEPSP was highly correlated with their BF relationship (Fig. 2B). TSMGBv induced LTP in matched neurons and LTDa in unmatched neurons. On the average, TSMGBv increased cortical fEPSP of matched neurons by 41.35 ± 25.36% from 310.31 ± 69.64 to 445.00 ± 150.87 µV (n = 15); the increase was significant (P < 0.001). TSMGBv decreased cortical fEPSP of unmatched neurons by 26.72 ± 11.58% from 270.46 ± 89.19 to 202.28 ± 88.03 µV (n = 25); the decrease was also significant (P < 0.001). The largest decrease in fEPSP was found when the BF difference in AI and MGBv neurons ranged from 2 to 4 kHz. The decrease in fEPSP amplitude was 29.88 ± 11.52% (n = 17) in neurons with a 2- to 4-kHz BF difference, which was significantly larger than those with a 4- to 10-kHz BF difference (20.02 ± 8.97%, n = 8, P < 0.05). The changes in cortical fEPSP exceeded 30 min for both matched and unmatched neurons, an average of 41.25 ± 11.99 min for 12 of 15 matched neurons and 42.15 ± 12.18 min for 20 of 25 unmatched neurons. There was no difference in the duration of the fEPSP changes between matched and unmatched neurons (P = 0.84). Three matched neurons and 5 unmatched neurons exhibited an incomplete recovery during the observation period.

In addition to changes in the amplitude of cortical fEPSP, we also examined changes in the latency and slope. The latency changed from 15.29 ± 4.23 to 13.78 ± 4.95 ms for matched neurons (n = 15, P < 0.01) and from 17.55 ± 4.29 to 19.95 ± 4.63 ms for unmatched neurons (n = 25, P < 0.001). The slope changed from 12.50 ± 4.18 to 17.99 ± 7.90 µV/ms for matched neurons (n = 15, P < 0.001) and from 10.36 ± 3.84 to 7.86 ± 4.18 µV/ms for unmatched neurons (n = 25, P < 0.001). The latency and slope changes were also systematically related to the BF difference between the recorded AI neurons and stimulated MGBv neurons (Fig. 2C, D).

To analyze the relationship between changes in fEPSP and RF, the RFs of AI neurons were measured 4 min before, as well as 4 and 50 min after the TSMGBv, with the electrode positioning unchanged. Figure 3 shows examples of one matched neuron and one unmatched neuron. TSMGBv potentiated cortical fEPSP of the matched neuron and its potentiation lasted >30 min (Fig. 3A, top panel). Along with the changes in fEPSP, this neuron showed increased auditory responses and stayed tuned to 19 kHz (Fig. 3A, lower panels). On the other hand, TSMGBv depressed cortical fEPSP of the unmatched neuron and its depression lasted >40 min (Fig. 3B, top panel). Along with the depression of fEPSP, the auditory responses of this neuron decreased and its BF shifted from 14 to 16 kHz (Fig. 3B, lower panels). On the average, TSMGBv increased the spike number of matched neurons from 19.59 ± 7.31 to 27.61 ± 11.15 (n = 15, P < 0.001), but decreased the spike number of unmatched neurons from 21.59 ± 8.85 to 15.44 ± 6.51 (n = 25, P < 0.001). TSMGBv did not change the BFs of matched neurons, but shifted the BFs of unmatched neurons toward that of stimulated MGBv neurons. These changes in spike number and BFs are similar to those induced by auditory learning/experience and focal activation of the MGBv (Jafari et al. 2007).

A critical question raised at this point is why high-frequency TSMGBv induced both LTP and LTDa in AI? We then used a multibarrelled glass electrode designed for acquiring measurements as well as for microiontophoretic injections of either saline, N-methyl-D-aspartate (NMDA) receptor antagonist APV, and/or...
γ-aminobutyric acid-A (GABA<sub>A</sub>) receptor antagonist BMI to the recorded neurons. After first sampling the control fEPSP, we made microiontophoretic injections throughout the recording session, including the period of TSMGBv. Since microiontophoresis of saline did not appear to influence ESMGBv-evoked fEPSP as well as TSMGBv-induced LTP (n = 7) and LTDa (n = 7), these results were pooled with the data collected using the tungsten electrodes. We also sampled the RFs of AI neurons at 5 min before drug application, 5 min before TS, and 50 min after TS or when the fEPSP returned to the pre-TSMGBv level.

Figure 4 provides 3 examples of the effects of APV and APV and BMI on ESMGBv-evoked fEPSP. Two examples depict neurons tuned to 14 kHz (identical to the stimulated MGBv BF, a matched neuron, Fig. 4Aa, b) and 12 kHz (different from the stimulated MGBv BF, an unmatched neuron, Fig. 4Ab, b1), respectively. The effects of APV on both neurons were the same, that is, fEPSP amplitudes and spike numbers were drastically reduced, but their BFs were not changed (Fig. 4Aa, b, #2). The TSMGBv clearly did not impact the fEPSP amplitude (Fig. 4Aa, after arrowhead), spike number, and BF (Fig. 4Ab, #3) of the unmatched neuron. In contrast, TSMGBv further decreased the fEPSP amplitude (Fig. 4Ab, after arrowhead) and spike number (Fig. 4Bb, #3) of the unmatched neuron. In addition, the BF of this neuron shifted toward the stimulated MGBv BF to 13 kHz in Figure 4Ab (#3 and gray line). The third example in Figure 4C depicts the effects of cortical application of APV and BMI in an unmatched neuron. It was obvious that fEPSP (Fig. 4Ca), spike number, and BF (Fig. 4Cb) were rarely altered before and after drug application or TSMGBv. The APV effects on 7 matched neurons and 20 unmatched neurons were studied. The effects of APV and BMI were observed in 7 unmatched neurons.

The cortical application of APV decreased fEPSP amplitude from 316.81 ± 75.15 to 193.57 ± 40.99 µV (n = 7, P < 0.001) in the matched neurons and from 253.28 ± 70.71 to 177.95 ± 51.33 µV (n = 20, P < 0.001) in the unmatched neurons. Following the onset of the APV injection, matched neurons required 13.71 ± 3.04 min and unmatched neurons required 15.27 ± 4.46 min to stabilize new levels. For the matched neurons, the fEPSP amplitude was 193.57 ± 40.99 µV before and 197.86 ± 40.19 µV after TSMGBv (n = 7). Since this was not statistically significant (P = 0.54), we concluded that TSMGBv did not induce LTP or LTDa. On the other hand, TSMGBv further decreased the fEPSP from 177.95 ± 51.33 to 72.94 ± 24.66 µV (n = 20, P < 0.001) in the unmatched neurons. The decrease was 59.32 ± 5.89%, which was significantly larger than that without APV (29.15 ± 13.37%, n = 17, P < 0.001) and allowed us to conclude that blocking the NMDA receptor enhanced TSMGBv-induced LTDa in unmatched neurons. Following TSMGBv, 3 of 20 unmatched neurons retained a lower fEPSP during the observation period. The remaining neurons in this group recovered at a rate of 48.00 ± 11.38 min, which was similar to that without APV (P > 0.05). These findings suggested that TSMGBv-induced LTD of matched neurons was NMDA-dependent, and that TSMGBv-induced LTDa of unmatched neurons also included the component of NMDA-mediated potentiation. Further examination showed that the simultaneous application of APV and BMI produced few changes in cortical fEPSP, which was 260.86 ± 80.88 µV before and 258.57 ± 79.25 µV after application of the drugs (n = 7, P > 0.05). Under the impact of APV and BMI, TSMGBv did not significantly alter the fEPSP amplitude, which was 258.57 ± 79.25 µV before and 256.43 ± 77.93 µV after TSMGBv (n = 7, P > 0.05). These findings confirmed the involvement of GABA<sub>A</sub> receptor-mediated inhibition.

Drug application altered not only the fEPSPs, but also the spike numbers. The impact of drug application was also significant in TSMGBv-induced changes in fEPSPs and spike numbers. The cortical application of APV decreased spike numbers from 24.67 ± 6.93 to 15.24 ± 4.15 (n = 7, P < 0.005) in matched neurons and from 21.56 ± 14.63 ± 5.99 (n = 20, P < 0.01) in unmatched neurons. Under the impact of APV, TSMGBv did not significantly change the spike numbers of matched neurons (15.24 ± 4.15 vs. 16.17 ± 4.79, n = 7, P > 0.05), but largely decreased those of unmatched neurons (14.63 ± 5.99 vs. 6.13 ± 2.71, n = 20, P < 0.001). The BFs of unmatched neurons also shifted toward that of stimulated MGBv neurons. The cortical application of APV and BMI did not alter spike numbers (21.05 ± 6.16 vs. 20.43 ± 6.16, n = 7, P > 0.05). This suggested that the effects of APV and BMI on neuronal activity were mostly balanced. Under the joint actions of APV and BMI, TSMGBv had little effect on the response magnitudes of cortical neurons. The spike numbers were 20.43 ± 6.16 before and 19.47 ± 5.48 after TSMGBv (n = 7, P > 0.05). TSMGBv also did not impact the BFs of cortical neurons.

As discussed above, the TSMGBv-induced changes in fEPSP, spikes, and BFs appeared correlated. This correlation was further analyzed (Fig. 5). The spike number increased when LTP occurred, that is, when cortical fEPSP increased, and the spike number decreased when LTDa occurred, that is, when cortical fEPSP decreased. The spike numbers were little changed when cortical fEPSPs were not changed. The changes in the spike numbers of cortical neurons were linearly correlated with that in cortical fEPSPs. Furthermore, this correlation was observed to be consistent and not influenced by drug application (Fig. 5A). The BF change is commonly used as an index of RF plasticity of cortical neurons. We therefore further analyzed the relationship of TSMGBv-induced changes in the BFs of cortical neurons and cortical fEPSPs. The BF shift was closely...
Secondly, auditory thalamocortical projections are the only neural substrate that provides the auditory cortex with precise sound information. Thirdly, our recent study demonstrates that the RFs of AI neurons shift to that of the stimulated MGBv neurons following focal ES_{MGBv} (Jafari et al. 2007), similar to those induced by auditory learning and experience. Finally, the influence of thalamocortical LTP is well evidenced in visual, somatosensory, and auditory systems (Heynen and Bear 2001; Scott et al. 2007; Chun et al. 2013). The changes in thalamocortical synaptic transmission, however, cannot account for simultaneous facilitation and inhibition, an essential phenomenon of the RF shift in auditory neurons. Cortical neural circuits must be considered (Galindo-León et al. 2009; Liu et al. 2011; Metherate 2011; Ojima 2011). During early development, the balance between cortical excitation and inhibition is important in determining the RFs of auditory cortical neurons (Dormann et al. 2010; Sun et al. 2010). In adult animals, focal electrical stimulation of the auditory cortex also leads to frequency-specific RF shifts between neighboring neurons (Maldonado and Gerstein 1996; Chowdhury and Suga 2000; Talwar and Gerstein 2001), indicating the importance of cortical lateral excitation and inhibition. The impact of cortical lateral inhibition and excitation is also demonstrated by pharmacological manipulation. In bats, the blockade of NMDA receptors largely attenuates the learning-induced RF shifts of cortical neurons (Ji et al. 2005). Focal administration of GABA_A receptor antagonist even reverses the direction of the cortical RF shift (Xiao and Suga 2002, 2004).

It would appear that thalamocortical projections incorporated with associated cortical lateral excitatory and inhibitory projections form a plausible circuit for frequency-specific cortical plasticity (Fig. 6, Jafari et al. 2007; Xiong et al. 2011). Our data demonstrated that conventional high-frequency TS of MGBv neurons led to long-term remodeling of ES_{MGBv}-induced cortical fEPSP. This remodeling is not as simplistic as suggested in previous reports where high-frequency TS alone appeared to induce LTP (Nelson and Turrigiano 2008; Feldman 2009; Chun et al. 2013). We found that most AI neurons exhibited LTDa rather than LTP following TS_{MGBv}. LTP was found in physiologically matched AI neurons that likely receive direct inputs from stimulated thalamocortical neurons, whereas LTDa was found in physiologically unmatched neurons that do not receive direct inputs from stimulated thalamocortical neurons. Our findings highlight 2 conclusions. One, an identical stimulus is capable of inducing both LTP and LTDa, the synaptic mechanism underlying learning and experience. In other words, an
acquired signal induces both facilitation and suppression in the auditory cortex (Bakin and Weinberger 1990; Gao and Suga 2000; Suga et al. 2000, 2002; Jafari et al. 2007; Dorrn et al. 2010; Sun et al. 2010). Secondly, LTP/LTDa induced by TSMGBv demonstrates tonotopy, the fundamental characteristic of the auditory system.

In line with numerous reports in the literature (Nelson and Turrigiano 2008; Feldman 2009; Smith et al. 2009; Chun et al. 2013), thalamocortical LTP of matched neurons is NMDA-dependent (Fig. 4A). The underlying mechanism for the LTDa of unmatched neurons appears more complex and different from conventional LTD, another form of excitatory synaptic plasticity induced by low-frequency TS (Habib and Dringenberg 2010). In our study, we induced cortical LTDa with a protocol that is typically used for LTP induction, that is high-frequency TS. As shown in Figure 6, unmatched neurons receive both inhibitory and excitatory inputs. This suggests that TSMGBv is able to potentiate both inhibition and excitation but as clarified by our results, the potentiation resulting from inhibitory inputs is greater than that from excitatory inputs. We therefore examined the TSMGBv-induced LTDa after the blockage of the NMDA and GABA_A receptors. We demonstrated that microiontophoresis of APV led to even greater TSMGBv-induced LTDa, whereas microiontophoresis of both APV and BMI eliminated the TSMGBv-induced LTDa (Fig. 4B,C), suggesting that the LTDa of unmatched neurons is a summated result of potentiation and depression. The potentiation is possibly due to the excitatory inputs of cortical glutamatergic neurons receiving inputs from the matched neurons or, alternatively, the collateral inputs of the stimulated thalamocortical neurons. What is clear to us is that the component of potentiation is NMDA-dependent. The component of depression is most likely due to intracortical pathways, such as cortical GABAergic interneurons, that receive the inputs from potentiated matched neurons or stimulated MGBv neurons. It is also evident that the TSMGBv-induced depression is not NMDA-dependent. We are not yet confident if the depression is simply a transformation of the potentiation of matched neurons via GABAergic interneurons or a direct potentiation of GABAergic synapses on unmatched neurons. Although the mechanism underlying TSMGBv-induced cortical LTD requires further clarification, the role of cortical lateral excitation and inhibition can be emphasized (Metherate 2011; Ojima 2011; Xiong et al. 2011). A recent study has demonstrated the critical role of intracortical pathways in learning-induced plasticity of the auditory cortex (Guo et al. 2013).

High-frequency TS of the thalamus also induced frequency-specific changes in the response magnitude and RF of cortical neurons. Matched neurons showed enhanced responses but no RF shift, whereas unmatched neurons showed decreased responses and RF shifts toward that of the stimulated thalamocortical neuron. The changes in response magnitude and RF shift of cortical neurons were highly correlated with that in thalamocortical fEPSP (Fig. 5). These data strongly suggest that RF plasticity of cortical neurons is in accordance with TSMGBv-induced cortical LTP and LTDa. Our data provide strong support for the long-standing assumption that thalamocortical LTP and LTD underlie input-specific RF plasticity of the auditory cortex, albeit the LTDa appears generated through polysynapses.

Thalamocortical projections, incorporated with associated cortical lateral excitatory and inhibitory projections, form a circuit within the central auditory system. This circuit allows functional changes to occur in a frequency-specific manner (Liu et al. 2011). Studies demonstrate that many other brain structures in the limbic system are involved in auditory plasticity, including the nucleus basalis (Bakin and Weinberger 1996; Kilgard and Merzenich 1998; Ji et al. 2001; Yan and Zhang 2005), raphe nucleus (Ji and Suga 2007), amygdala (Zheng et al. 2008; Chavez et al. 2013), pedunculopontine tegmental nucleus (Luo et al. 2011; Luo and Yan 2013), and ventral tegmental area (Bao et al. 2001). Collaboration of the thalamocortical circuit with associated nuclei in the limbic systems is essential for learning-induced and experience-dependent neural plasticity in the central auditory system (Xiong et al. 2009).

In summary, the centripetal pattern characterized by the RF shift in auditory plasticity is observed in several species of mammals (Bakin and Weinberger 1990; Gao and Suga 1998; Kilgard and Merzenich 1998; Sakai and Suga 2001; Valentine and Eggermont 2003; Pantev et al. 2003; Yan and Zhang 2005; Ma and Suga 2007; Zhou and Jen 2007; Bajo et al. 2010) and in different types of tuning (Gao and Suga 1998; Yan and Zhang 2005; Ma and Suga 2007; Zhou and Jen 2007; Tang and Suga 2008, 2009). Centripetal plasticity of the auditory cortex can be induced by different protocols including repetitive acoustic stimulation (Gao and Suga 1998), auditory fear conditioning (Bakin and Weinberger 1990; Gao and Suga 1998), electrical stimulation of the auditory cortex (Chowdhury and Suga 2000; Talwar and Gerstein 2001), thalamus (Jafari et al. 2007), and midbrain (Zhang and Suga 2005), electrical stimulation of the cholinergic nuclei paired with a tone (Bakin and Weinberger 1996; Kilgard and Merzenich 1998; Ma and Suga 2003; Yan and Zhang 2005) and the exposure of some animals to particular sounds (Zhang et al. 2001; Pantev et al. 2003). Our current findings may help define a common mechanism underlying input-specific plasticity of the auditory cortex across species not only in the frequency domain, but also in the time and intensity domains.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

Funding
This work was supported by grants from the Canadian Institute of Health Research (MOP164961 and MOP274494), the Natural Sciences and Engineering Research Council of Canada (Discovery Grant), the Campbell McLaurin Chair for Hearing Deficiencies, and Alberta Innovates—Health Solutions.

Notes
Conflict of Interest: None declared.

References


