The Pedunculopontine Tegmental Nucleus: A Second Cholinergic Source for Frequency-Specific Auditory Plasticity

Feng Luo, Xiuping Liu, Carol Wang, and Jun Yan

Department of Physiology and Pharmacology, Hotchkiss Brain Institute, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada; and Health Science Centre, Hebei University, Baoding, Hebei, China

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Luo F, Liu X, Wang C, Yan J. The pedunculopontine tegmental nucleus: a second cholinergic source for frequency-specific auditory plasticity. J Neurophysiol 105: 107–116, 2011. First published October 27, 2010; doi:10.1152/jn.00546.2010. Cholinergic modulation is essential for many brain functions and is an indispensable component of the prevalent models attempting to understand the neural mechanisms responsible for learning-induced auditory plasticity. Unlike the cholinergic basal forebrain, the cholinergic pedunculopontine tegmental nucleus (PPTg) has received little attention. This study was designed to confirm whether the PPTg enables frequency-specific plasticity in the ventral division of the medial geniculate body of the thalamus (MGBv). Using the mouse model, we paired electrical stimulation of the PPTg with tone stimulation to help define the role of the PPTg. The receptive fields of MGBv neurons were examined before and after the paired stimulation; they were quantified in this study by best frequency (BF), response threshold, dynamic range, and spike number. We found that the electrical stimulation of the PPTg together with a tone presentation shifted the BFs of MGBv neurons upward when the frequency of the paired tone was higher than that of the control BF. Similarly, the BFs shifted downward when the frequency of the paired tone was lower than that of the control BF. The BFs of MGBv neurons, however, remained unchanged when the frequency of the paired tone was the same as that of the control BF. There was a linear relationship between the BF shift of MGBv neurons and the difference between the frequency of the paired tone and the control BF of MGBv neurons. High frequency specific changes were also observed in the response threshold, dynamic range, and spike number. This frequency-specific plasticity was largely eliminated by the microinjection of the muscarinic receptor antagonist atropine into the MGBv before the paired stimulation. Our findings suggest that the PPTg, like the cholinergic basal forebrain, is an important cholinergic source that enables frequency-specific plasticity in the central auditory system.

INTRODUCTION

Sensory plasticity allows novel and/or behaviorally relevant information to be retained in the brain through learning and experience (Dahmen and King 2007; Feldman 2009; Keuroghlian and Knudsen 2007; Kral and Eggermont 2007). Studies in both animal and human subjects confirm that auditory learning and experience induce frequency-specific plasticity in the auditory cortex and subcortical nuclei such as the thalamus (Edeline and Weinberger 1991; Gao and Suga 1998; Ji et al. 2005; Lennartz and Weinberger 1992; Polley et al. 2006; Zhang et al. 2001). Auditory plasticity is typically demonstrated by a shift of receptive field toward the frequency of the acquired sound or through an enhanced neural representation thereof.

It is well established that the frequency-specific auditory plasticity induced by auditory learning is eliminated when the muscarinic acetylcholine receptor (mAChR) is blocked (Gao and Suga 2000; Ji et al. 2005; Lennartz and Weinberger 1992; Recanzone et al. 1993; Thiel et al. 2002). Cholinergic modulation is consequently regarded as an essential component of auditory plasticity (Suga 2008; Thiel 2007; Weinberger 2004; Yan 2003). The identification of the cholinergic nucleus basalis (NB) of the basal forebrain represents yet another milestone (Bakin and Weinberger 1996; Kilgard and Merzenich 1998; Ma and Suga 2003; Yan and Zhang 2005). That the cholinergic basal forebrain cannot be solely accountable for all of the cholinergic effects on learning-induced auditory plasticity, however, is supported by several notable observations. First, the cholinergic neurons of the basal forebrain project only to the cerebral cortex (Mesulam et al. 1983). Second, mAChRs are not only distributed in the cortex but are also found in subcortical nuclei such as the thalamus (Levey 1996; Levey et al. 1991). Finally, an NB lesion or cortical application of the mAChR antagonist only partially eliminates the subcortical auditory plasticity induced by auditory learning (Ji et al. 2001; Ma and Suga 2003). These findings support the premise that another cholinergic source, likely influencing the modulation of subcortical neurons, must be involved in learning-induced auditory plasticity.

There are six groups of cholinergic neurons in the brain (Mesulam et al. 1983). Only two groups (groups 4 and 6) are anatomically positioned. Group 4 is found in the NB of the basal forebrain and group 6, in the pedunculopontine tegmental nucleus (PPTg) of the brain stem. The PPTg is a source of interest because of its direct cholinergic projections to subcortical nuclei, including the thalamus (Everitt and Robbins 1997; Mesulam et al. 1983), and because of its involvement in several brain functions, including arousal, learning, memory, and cognition (Inglis and Winn 1995; Steckler et al. 1994; Steriade and McCarley 1990). Behavioral studies have demonstrated that the PPTg shows increased activity during learning (Ivleva and Timofeeva 2003; Koyama et al. 1994; Richardson and DeLong 1991). It has also been amply demonstrated that a PPTg lesion impairs learning (Bechara and van der Kooy 1989; Della et al. 1991; Fujimoto et al. 1989, 1992; Inglis et al. 1994, 2001; Olimstead et al. 1998). We therefore hypothesize that the PPTg is a second cholinergic source that enables frequency-specific auditory plasticity through its modulation of the auditory thalamus.
To test this hypothesis, we examined in mice how the neuronal receptive fields of the ventral division of the medial geniculate body (MGBv) were altered by the electrical stimulation of the PPTg (ES_PPTg) paired with a tone (tone-ES_PPTg). Our data suggested that tone-ES_PPTg induced a frequency-specific and mAChR-sensitive change in the receptive fields of MGBv neurons.

**METHODS**

**Animals and surgery**

Forty C57BL/6 female mice (Charles River Laboratories, Wilmington, MA), aged 4–6 wk and weighing 13.8–22.6 g (18.0 ± 2.4 g), were used in this study. All protocols and procedures were designed using the Guide to the Care and Use of Experimental Animals published by the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University of Calgary. Animals were anesthetized with a mixture of ketamine and xylazine (200 mg/kg, 10 mg/kg, administered intraperitoneally; Bioniche Animal Health, Belleville, Ontario, Canada). The anesthetic status was monitored by pinching the subject’s hind paw with forceps approximately every 40 min. An additional dose of ketamine (40 mg/kg) and xylazine (2 mg/kg) was administered when the animal responded to pinching. Under anesthesia, the animal’s head was immobilized in a custom-made head holder by rigidly clamping between the palate and nasal/frontal bones. The mouth bar was adjusted to align bregma and lambda points in one horizontal plane. After the skull was surgically exposed, two holes (1–2 mm in diameter) used for electrode placement were made on the left skull above the MGBv (3.1 mm posterior to the bregma, 1.9 mm left to the midline) and PPTg (4.3–4.9 mm posterior to the bregma, 0.8–1.5 mm left to the midline) (Franklin and Paxinos 1996). The dura mater was carefully removed. Two additional holes (1 mm in diameter) were made adjacent to bregma and lambda on the right skull for electroencephalogram (EEG) recordings. The animal’s body temperature was maintained at 37°C by a feedback-controlled heating pad.

**Acoustic stimulation**

Tone bursts (60-ms duration with 5-ms rise and fall times) were digitally synthesized and converted into analog sinusoidal waves by an Enhanced Real-time Processor (RP2; Tucker-Davis Technologies [TDT], Alachua, FL). The signals were then fed into an electrostatic speaker through a digital attenuator (PA5; TDT) and an electrostatic speaker driver (ED1; TDT). The speaker was placed at 45° to the sagittal plane and 18 cm away from the mouse’s right ear. Speaker output was calibrated at this position using a condenser microphone (Model 2520; PCB Piezotronics/Larson-Davis Laboratories, Depew, NY) and a microphone preamplifier (Model 2200C; PCB Piezotronics/Larson-Davis Laboratories). The amplitude of tone bursts was expressed as decibel sound pressure level (dB SPL, referenced to 20 μPa). Frequencies and amplitudes of tone bursts were automatically or manually varied by BrainWare data acquisition software (TDT). The responses of neurons to a series of tones with various frequencies and amplitudes (frequency–amplitude scan [FA-scan]) were sampled for the off-line construction of the neuronal receptive field, the excitatory response area (tone frequency vs. amplitude) of an auditory neuron. In the FA-scan, tone frequency ranged from 3 to 40 kHz in an increment of 1 kHz, whereas tone amplitude ranged from −100 to 0 dB in increments of 5 dB. Therefore one FA-scan consisted of 799 frequency–amplitude blocks including an additional block without stimulus. The tones of various frequencies and amplitudes were randomly delivered in intervals of 250 ms using BrainWare data acquisition software. Each tone (FA-scan) was repeated five times.

**EEG recording and electrical stimulation of the PPTg**

Two silver electrodes were placed on the dura, close to the bregma and lambda, and connected to an amplifier (Grass P55; Astro-Med, West Warwick, RI) for EEG recording. Electrical signals were amplified ×10,000 and filtered with a band-pass of 3–100 Hz. The output signals were fed into an oscilloscope for monitoring and recorded with a SciWork data acquisition system (DataWave, Atlantic Beach, FL). The EEG was continuously monitored during the electrode penetration to the PPTg. It is known that the ES_PPTg changes the EEG from large and low-frequency waves to small and high-frequency waves, i.e., EEG desynchronization (Dringenberg and Olsmund 2003; Rasmussen et al. 1994). EEG desynchronization was therefore used to confirm effective PPTg activation.

A concentric bipolar electrode (OD = 125 μm) was dorsoven-trally inserted into the PPTg. Electrical stimulation consisted of a train of electrical pulses (0.2 ms long, monophasic and constant-current square wave, 120 Hz, 160-ms duration) generated by a Grass S88 stimulator and a Grass CCU1 constant-current unit (Astro-Med). The negative current was delivered through the central pole of the electrode. The electrode’s position was adjusted until maximal EEG desynchronization was attained following electrical stimulation (Fig. 1A). Once the position of the electrode was confirmed (2.5–3.5 mm below the brain surface), the threshold current for EEG desynchronization was determined by decreasing the current level. The stimulation current for subsequent experiments was set at 10 μA above the threshold current level, which ranged from 93 to 243 μA (134.6 ± 28.6 μA) in this study.

**MGBv recording and microinjection**

An electrical electrode was used for MGBv recording and microinjection. The assembly consisted of two tungsten electrodes for recording and a glass electrode for microinjection. The tungsten electrodes, which had an impedance of 2 MΩ and were separated by 100 μm, were connected to the data acquisition system (see following text). The tip of a glass electrode (~20 μm in diameter) was positioned between the two tips of the tungsten electrodes. The glass electrode was filled with either atropine (0.05 mM atropine sulfate dissolved in 0.9% saline; Sigma-Aldrich, St. Louis, MO) or 0.9% saline as used in the control and connected to the output tube of the microinjection pump (Neurophore BH-2; Harvard Apparatus, Hollis-ton, MA). The assembled electrodes were dorsoven-trally inserted into the MGBv using stereotaxic coordinates. Once a tone-evoked neuronal response was observed (~3 mm below the brain surface), the electrode was advanced until the tone-evoked auditory responses disappeared. This point represented the ventral boundary of the MGBv. The electrode was then slowly withdrawn by 50–100 μm and the responses of MGBv neurons to the FA-scan were recorded. Histological examination of lesion marks confirmed electrode placement within the MGBv (Jafari et al. 2007). Following the sampling of receptive fields with the FA-scan, 0.15–0.20 μl atropine or saline was injected into the MGBv within a time period of about 30 min. The receptive fields of MGBv neurons were then sampled twice: 10 min after drug injection and prior to subsequent experiments.

**Recording of action potentials**

The tungsten electrodes of our electrode assembly were connected to an RA-16PA preamplifier and RA16 multichannel amplifier (TDT) for data collection. Electrical signals were amplified ×10,000 and filtered with a band-pass of 300 Hz to 10 kHz. The output of the amplifier fed signals to an oscilloscope and were digitally monitored. The time and waveform of action potentials above a trigger level were saved in computer hard disk for off-line processing. The trigger level was defined as the voltage required to isolate spikes from background noise during data acquisition.

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Experimental groups, protocols, and data acquisition

We designed three experimental groups to clarify the role of the PPTg on frequency-specific plasticity in the MGBv. Group 1 examined the effect of the paired tone-ESPPTg on the receptive fields of MGBv neurons (18 mice). In this group, tone presentation and the ESPPTg were delivered at a rate of 1 Hz and ESPPTg was synchronized at the onset of the tone. Group 2 examined how the MGBv plasticity evoked by the paired tone-ESPPTg was affected by the local application of atropine (14 mice). Group 3 examined the effect of unpaired tone-ESPPTg on the receptive fields of MGBv neurons (8 mice). In this group, ESPPTg and tone were randomly delivered in a 1-s time frame. Identical amounts (0.15–0.20 μl) of 0.9% saline were injected into the brain at 4.8 mm posterior to the bregma. An arrow indicates a lesion mark in the PPTg. Dhc, dorsal hippocampal commissure; SC, superior colliculus; ICx, external nucleus of the inferior colliculus; AQ, cerebral aqueduct; PAG, periaqueductal gray; DR, dorsal nucleus raphe; VTN, ventral tegmental nucleus; NLL, nucleus of the lateral lemniscus; PRNr, pontine reticular nucleus; PSV, principal sensory nucleus of the trigeminal; NTB, nucleus of the trapezoid body; POR, periolivary region of the superior olivary complex.

Both immediately and 30 min after the microinjection of saline or atropine to the MGBv. The animal then received either a paired or unpaired tone-ESPPTg at a rate of 1 Hz for 6 min. In group 1, five mice received ESPPTg alone (at 1 Hz for 6 min) before the tone-ESPPTg. For tone-ESPPTg, the amplitude of the paired tone (PT) was set at 20 dB above the minimum threshold (MT), the lowest response threshold of a given neuron to all the frequencies. The PT frequency (PTF) was set in the range of 8 kHz below and above the best frequency (BF), the frequency at which a given neuron showed the lowest response threshold. The receptive fields of MGBv neurons were then recorded immediately after the tone-ESPPTg and every 20 min thereafter until the changes evoked by tone-ESPPTg recovered by a minimum of 50%.

Data processing

Single units were isolated using a computer-based spike-sorting technique (Luo et al. 2008; Yan and Ehret 2002). The receptive field was the excitatory response area (frequency vs. amplitude of tone) of a given neuron; it was constructed based on the responses of a single unit to the FA-scan. This study used four quantitative values to examine the receptive field, including BF, response thresholds to the BF (before and after tone-ESPPTg), dynamic range (DR), and spike number at 5 dB above the MT.

The response threshold to a particular frequency, determined according to tone-evoked spikes, was the lowest tone amplitude that evoked at least one spike. If spontaneous activity interfered with spike counting, the tone-evoked responses were typically assessed from a peak 5–60 ms following the tone stimulus. A peak or a sharp increase that appeared outside of the window was considered a spontaneous burst discharge and discarded.

The DR was defined as the dB range from the MT to the largest response magnitude (Fig. 3, A). For neurons with monotonic rate-intensity functions, the DR was the dB range between the MT and the tone amplitude that evoked 80% of the largest response magnitude (Fig. 3, C–E; Yan and Ehret 2002).

Study members who performed off-line data analysis were blind to experimental protocols. Plastic changes of MGBv neurons were quantified by the difference in the BF, MT, DR, and spike number before and after treatments. The maximal changes in BF shift after treatments were used for the comparative analysis with control data.

Data were expressed as means ± SD. The differences between groups of data were evaluated using paired t-test or the chi-square test. A value of P < 0.05 was considered statistically significant.

Lesion histology

Once the electrophysiological experiment was completed and with the animal still under deep anesthesia, a lesion was made in the recording site with a DC current of 10 μA for 10 min. The animal was then killed with an intracardiac perfusion of 50 ml physiological saline followed by a mixture of 4% paraformaldehyde and 0.1 M phosphate buffer (pH 7.4). The mouse brain was removed from the cranium and held in the paraformaldehyde solution for 24 h. It was next transferred into a 4% paraformaldehyde solution containing 20% sucrose and stored in a 4°C refrigerator. Embedded with OCT (optimal cutting temperature), coronal sections of the brain were made every 40 μm with a cryostat. Brain sections were mounted onto glass slides and stained using the Nissl method. The electrolytic lesion point was examined under a light microscope. Data were excluded if the lesion mark was outside of the PPTg. Figure 1B is a typical example of a coronal section and lesion.

RESULTS

As previously reported (Rasmusson et al. 1994), our data confirmed that ESPPTg led to EEG desynchronization when the
stimulating electrode was positioned in the PPTg (Fig. 1A). The EEG typically changed from low-frequency and large-amplitude waves to high-frequency and small-amplitude waves. Experiments continued only when EEG desynchronization was observed. On average, the frequency and amplitude of the EEG changed from 1.1 ± 0.5 Hz and 220.4 ± 85.5 μV to 2.2 ± 0.7 Hz and 95.3 ± 39.4 μV (n = 40), respectively. The duration of EEG desynchronization was 11.2 ± 2.9 s, ranging from 8.5 to 14.0 s. In total, 101 neurons were sampled in the MGBv. The BFs of MGBv neurons ranged from 11 to 29 kHz (16.7 ± 2.1 kHz) and the MTs ranged from 11.6 to 26.7 dB SPL (17.7 ± 3.8 dB SPL); they were within the normal hearing range of this mouse species (Luo et al. 2009; Zhang et al. 2005a).

Three examples of MGBv neurons before and after tone-ESPPTg

Figure 2 illustrates changes in the receptive field of an MGBv neuron following ESPPTg alone and tone-ESPPTg. The control BF and MT of this neuron were 19 kHz and 19.2 dB SPL, respectively (Fig. 2A). ESPPTg alone decreased the MT by 5 dB but rarely influenced the shape of the receptive field and the BF (Fig. 2B). An obvious change was observed following tone-ESPPTg. When paired with a 26-kHz tone, ESPPTg elevated the response threshold to 19 kHz (control BF), from 14.2 to 24.2 dB SPL and decreased the response threshold to 23 kHz (shift BF) from 26.1 to 11.1 dB SPL (Fig. 2C). The latter was even lower than the threshold to the control BF. The BF of this neuron shifted from 19 to 23 kHz. The receptive field mostly returned to control level 3 h after tone-ESPPTg (Fig. 2D).

The other two neurons in Fig. 3, A and B illustrate the effect of tone-ESPPTg on the receptive fields (the area encompassed by the curves connecting the response threshold to frequencies) when the PTF was either identical to or lower than the control BF. The other two neurons in Fig. 3, A and B illustrate the effect of tone-ESPPTg on the receptive fields (the area encompassed by the curves connecting the response threshold to frequencies) when the PTF was either identical to or lower than the control BFs of these two neurons. The control BF and MT of the neuron in Fig. 3A were 15 kHz and 20.4 dB SPL. ESPPTg paired with a 15-kHz tone increased the responses of this neuron but did not change either the BF or the DR (Fig. 3, A and C). The second neuron in Fig. 3B originally tuned to 16 kHz with an MT of 21.6 dB SPL. ESPPTg paired with an 8-kHz tone decreased its response threshold to 13 kHz to 21.7 dB SPL and increased its response threshold to 16 kHz to 31.6 dB SPL. Due to the differential changes in response thresholds, the BF of this neuron shifted from 16 to 13 kHz. The DR consequently decreased by 9 dB in response to 16 kHz (the control BF, Fig. 3D), but increased by 11 dB in response to 13 kHz (the shift BF, Fig. 3E).

The responses of these three neurons in Figs. 2 and 3 indicate that tone-ESPPTg induced a remarkable change in the receptive fields of MGBv neurons when the MGBv BF was different from the PTF; the change appeared related to the PTF.

Effects of ESPPTg alone

The effects of ESPPTg alone on MGBv neurons were examined in 14 neurons. There were no observable changes in BF. The averaged spike number increased from 22.6 ± 8.1 to 29.7 ± 13.5 (n = 14, P = 0.03, paired t-test), the MT slightly decreased from 20.1 ± 3.2 to 18.5 ± 4.8 dB SPL (n = 14, P = 0.11, paired t-test), and the DR showed little change from 53.0 ± 9.9 to 51.5 ± 11.8 dB (n = 14, P = 0.70, paired t-test).

Frequency-specific shift in BFs of MGBv neurons after tone-ESPPTg

The BF shift of all MGBv neurons sampled in group 1 (with saline injection) was analyzed as the function of the control BF, PTF, and the difference between them. Following the tone-ESPPTg, the BFs of MGBv neurons could possibly shift higher.
lower, or not at all. We found that the BF shift showed little correlation to either the control BF of MGB neurons (Fig. 4A, \( P = 0.34 \)) or the PTF (Fig. 4B, \( P = 0.77 \)). In contrast, a linear correlation emerged between the BF shift of MGBv neurons and the difference between the control BF and the PTF (Fig. 4C, \( P < 0.01 \)). MGBv BF shifted upward when the PTF was higher than the control BF but downward when the PTF was lower than the control BF. MGBv BF remained unchanged when the PTF was the same as the control BF.

**Frequency-specific changes in response thresholds, dynamic ranges, and spike numbers after tone-ESPPTg**

In addition to the shift in MGBv BF, the tone-ESPPTg also led to the changes in the response threshold, DR, and spike number. These changes appeared to be associated with the BF shift. Consequently, these parameters were also examined in both control and shifted BFs.

The response thresholds to control and shifted BFs were actually the MTs before and after tone-ESPPTg, respectively. The difference between them represented the change in MT evoked by tone-ESPPTg. A simple analysis of the averaged MTs showed a significant change in the MT of MGBv neurons, 19.7 ± 3.4 dB SPL before tone-ESPPTg and 21.2 ± 3.8 dB SPL after tone-ESPPTg (\( n = 42, P = 0.13, \) paired \( t \)-test). A frequency-dependent change in the MTs of MGBv neurons following tone-ESPPTg was confirmed by the percentages of MGBv neurons in which MT increased, decreased, or remained unchanged. Of the 33 MGBv neurons with a BF shift, 23 (69%) showed an increase in MT and 10 (31%) showed a decrease in MT following tone-ESPPTg. In contrast, of 9 MGBv neurons with no BF shift, 8 (89%) showed an unchanged or decreased MT and only one (11%) showed an MT increase. All of the results were statistically significant (\( P < 0.01, \) chi-square test).

Further analysis showed a systematic change in response thresholds in relation to the BF shift. For the neurons that had no BF shift, tone-ESPPTg rarely changed the response threshold to the BF (\( n = 9, -1.1 ± 2.2 \text{ dB}, P > 0.05 \)). For the neurons with a BF shift, tone-ESPPTg increased the response threshold to the control BF but decreased the response threshold to the shifted BF. The degree of threshold change increased in response to an increase in the shifting range of the MGBv BF (Fig. 5a). On average, tone-ESPPTg increased the response threshold to the control BF by 5.8 ± 4.4 dB, from 18.2 ± 3.2 to 24.2 ± 4.4 dB SPL (\( n = 33, P < 0.01, \) paired \( t \)-test). Significantly, tone-ESPPTg decreased the response threshold to the shifted BF by 6.4 ± 4.2, from 23.6 ± 3.7 to 17.2 ± 4.6 dB SPL (\( n = 33, P < 0.01, \) paired \( t \)-test).

The change in DR evoked by tone-ESPPTg was similar to the change in response threshold. Tone-ESPPTg rarely changed the DR of neurons, 1.2 ± 1.7 dB (\( n = 9, P > 0.05, \) paired \( t \)-test), without a BF shift. For neurons with a BF shift, tone-ESPPTg decreased the DR at the control BF by 6.7 ± 7.9 dB, from 58.8 ± 10.4 to 51.7 ± 9.3 dB (\( n = 33, P < 0.01, \) paired \( t \)-test). On the other hand, the DR at the shifted BF increased by 6.5 ± 6.5 dB, from 50.6 ± 8.9 to 56.6 ± 8.2 dB (\( n = 33, P < 0.01, \) paired \( t \)-test).

Similar responses were also observed in the change in spike number. Tone-ESPPTg led to an increase, decrease, or no change in spike number of MGBv neurons. The spike number of neurons with no BF shift was scarcely changed (0.4 ± 6.9, \( n = 9, P > 0.05, \) paired \( t \)-test) by tone-ESPPTg. For neurons...
with a BF shift, tone-ESPPTg decreased the spike number in response to the control BFs by 6.0/110.06 9.2, from 19.8/110.06 11.5 to 13.1/110.06 11.6 (n = 33, P < 0.01, paired t-test). The spike number in response to the shifted BF, however, increased by 7.7/110.06 5.7, from 15.7/110.06 5.1 to 23.5/110.06 6.1 dB (n = 33, P < 0.01, paired t-test).

Effects of local application of atropine on the BF shift of MGBv neurons evoked by tone-ESPPTg

When the MGBv was treated with atropine, tone-ESPPTg also induced a BF change in MGBv neurons. The shifting range was smaller (Fig. 6, n = 26) than that in the neurons treated with saline (Fig. 4C, n = 42). Of 26 MGBv neurons sampled in this group, tone-ESPPTg failed to induce BF shifts in 15 (58%) neurons. Eleven additional neurons displayed a BF shift but to a lesser degree. An analysis of the regression slopes (Figs. 4C and 6) indicates that MGBv application of atropine reduced the range of the BF shift by 66%.

FIG. 4. The frequency-specific shifts in the BFs of MGBv neurons induced by paired tone-ESPPTg. A: the BF shift is plotted as a function of the control BF. B: the BF shift is plotted as a function of the paired-tone frequency (PTF). C: the BF shift is plotted as a function of the differences between MGBv BF and the PTF. Solid lines represent linear regressions (A, y = 0.66 ± 0.06x, P = 0.34; B, y = 1.79 ± 0.01x, P = 0.77). Dashed lines represent the zero points of abscissa and ordinate. Diff., difference; n, number.

FIG. 5. Frequency-specific changes in response threshold (A), dynamic range (B), and spike number (C) following tone-ESPPTg stimulation. The scatterplot and the average changes (mean ± SD) at the original BF (unfilled circles, n = 42) and shifted BF (filled circles, n = 42) are plotted as a function of the BF shifts. The dashed lines represent the zero points of abscissa and ordinate. Con., control.

FIG. 6. Application of atropine into the MGB largely eliminated the frequency-specific shifts induced by tone-ESPPTg. The BF shift is plotted as a function of the differences between MGBv BF and the PTF. The solid line represents linear regression and the dashed lines represent the zero points of abscissa and ordinate. Diff., difference; n, number.
Effects of an unpaired presentation of tone and ESPPTg on the BF shifts of MGBv neurons

In auditory fear conditioning, the timing of a conditioned tone and an unconditioned stimulus is critical for inducing frequency-specific plasticity of the auditory cortex (Bakin and Weinberger 1990, 1996). Experimental group 3 was designed to confirm whether the pairing of ESPPTg and tone presentation was essential for the induction of the frequency-specific BF shifts. Identical to the paired tone-ESPPTg stimulation, the unpaired tone-ESPPTg shifted the BF of the MGBv neuron upward when the PTF was higher than the control BF. It shifted downward when the PTF was lower than the control BF (Fig. 7). The shift in MGBv BFs was linearly correlated to the difference between the control BF of the MGBv neuron and the PTF ($n = 18$, $P < 0.01$). The regression slope of the BF shift was similar to that induced by the paired tone-ESPPTg (0.32 vs. 0.31, Figs. 4C and 7).

DISCUSSION

Cholinergic modulation via mAChR is an important component of frequency-specific auditory plasticity induced by learning or experience. Studies from 1996 until now have confirmed the critical role of the NB in frequency-specific plasticity in the auditory cortex (Bakin and Weinberger 1996; Kilgard and Merzenich 1998; Ma and Suga 2003; Yan and Zhang 2005). Despite some discrepancy in two contemporary models, both consider cholinergic modulation a crucial component and the cholinergic basal forebrain an important neural substrate (Suga and Ma 2003; Weinberger 2004). Our study presents new insight into these models with the physiological finding that tone-ESPPTg induces frequency-specific changes in the receptive fields of the MGBv neurons (Fig. 8).

Frequency-specific plasticity of MGBv neurons after tone-ESPPTg

The main feature of the plasticity of MGBv neurons evoked by tone-ESPPTg was the large-scale change in the receptive field that was highly specific to the paired-tone frequency (PTF); significantly, the tone-ESPPTg shifted the MGBv BF toward the PTF (Figs. 2, 3, A and B, 4C, and 7). The BF shift appeared to result from the threshold decrease in response to the shifted BF and threshold increase in response to the control BF (Fig. 5A). Related changes were also seen in the DR and spike number in response to the shifted BF and decreased in response to the control BF (Fig. 5, B and C). This pattern of MGBv plasticity is consistent with the cortical plasticity evoked by electrical stimulation of the NB of the basal forebrain paired with a tone in the same mouse species (Yan and Zhang 2005) as well as in studies involving other species (Bakin and Weinberger 1996; Ma and Suga 2003). The highly specific plasticity of MGBv neurons evoked by tone-ESPPTg was largely reduced by application of atropine into MGBv (Fig. 6). Our findings suggest that the PPTg enables the frequency-specific plasticity of the MGBv through cholinergic modulation and the mAChR. This provides compelling evidence that the PPTg may be a second cholinergic neural substrate that facilitates learning-induced auditory plasticity. This is illustrated in Fig. 8 and discussed in detail in the following text.

A neural model for learning-induced auditory plasticity and the role of PPTg

Auditory fear conditioning, a common experimental approach for studying associative learning, consists of a condi-

FIG. 7. The BF shift after unpaired tone-ESPPTg is plotted as a function of the difference between MGBv BF and PTF. ESPPTg and tone were randomly delivered at a rate of 1 Hz in unpaired tone-ESPPTg. The solid line represents linear regression and the dashed lines represent the zero points of the ordinate and abscissa. Diff., difference; $n$, number.
tioned tone stimulus (CS) and an unconditioned aversive stimulus (US) such as an electrical foot shock. The CS is transmitted to the auditory thalamus and cortex through the auditory ascending pathway and the US is transmitted to the somatosensory thalamus and cortex through the somatosensory ascending pathway. When the CS and US are paired, the associated information or learning is retained in the medial division of the MGB/posterior intralaminar complex (PIN) and/or amygdala. The amygdala activates additional neural structures in the limbic system. In terms of the cholinergic system, the NB of the basal forebrain is activated. The highly specific plasticity of the auditory cortex is induced by cholinergic modulation of the responses of cortical neurons to the CS tone and, subsequently, to the non-CS tone. The frequency-specific cortical plasticity can be forwarded down to the auditory thalamus and midbrain through the corticofugal system (Zhang and Yan 2008; Zhang et al. 2005b).

The preceding text is a brief summary of previous models proposed by Drs. Weinberger, Suga, and LeDoux (Blair et al. 2001; Suga and Ma 2003; Weinberger 2004). For reasons listed in the INTRODUCTION, previous models are incomplete in terms of the engagement of the cholinergic system. We have therefore proposed that the PPTg should be considered a second cholinergic source (Xiong et al. 2009), which is strongly supported by data from this study. Similar to the NB of the basal forebrain, the PPTg also receives inputs from the amygdala and sends cholinergic projections to the thalamus (Fibiger and Semba 1992; Steckler et al. 1994).

Since the PPTg and the NB exhibit increased activity during learning (Ingls and Winn 1995; Richardson and DeLong 1991) and are mutually influenced through their reciprocal projections (Jones and Cuello 1989; Semba and Fibiger 1992; Steckler et al. 1994), the findings yielded in the present study allow us to propose a more comprehensive neural model for learning-induced auditory plasticity (Fig. 8). During learning, both the NB and PPTg are activated. The NB is responsible for the cholinergic modulation of the cortical neurons and the PPTg is responsible for the cholinergic modulation of the thalamic neurons. When a sound is processed in the auditory system, increased cholinergic activity in the auditory cortex and thalamus promotes sound-specific plasticity in these two important auditory information processing centers. The sound-specific plasticity in the auditory cortex and thalamus is eventually coordinated through the tonotopic loops that consist of thalamocortical and corticofugal projections (Xiong et al. 2009).

Our data thus far confirm that the PPTg is capable of inducing the frequency-specific plasticity of the auditory thalamus via mAChR. More investigations, particularly behavioral studies, are required to substantiate that the PPTg is indeed a neural substrate for learning-induced auditory plasticity.

The matter of the pairing of the ES\textsubscript{PPTg} and tone presentation

It is well known that the pairing of CS and US is critical for the establishment of fear conditioning and learning-induced auditory plasticity (Bakin and Weinberger 1996). One may question why the pairing of tone and ES\textsubscript{PPTg} was not required in the present study involving the PPTg.

An issue that must be clarified here is that activation of either the PPTg or NB by electrical stimulation is different from that by fear conditioning. Artificial activation of the PPTg or NB bypasses the neural pathways that are required for the integration of CS and US information in the medial division of the MGB/PIN and the amygdala (Fig. 8; Blair et al. 2001; Suga and Ma 2003; Weinberger 2004). The critical factor for the induction of frequency-specific auditory plasticity evoked by PPTg or NB activation appears to be that the information of the paired tone should arrive at the MGBv or auditory cortex within the period of elevated acetylcholine (ACh) level, i.e., the period of EEG desynchronization. In the present study, the PPTg was stimulated every second and the EEG desynchronization by each PPTg stimulation lasted around 10 s (Fig. 1A). This means that the information of the paired tone always arrived at the MGBv during the period of EEG desynchronization irrespective of whether the tone was paired or not paired with PPTg activation. The stimulus paradigm used and EEG desynchronization observed in this study were the same as those in our previous study of the NB. The results are identical: the NB stimulation paired with a tone induces frequency-specific plasticity in the auditory cortex regardless of the paired tone being synchronously or randomly delivered (Yan and Zhang 2005). Our findings concur with another study in rat that showed frequency-specific cortical plasticity can be induced when the paired tone is delivered either before or after the NB activation (Kilgard and Merzenich 1998). Therefore the arrival of the tone at the time when the ACh level has increased appears to be significant. This is also supported by the finding that external injection of ACh together with a tone presentation induces frequency-specific plasticity in the auditory cortex (Chen and Yan 2007).

Potential involvement of the auditory cortex and midbrain

The local application of the mAChR antagonist atipamezole into thalamus largely reduced the shifts of MGBv BFIs (Fig. 6), suggesting mAChR dependence. Significantly, MGBv application of atipamezole did not completely abolish the frequency-specific BF shift of MGBv neurons following tone-ES\textsubscript{PPTg} (Fig. 6). The remaining plastic changes in the MGBv raise two possibilities. One possibility is that the plasticity may also occur in the auditory midbrain. A recent anatomical study confirms that the PPTg also sends cholinergic projections to the auditory midbrain (Schrofied 2010). Activation of the PPTg may increase the ACh level in the auditory midbrain and induce frequency-specific plasticity of midbrain neurons when the ES\textsubscript{PPTg} paired with a tone. If this is the case, the plastic changes of midbrain neurons can simply be forwarded up to the thalamus through colliculothalamic projections. A second possibility is that the frequency-specific plasticity may also occur in the auditory cortex. Although the PPTg itself does not have direct cholinergic projections to the cortex, it innervates the NB of the basal forebrain, a major source of cholinergic fibers in the cortex (Bertorelli et al. 1991). A previous study demonstrates that activation of the PPTg with a larger current (400 \(\mu\)A) increases the ACh level in the cortex through the NB of the basal forebrain (Rasmussen et al. 1994). Increased cortical...
levels of ACh are capable of inducing a frequency-specific BF shift in the auditory cortex as well as in the MGBv through corticofugal projections if a tone stimulation is presented (Chen and Yan 2007; Yan and Zhang 2005; Zhang and Yan 2008). Therefore the plastic changes of MGBv neurons observed in the present study could be partially attributed to the plasticity occurring in the auditory cortex and midbrain, as facilitated through the tonotopic loops in the cortex, thalamus, and midbrain (Xiong et al. 2009). Our preliminary data in this area of investigation (unpublished data) support this premise.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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