Auditory midbrain processing is differentially modulated by auditory and visual cortices: An auditory fMRI study

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The cortex contains extensive descending projections, yet the impact of cortical input on brainstem processing remains poorly understood. In the central auditory system, the auditory cortex contains direct and indirect pathways (via brainstem cholinergic cells) to nuclei of the auditory midbrain, called the inferior colliculus (IC). While these projections modulate auditory processing throughout the IC, single neuron recordings have samples from only a small fraction of cells during stimulation of the corticofugal pathway. Furthermore, assessments of cortical feedback have not been extended to sensory modalities other than audition. To address these issues, we devised blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) paradigms to measure the sound-evoked responses throughout the rat IC and investigated the effects of bilateral ablation of either auditory or visual cortices. Auditory cortex ablation increased the gain of IC responses to noise stimuli (primarily in the central nucleus of the IC) and decreased response selectivity to forward species-specific vocalizations (versus temporally reversed ones, most prominently in the external cortex of the IC). In contrast, visual cortex ablation decreased the gain and induced a much smaller effect on response selectivity. The results suggest that auditory cortical projections normally exert a large-scale and net suppressive influence on specific IC subnuclei, while visual cortical projections provide a facilitatory influence. Meanwhile, auditory cortical projections enhance the midbrain response selectivity to species-specific vocalizations. We also probed the role of the indirect cholinergic projections in the auditory system in the descending modulation process by pharmacologically blocking muscarinic cholinergic receptors. This manipulation did not affect the gain of IC responses but significantly reduced the response selectivity to vocalizations. The results imply that auditory cortical gain modulation is mediated primarily through direct projections and they point to future investigations of the differential roles of the direct and indirect projections in corticofugal modulation. In summary, our imaging findings demonstrate the large-scale descending influences, from both the auditory and visual cortices, on sound processing in different IC subdivisions. They can guide future studies on the coordinated activity across multiple regions of the auditory network, and its dysfunctions.

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Introduction

Sensory cortices contain extensive descending projections to subcortical nuclei (Winer, 2006), as well as widespread connectivity between different sensory modalities (Falchier et al., 2002). It is hence plausible that subcortical nuclei integrate cortical feedback from multiple sensory modalities, for the purpose of enhancing detection, identification, or localization of external objects. Specifically, in the auditory system, besides the ascending projections that transmit information from the ear to higher levels for perception (Malmierca, 2003), there are extensive descending projections from the auditory cortex (AC) to nuclei of the auditory midbrain, called the inferior colliculus (IC) (Bajo and Moore, 2005; Bajo et al., 2007; Coomes et al., 2005; Schofield and Motts, 2009; Schofield et al., 2011), which is a compulsory relay for all ascending auditory information from multiple brainstem nuclei (Malmierca, 2003) and the origin of several important auditory processing properties (Nataraj and Wenstrup, 2005; Woolley et al., 2005). At the same time, cortices of other sensory modalities, such as the visual cortex (VC), send direct projections to both the AC (Budinger et al., 2006; Campi et al., 2010) and the IC (Cooper and Young, 1976; Dong, 2008). However, until recently, the relative influence of AC and VC projections on auditory processing within the IC has not been investigated.

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In the auditory system, there are direct cortex-to-collicular or cortico-collicular projections from several AC fields to all subdivisions of the IC (Bajo and Moore, 2005; Bajo et al., 2007; Schofield, 2009). There is also an indirect pathway from AC to cholinergic pontomesencephalic tegmentum neurons, which, in turn, project to the IC (Schofield et al., 2011). The direct and indirect projections are likely to have a profound impact on the sound-evoked response properties throughout the IC. However, previous electrophysiological studies investigating the functional implications of these projections could only sample a small fraction of IC neurons (Bajo and King, 2012). Although they indicate that the sensitivity of IC neurons to basic acoustic cues, e.g. frequency (Yan et al., 2005; Yan and Suga, 1998; Zhang et al., 1997), intensity (Yan and Ehret, 2002), duration (Ma and Suga, 2001), and location (Nakamoto et al., 2008), can be altered by electrically stimulating or cryogenically inactivating some AC neurons, the exact large-scale functional influences on the coordinated activity in different IC subnuclei is not easily extrapolated from single neuron recordings. Meanwhile, it is unclear how the corticocollicular projections modulate the IC responses to complex sounds, such as species-specific vocalizations, which can play important roles in communicating information or facilitating behavioral responses for many species (Portfors, 2007; Woolley and Portfors, 2013). In addition, there has been no attempt to differentiate the relative contribution of direct and indirect projections in modulating IC responses to different types of sounds. Lastly but more importantly, while evidence of the widespread connectivity from the VC to multiple levels of the auditory pathway has accumulated (Budinger et al., 2006; Campi et al., 2010; Cooper and Young, 1976; Dong, 2008) and interactions between the two sensory modalities at cortical level have been examined in many studies (Bisley and King, 2009; Finney et al., 2001; Lomber et al., 2010; Petrus et al., 2014; Wallace et al., 2004), how the visual feedback crossmodally influences IC auditory processing has not been investigated.

In this study, we employed blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) (Ogawa et al., 1990) to characterize the functional roles of cortical feedback throughout the auditory midbrain, particularly the differential contribution of auditory and visual modalities. BOLD fMRI is a non-invasive technique that can measure the hemodynamic responses (Kim and Ogawa, 2012) as neural correlates (Logothetis et al., 2001; Mukamel et al., 2005) throughout a processing area with relatively high spatial and temporal resolutions. BOLD fMRI has been successfully used to noninvasively measure stimulus-evoked activity in the auditory system of humans (Barton et al., 2012; De Martino et al., 2013; Ress and Chandrasekaran, 2013; Sigalovsky and Melcher, 2006) as well as animals, such as primates (Baumann et al., 2011; Kayser et al., 2007; Tanji et al., 2010) and songbirds (Boumans et al., 2008; Poirier et al., 2009; Van Meir et al., 2005; Voss et al., 2007). More recently, fMRI has been demonstrated as a powerful tool for investigating the auditory functions in small rodents (Cheung et al., 2012a; Lau et al., 2015b; Yu et al., 2009), particularly the processing of basic acoustic components in the midbrain (Cheung et al., 2012a,b; Gao et al., 2014, 2015; Lau et al., 2013, 2015a;b; Zhang et al., 2013a,b). To examine the influence of cortically originating descending projections on sound-evoked IC responses (both noise and species-specific vocalizations), BOLD imaging was performed in bilateral AC-ablated (ACA) and VC-ablated (VCA) rats with comparison to age-matched normal (NM) control animals. We also probed the potential role of the indirect cholinergic pathway in this descending modulation process by pharmacologically blocking it with systemic injection of atropine, an antagonist of muscarinic acetylcholine receptors (Habbicht and Vater, 1996). Loss of the AC led to a marked increase in sound-evoked responses and a diminished response selectivity to forward species-specific vocalizations (versus temporally reversed ones), indicating that the input normally exerts a suppressive influence on gain but a facilitative one on specific responses to natural vocalizations. In contrast, loss of the VC led to decreased sound-evoked responses and slightly decreased response selectivity, demonstrating that this modality normally increases gain and facilitates the specific responses. These results revealed a large-scale influence of descending projections on IC processing, both within and across sensory modalities.

Materials and methods

Cortical ablation surgery

All animal experiments were approved by the Committee on the Use of Live Animal in Teaching and Research of the University of Hong Kong. Adult male rats (Sprague–Dawley strain, 250 g) underwent surgery. After induction of anesthesia using 2% isoflurane, skull windows were opened over the bilateral auditory or visual cortex. The tissue in the primary and secondary auditory cortex or the anterior part of the entire primary and partial secondary visual cortex was ablated without injuring the white matter underneath (Fig. 1). The ablated area was then filled with biodegradable hemostatic sponge (Surgicell®) and skin incision was sutured. The animals were allowed to recover at 37 °C before returned to the feeding cage. They underwent fMRI experiments 2 weeks later. Note that the ACA and VCA surgeries both involved more than 30 animals. The location and size of the ablated areas were verified in each animal using anatomical MRI and 12 animals whose ablated areas were bilaterally symmetric and restricted within the intended AC/VC region were employed in the following fMRI experiments. The resulted sample size was NM: n = 12, ACA: n = 12, VCA: n = 12 (Table 1).

Preparation for fMRI

Animals were prepared for fMRI experiments as described in our previous studies (Chan et al., 2010; Cheung et al., 2012a,b; Gao et al., 2014, 2015; Lau et al., 2013, 2015a,b; Zhang et al., 2013a,b; Zhou et al., 2014). Briefly, rats were initially anesthetized with 3% isoflurane and then mechanically ventilated via oral intubation. They were placed on a holder in the prone position with a tooth bar to restrict head motion. Throughout the course of MR scanning, light anesthesia was maintained with 1% isoflurane. Animal heart rate, respiration rate, oxygen saturation, and rectal temperature were continuously monitored by sensors (SA instruments) and kept in normal ranges (heart rate: 380–420; respiration rate: 56–60; oxygen saturation: ≥95%; rectal temperature: 36.5–37.5 °C).

fMRI data acquisition

All MR experiments were performed on a 7 T MRI scanner (PharmaScan 70/16, Bruker Biospin GmbH) using a transmit-only birdcage coil in combination with an actively decoupled receive-only surface coil. Two fMRI experiments were performed to measure the gain of response and the selectivity to species-specific vocalizations, respectively, in IC and a set of projection nuclei to the IC, the nuclei of the lateral Lemniscus (LL, Fig. 2a) (Mallemierca, 2003). Scout images were first acquired to determine the coronal and sagittal planes of the brain. Then in the response gain experiment, a single coronal slice with 1.0 mm thickness was positioned to cover the center of the IC (at Bregma −8.5 mm, Fig. 2b), while in the vocalization experiment, 8 slices with 1.0/0.2 mm thickness/gap were positioned, with the 3rd and 4th covering the full IC (at Bregma −9.1 mm and −7.9 mm, Fig. 2c). T2-weighted images were acquired as anatomical reference using a Rapid Acquisition with Refocused Echoes (RARE) sequence (FOV = 32 × 32 mm², data matrix = 256 × 256, RARE factor = 8, TE/TR = 36/4200 ms). In the response gain experiment, the fMRI measurements were obtained using a single-slice balanced Steady-State Free Precession (bSSFP) sequence (FOV = 32 × 32 mm², data matrix = 64 × 64, flip angle = 19°, TE/TR = 1.9/3.8 ms, average = 4, temporal resolution = 1000 ms, no. of time points = 880). In the vocalization experiment, they were obtained using a multi-slice single-shot Gradient-Echo Planar-Imaging (GE-EPI) sequence (FOV = 32 × 32 mm², data matrix = 64 × 64, flip angle = 45°, TE/TR = 15.4/17.2 ms, average = 4, temporal resolution = 670 ms, no. of time points = 230).
In a block-design paradigm (Fig. 2d) that was made up of seven and Vaseline, to reduce the acoustic noise of scanner reaching the ears. Supplementary Materials). The other ear was occluded with cotton and a 6.5 cm soft tube into one of the animals’ ears (see illustration in individual experimental setup with stimulation delivered into the right ear of animals. The vocalization experiment was performed in another setup 1 week after the response gain experiment, in which the stimulation was presented to the left ear. Different sides were stimulated here to ensure the ear fresh from stimulation. Note that before each experiment, the auditory stimulation was calibrated outside the MRI magnet. The output waveform of each sound was measured by a recorder (FR2, Fostex, Japan) that was placed at ~2 mm from the tip of the flexible tube (Gao et al., 2014, 2015; Lau et al., 2015a,b). The variance of the SPL of each sound was maintained less than 2 dB.

### Auditory stimulation paradigms

Auditory stimulation was controlled by a computer and produced by a high-frequency magnetic speaker (MF1, TDT) driven by an amplifier (SA1, TDT) (Gao et al., 2014, 2015; Lau et al., 2015a,b). Monaural stimulation was delivered through a custom-made 165 cm long rigid tube and a 6.5 cm soft tube into one of the animals’ ears (see illustration in Supplementary Materials). The other ear was occluded with cotton and Vaseline, to reduce the acoustic noise of scanner reaching the ears.

In the response gain experiment, a broadband noise was presented in a block-design paradigm (Fig. 2d) that was made up of seven sound-on (80 s) periods and eight sound-off (40 s) periods. The total sound pressure level (SPL) of the noise was linearly ramped up from 10 dB to 90 dB during the first 40 s of the sound-on period and then ramped down to 10 dB in the next 40 s (see noise spectrum in Supplementary Materials). The SPL was varied to examine the dependence of BOLD responses on the SPL, and accordingly, a prolonged sound-on period was used to accommodate the slow hemodynamic process. In each animal, this paradigm was repeated twice.

In the vocalization experiment, a rat vocalization in the 22 kHz category (Portfors, 2007) (obtained online from http://www.avisoft.com/rats.htm, the “22 kHz rat calls” recorded from Wistar rats) and its temporal reversion (see sound spectrograms in Supplementary Materials) were presented in a standard block-design paradigm that was made up of six sound-on (20 s) periods and seven sound-off (40 s) periods (Fig. 2e). The forward and reversed vocalizations were alternatively used in different sound-on periods, and during each one, the vocalization (length 1.2 s, plus silence 0.8 s afterwards) was repeated ten times at 60% duty cycle. In each animal, this paradigm was repeated six times. In three of them, the forward vocalization was presented first and in the other three, the reversed one first. Note that the forward and reversed vocalizations contain identical long-term frequency spectrum except reversed timing.

For each animal, the response gain experiment was performed in an individual experimental setup with stimulation delivered into the right ear of animals. The vocalization experiment was performed in another setup 1 week after the response gain experiment, in which the stimulation was presented to the left ear. Different sides were stimulated here to ensure the ear fresh from stimulation. Note that before each experiment, the auditory stimulation was calibrated outside the MRI magnet. The output waveform of each sound was measured by a recorder (FR2, Fostex, Japan) that was placed at ~2 mm from the tip of the flexible tube (Gao et al., 2014, 2015; Lau et al., 2015a,b). The variance of the SPL of each sound was maintained less than 2 dB.

### Atropine manipulation

Response gain and vocalization fMRI experiments as described above were performed in two groups of normal adult male rats (n = 6 in each group, male, 250 g, Table 1), respectively, before and after intravenous injection of atropine, an antagonist of muscarinic acetylcholine receptors. After the pre-injection fMRI, atropine in saline (50 mg/ml) was injected (50 mg/kg) (Sutherland et al., 1982) via a tail vein catheter while the animals remained still inside the MRI scanner. The animals were allowed to
...the physiological conditions to recover. Then they underwent the same fMRI procedure again.

**fMRI data analysis**

In each experiment, the fMRI images from each animal were realigned and co-registered to a representative animal using SPM8 (Wellcome Department of Imaging Neuroscience, University College London, UK). Linear detrending was then performed in a voxel-wise manner.

For the response gain experiment, data from repeated trials were averaged, resulting in a single dataset per animal. For processing, the standard general linear model (GLM) analysis function provided in SPM8, which is widely used for conventional block-design paradigms, including in our previous auditory fMRI studies (Gao et al., 2014, 2015; Lau et al., 2015a,b), was not employed. Instead, to detect the expected linear BOLD signal change (Fig. 2d) and calculate the gain, the temporal correlation coefficient (CC) between the broadband noise SPL envelope and the BOLD signal profile in each voxel was calculated (Cheung et al., 2012a; Lau et al., 2013). Here a hemodynamic delay in the range of 3–8 s (at 1 s step) was included in the SPL envelope, and for each voxel, the one that resulted in the highest CC value was used. After this, activated voxels were identified by CC > 0.111 (corresponding to p < 0.001, uncorrected). This resulted in an activation map for each animal and these maps were averaged to obtain a mean activation map for each group.

Subsequently, four regions-of-interest (ROIs) in different IC/LL subdivisions were defined by consulting the Paxinos and Watson rat brain atlas. The BOLD signal profiles in each ROI were averaged across voxels and blocks (each block covering a 120 s period from 10 s before to 30 s after a sound-on period) and then scaled (by the mean signal intensity of the first 10 s) to calculate the BOLD signal change in percentage. The area under the BOLD signal profile was calculated by integrating the magnitude of the BOLD signal changes across the stimulation period, as a metric of response gain. The gain was compared between experimental groups using unpaired Student’s t-test with post hoc Holm–Bonferroni correction. To examine whether there were changes in the kinetics of the BOLD responses, the BOLD signal change was normalized by the gain and the rising and falling slopes were quantified by linearly fitting the 25th to 50th points and the 60th to 85th points, respectively. The slopes were similarly compared between experimental groups.

For the vocalization experiment, data from the forward-first and reversed-first trials were separately averaged, resulting in two datasets per animal. Each dataset was applied the standard GLM analysis (SPM8) (Gao et al., 2014, 2015; Lau et al., 2015a,b) to calculate the response coefficient (β) maps for both forward and reversed vocalizations. Activated voxels were identified with a following Student’s t-test (t > 3.13, corresponding to p < 0.001, uncorrected) on the β values. Since similar activation maps for both the forward and reversed vocalizations were obtained from the forward-first and reversed-first trials (Supplementary Materials), the β maps from the two datasets were further averaged, resulting in a single β map for forward vocalization and a single β map...
for reversed vocalization in each animal. These maps were averaged across animals to obtain mean $\beta$ maps for each group.

Following this, the difference between the $\beta$ maps for forward and reversed vocalizations was calculated to analyze response selectivity. Four ROIs in different IC/LL subdivisions were similarly defined by consulting the Paxinos and Watson rat brain atlas. The averaged $\beta$ value in each ROI was calculated and compared between forward and reversed vocalizations (paired Student's $t$-test with post hoc Holm–Bonferroni correction) to examine which IC/LL subdivisions exhibit response selectivity. The $\beta$ value difference between forward and reversed vocalizations, as a metric of response selectivity, was compared between experimental groups using unpaired Student’s $t$-test with post hoc Holm–Bonferroni correction.

Results

Auditory and visual cortices differentially modulate IC and LL gains

To examine the relationship between sound level and evoked response magnitude (i.e., gain), the sound pressure level (SPL) of a broadband noise stimulus to one ear was linearly modulated from 10 to 90 and then to 10 dB within 80 s, repeating every 120 s for seven times (Fig. 2d). The BOLD signals in the contralateral IC and LL (Fig. 3a) were observed to increase and then decrease, in an approximately linear fashion, with a hemodynamic delay of about 4 s (Fig. 3b and c). Note that no significant responses were observed in the ipsilateral IC or LL. The gain of the BOLD response, represented by the integrated magnitude of the BOLD signal change across the stimulation period, was quantified for nuclei of the IC and in LL (Fig. 3c).

Following AC ablation, the response was significantly increased in the central nucleus of the IC (CNIC, $p < 0.01$, Fig. 3c). In the dorsal cortex of the IC (DCIC), the response exhibited a clear trend of increase ($p < 0.05$ but did not pass the Holm–Bonferroni correction). However, no change was observed in the external cortex of the IC (ECIC). Since BOLD signals are highly correlated with neuronal firing rates (Logothetis et al., 2001; Mukamel et al., 2005), these results indicate that, in the absence of AC feedback, the firing rates of CNIC (and DCIC) neurons would become higher. The response gain in the dorsal nucleus of the LL (DNLL) also showed a trend of increase after AC ablation ($p < 0.05$ but did not pass the Holm–Bonferroni correction), consistent...
with the effect observed in its target, the CNIC. This suggests that AC co-modulates processing throughout the auditory brainstem.

VC ablation induced an effect opposite to that found for AC ablation. The gain of the BOLD response in the CNIC (and DCIC) was decreased after VC ablation (CNIC: \( p < 0.01 \); DCIC: \( p < 0.05 \) but did not pass the Holm–Bonferroni correction, Fig. 3c). This suggests that feedback from the VC may ordinarily serve to increase the dynamic range of the CNIC (and DCIC). In the ECIC, no apparent change was observed, similar to AC ablation. In the DNLL, the gain exhibited a clear trend of decrease after VC ablation (\( p < 0.05 \) but did not pass the Holm–Bonferroni correction), again consistent with the effect observed in its target (i.e., the IC), suggesting that VC also co-modulates processing throughout the auditory brainstem. Note that the BOLD signal profiles, after normalization by their respective gains (i.e., areas under the profiles), were nearly identical across the three groups, and no significant difference was observed for the rising or falling slopes (Supplementary Materials). This indicates that AC or VC ablation altered the gain of IC auditory responses, but not their kinetics.

Cortical modulation of response selectivity to species-specific vocalizations

To examine the impact of descending projections on natural stimulus processing, rat forward vocalizations and the same but temporally reversed vocalizations were presented to one ear in a block-design paradigm (Fig. 2e), and contralateral BOLD responses were acquired and analyzed (Fig. 4 and see BOLD signal profiles in Supplementary Materials). Again, no significant responses were observed in the ipsilateral side. In control animals, the BOLD response in the IC was stronger to the forward than to the reversed vocalization, demonstrating response selectivity (Fig. 4a) to the forward one. This selectivity was not observed in the LL, suggesting that it first emerges in midbrain. AC ablation generally reduced this selectivity (Fig. 4a), indicating that specific responses require descending feedback that originates in the AC. Ablation of the VC had little influence on response selectivity, suggesting that the cross-modal input does not contribute to the selectivity examined in this study.

Response selectivity was most prominent in the ECIC (Fig. 4b and c), and the influence of AC ablation was explained by nonequivalent changes of the BOLD responses to the forward and reversed vocalizations. Specifically, the averaged BOLD signal evoked by the forward vocalization was higher than the reversed one in all three IC subdivisions, but most significantly in the relatively large ECIC (ECIC: \( p < 0.001 \); CNIC: \( p < 0.01 \); DCIC: \( p < 0.05 \) but did not pass the Holm–Bonferroni correction). In the DNLL, the averaged BOLD signals evoked by the two vocalizations were nearly identical. Following AC ablation, the BOLD responses to the vocalizations were all increased, consistent with a general gain effect. However, in AC-ablated animals, the IC BOLD signal evoked by the forward vocalization increased less than that by the reversed one, leading to the decrease of the difference between them (\( p < 0.01 \), see Supplementary Materials), i.e. the reduction of the response selectivity to the forward one. Following VC ablation, the vocalization-evoked BOLD responses were all decreased, consistent with a change of gain, but the selectivity was not changed significantly (see Supplementary Materials).

Fig. 4. Ablation of auditory or visual cortices modulates IC response selectivity to species-specific vocalizations. (a) The activation (β) maps obtained from standard general linear model (GLM) analysis for the forward and reversed vocalizations and the difference (Δβ) between them in the NM, ACA, and VCA groups. Activated voxels with \( t > 3.13 \) (corresponding to \( p < 0.001 \), uncorrected) are shown by the heat map, and in the difference map, \( Δβ \) is further threshold at 0.3. They were detected in the contralateral IC and LL. The stronger response to the forward than the reversed vocalization demonstrates the response selectivity to the forward one in the IC (not LL). Auditory cortical ablation decreased the IC response selectivity while visual cortical ablation caused a much smaller effect. (b) Four analysis ROIs defined in the ECIC, CNIC, DCIC, and DNLL (left side) by consulting the Paxinos and Watson rat brain atlas (right side). The large ECIC and CNIC ROIs contain voxels from both slices, while the small DCIC ROI from only Bregma – 9.1 mm. (c) Comparison between the BOLD responses (mean \( t \) values) to forward and reversed vocalizations in each ROI (paired Student’s \( t \)-test with post hoc Holm–Bonferroni correction). Response selectivity to forward vocalization was seen in all three IC subdivisions but most prominently in the relatively large ECIC (ECIC: \( p < 0.001 \); CNIC: \( p < 0.01 \); DCIC: \( p < 0.05 \) but did not pass correction). Auditory cortical ablation generally increased the response to forward vocalization less than to the reversed one, therefore reducing the IC response selectivity (significantly in ECIC: \( p < 0.01 \), see Results and Supplementary Materials). Visual cortical ablation generally decreased responses to both forward and reversed vocalizations but did not change the response selectivity. The results are presented as means ± standard error of the mean and ** stands for \( p < 0.01 \), *** for \( p < 0.001 \), and n.s. for not significant.
Fig. 5. The effect of blocking the indirect cholinergic pathway from the AC to the IC (by atropine injection) on sound-evoked responses in the IC of normal animals. (a) Averaged activation (CC) maps for the broadband noise (as shown in Fig. 3a) and BOLD signal profiles in different IC/LL subdivisions (based on ROIs defined in Fig. 3b) before and after atropine injection. Atropine did not apparently alter the response gains. (b) The activation (β) maps for the forward and reversed vocalizations and their difference (as shown in Fig. 4a) before and after atropine injection. IC response selectivity to forward vocalization was almost eliminated by atropine. (c) Comparison between the BOLD responses to the forward and reversed vocalizations in different IC/LL subdivisions (based on ROIs defined in Fig. 4b, paired Student’s t-test with post hoc Holm–Bonferroni correction). Atropine mainly reduced the response to the forward vocalization, leading to minimal response selectivity (significantly in ECIC: p < 0.01, see Results and Supplementary Materials). The results are presented as means ± standard error of the mean and ** stands for p < 0.01 and n.s. for not significant.
Role of the cholinergic pathway in auditory cortical modulation

AC could influence IC function via direct corticocollicular projections or the indirect cholinergic pathway. Since both pathways were presumably inactivated by AC ablation, an initial effort to assess the contribution of the indirect cholinergic pathway was made by measuring sound-evoked responses before and after injecting the muscarinic acetylcholine receptor antagonist, atropine (Habicht and Vater, 1996). The noise-evoked BOLD responses were not affected by atropine (Fig. 5b), either in the IC or LL, suggesting that gain modulation was mediated primarily via direct corticocollicular projections. In contrast, atropine had a significant effect on vocalization-evoked IC responses (Fig. 5c) and c and see BOLD signal profiles in Supplementary Materials). The response selectivity to the forward vocalization, which was most prominent (p < 0.01) in the ECIC before injection, was nearly abolished following atropine injection (p > 0.01, Fig. 5b and c and Supplementary Materials). This effect was largely attributable to a drastic decrease of the BOLD response to the forward vocalization. These results suggest that the cholinergic pathway contributes to midbrain response selectivity to species-specific vocalizations, but not the gain. They also point to future studies for more comprehensive investigation of the differential roles of the two types of pathways in corticofugal modulation.

Discussion

Descending projections from AC modulate sensory processing in the thalamus (Antunes and Malmierca, 2011; Zhang et al., 1997), midbrain (Anderson and Malmierca, 2013; Ma and Suga, 2001; Nakamoto et al., 2008; Yan and Ehret, 2002; Yan et al., 2005; Yan and Suga, 1998; Zhang et al., 1997), ventral brainstem (Luo et al., 2008), and cochlea (Liu et al., 2010; Xiao and Suga, 2002). Despite the wealth of positive evidence, it is challenging to establish how these effects are integrated across a broad range of targets. Furthermore, while it has been assumed that descending control exerts the largest effect within sensory modality, the vast interconnectivity of sensory cortices suggests that a cross-modal influence could be profound. Here, we examined both of these issues using BOLD fMRI to monitor the entire auditory midbrain, a center in which all ascending information is encoded (Malmierca, 2003). The results suggest that AC projections exert a net suppressive influence on specific IC subnuclei and the LL, while VC projections provide a facilitatory influence. The AC influence on gain was associated with enhanced response selectivity to species-specific vocalizations, most prominently in the ECIC. Together, these results indicate that IC processing emerges through integration of descending input from both auditory and visual centers.

Auditory and visual cortical modulation of response gain in IC

Corticocollicular efferents contact all IC subdivisions, with the CNIC receiving less projections than the DCIC and ECIC (Bajo and Moore, 2005; Bajo et al., 2007; Schofield, 2009). This suggests that descending projections from AC should influence processing throughout the IC. In this fMRI study, the AC input was observed to suppress the gain in the CNIC and DCIC, but not influence the gain in the ECIC. This finding may suggest that cortical projections to different IC subdivisions play different functional roles, potentially to mediate different aspects of auditory processing in the IC. The suppression likely resulted from the inhibitory feedback that has previously been shown to reduce the dynamic range and response magnitude of IC neurons (Yan and Ehret, 2002). In particular, the noise stimulus that we used would be expected to recruit inhibitory feedback from AC to IC neurons with unmatched best frequencies (Yan and Ehret, 2002; Yan et al., 2005; Yan and Suga, 1998). This gain modulation mechanism may contribute to a number of functions in the auditory system. For example, it may allow the IC neurons to adjust their responses adaptively to the statistics of sound level within specific acoustic environments (Dean et al., 2005). It may also optimize IC neuron dynamic range to facilitate the perception of specific sounds, such as low-level communication sounds, in the presence of environmental noise (Rabinowitz et al., 2013). The result showed that gain modulation also occurred in LL nuclei that project to the IC, indicating that the descending influence of AC may exert a coherent influence on many auditory brainstem nuclei. This would be consistent with the existence of AC descending projections that target other structures along the auditory pathway (Coomes and Schofield, 2004; Schofield and Coomes, 2005a,b; Winer, 2006; Winer et al., 2001).

A significant finding of the present study was that VC provides strong modulatory drive to the auditory midbrain. Furthermore, the functional influence of the VC is opposite to that of the AC. Ablation of the VC reduced the gain of IC responses, indicating that descending input from the VC normally increases the responsiveness of IC neurons to auditory stimuli. This influence is possibly mediated by direct projections from the VC to the IC (Cooper and Young, 1976; Dong, 2008). Notably, the reduction of the BOLD responses to both the broadband noise and the vocalizations was most evident in the medial side of the IC (mainly corresponding to CNIC), where some of the voxels no longer showed statistically significant responses (Figs. 3a and 4a). This observation is consistent with anatomical evidence that the VC projections to the IC terminate densely in this region (Dong, 2008). It is also possible that the AC (Budinger et al., 2006; Campi et al., 2010) or the superior colliculus (Coleman and Clerici, 1987) relays the visual drive to the IC.

In cortex, cross-modal interactions are commonly observed in the normal brain or after sensory input deprivation (Bizley and King, 2009; Finney et al., 2001; Lomber et al., 2010; Petrus et al., 2014; Wallace et al., 2004) and may be mediated by specific corticocortical projections (Budinger et al., 2006; Campi et al., 2010; Falchier et al., 2002). Our findings demonstrate that these cross-modal interactions may impact early stages of the sensory processing, due to the large descending projections from cortex. The capability to synthesize cortical feedback from multiple senses may improve the accuracy of information encoding. For example, the encoding of sound location in the brainstem auditory pathway may be facilitated with the guidance of visual input (Groh et al., 2001; Custers and Groh, 2012; Gutfreund et al., 2002; Porter et al., 2007). Meanwhile, it may improve the sensitivity to salient congruent stimuli, such as infants’ sensitivity to space–pitch associations before acquiring languages (Dolscheid et al., 2014).

IC response selectivity to species-specific vocalizations and the role of cortical feedback

Vocalizations are important for conspecific aural communication throughout the animal kingdom (Simmons et al., 2003). Understanding the neural mechanisms underlying the learning, production, and representation of species-specific vocalizations may present important insights for understanding complex sound (e.g. speeches) processing in humans (Rauschecker and Scott, 2009). Rat can emit three types of vocalizations depending on the animal’s age, affective state, and its environmental conditions (Portfors, 2007). The current study examined the “22 kHz vocalizations” (in normal, cortex-ablated, as well as atropine-injected rats), which are usually emitted when the animals are in anticipation of inescapable aversive stimuli (Brudzynski, 2013; Portfors, 2007). In an additional experiment, we further examined and observed the midbrain response selectivity to “40 kHz” and “50 kHz vocalizations” (forward versus reversed) in a separate normal rat using similar BOLD fMRI setup (see Supplementary Materials). Whether the cortices influence the midbrain response selectivity to these vocalizations versus their temporal reversion will be examined in our future studies. Note that our recent fMRI study has demonstrated the BOLD responses to “50 kHz vocalizations” throughout the normal rat auditory system (Gao et al., 2015).

Our fMRI results clearly reveal the large-scale and strong response selectivity in the auditory midbrain to forward vocalizations versus their temporal reversion, despite their identical long-term frequency spectrum (Pincherli Castellanos et al., 2007). The present results are in
agreement with electrophysiological studies showing that the auditory midbrain is the first region in the ascending auditory pathway to display response selectivity to vocalizations and that the selectivity is shaped by inhibition (Pollak, 2013; Woolley and Portfors, 2013). The descending pathway from AC recruits inhibition to the IC, which is nonequivalent for forward and reversed vocalizations (Fig. 4c), thereby enhancing the representation of the biologically relevant forward vocalizations in the brainstem. This will possibly facilitate the animals’ perception of information conveyed by these sounds, such as the mood of their companions/mother/pups, their group status, or environmental conditions (Portfors, 2007). Our results further showed that vocalization response selectivity was most prominent in the ECIC, and AC ablation had its greatest effect on this subdivision, consistent with a prominent corticocochlear projection (Bajo and Moore, 2005; Bajo et al., 2007; Schofield, 2009). Taken together with the fact that AC ablation did not change of the gain in the ECIC, the finding here suggests that cortex modulates different aspects of auditory processing in the midbrain through different mechanisms.

**Auditory cortical modulation via direct and indirect pathways**

The auditory cortex innervates the cholinergic neurons in the pontomesencephalic tegmentum, which are the major source of acetylcholine to the auditory midbrain and thalamus, and a substantial source to the cochlear nucleus (Schofield et al., 2011). The divergent projections of the pontomesencephalic tegmentum neurons suggest that the AC can have complex and widespread effects on lower auditory centers via this indirect pathway. The present study has made an initial effort to understand the functional implications of these projections on auditory midbrain processing. Pharmacological blockade of the muscarinic cholinergic pathway (i.e. atropine injection) did not affect the gain (Fig. 5a) but did significantly reduce the response selectivity to the vocalizations in the IC (Fig. 5b and c). This is consistent with previous reports showing that activating the cholinergic neurons in the pontomesencephalic tegmentum induces an emission of the same category of vocalizations (Brudzynski, 2001). Taken together with the effects induced by AC ablation, the results may suggest that the AC influence on processing basic acoustic cues, such as sound level, is mediated primarily by the direct corticocochlear projections, whereas the influence on responses to natural vocalizations is mediated by both. By employing both direct projections and the indirect cholinergic pathway, the cortical descending inputs may essentially improve the efficiency of detecting and encoding these behaviorally relevant sounds in the auditory midbrain and reduce irrelevant information that is later relayed to the AC (Holmstrom et al., 2010).

Nevertheless, future investigations are necessary to comprehensively assess the differential roles of the direct and indirect pathways in auditory corticofugal modulation. In addition to the muscarinic acetylcholine receptors, the potential effect of nicotinic acetylcholine receptors should also be examined, as both types are found across the IC (Morley and Happe, 2000). The effects of acetylcholine agonist/antagonist itself on the BOLD signals should be controlled. The potential effects of acetylcholine transmission on other brain areas should be examined (Mooney et al., 2004; Vidal and Changeux, 1993). Meanwhile, it should be noted that other auditory nuclei may also mediate the AC feedback to the IC (Coomes and Schofield, 2004; Coomes Peterson and Schofield, 2007; Schofield and Coomes, 2005a,b), although their contributions are presumably smaller than the direct corticocochlear projections.

**Implications for future work**

The present findings can guide future studies on the coordinated activity of the auditory neuraxis, and its dysfunction in hearing disorders. For example, the functional connectivity between the AC and the IC is significantly reduced in hearing-impaired tinnitus patients (Boyen et al., 2014). Whether this is associated with a dysfunction of AC feedback remains to be investigated. Meanwhile, by integrating the functional impact of the descending inputs, an improved understanding of brainstem auditory processing may facilitate the design and use of brainstem implants for the restoration of hearing (Geleoc and Holt, 2014; Lim and Lenarz, 2015). Finally, our findings have implications for the development of AC stimulation strategies to induce appropriate changes in brainstem circuitry, which may serve as therapeutic interventions to facilitate speech perception or suppress tinnitus (Zhang, 2013).

On the other hand, limitations of the current study should be noted for future work. First, this study employed a light anesthesia (1.0% isoflurane) during fMRI to prevent imaging artifacts caused by animal movement. Although our previous fMRI studies show that both the AC and VC respond to external stimuli under such condition (Cheung et al., 2012a; Lau et al., 2015b; Zhang et al., 2013a; Zhou et al., 2012), electrophysiological studies suggest that anesthetics can affect the neural responses in the cortex (Schumacher et al., 2011; Ter-Mikaelian et al., 2007). Therefore, the cortical modulation effects revealed by this study may not be entirely equivalent to those when the animals are awake. Future studies may examine awake animals to further understand the difference (Liang et al., 2011). Second, this study employed prolonged auditory stimulation, which could result in adaptation in IC neurons. How adaptation influences the measured BOLD fMRI signals and subsequently the difference among different groups was unclear. Future studies may investigate this issue in more detail. Third, this study adopted a gross ablation model (i.e. AC and VC ablation) in coordination with the large-view fMRI technique to investigate the general and large-scale cortical modulation effects. Ablation eliminated both ascending and descending signaling in all cell types of the AC or VC. Meanwhile, plastic changes in other areas of the brain may occur during the period of recovery and may have mediated the changes of IC BOLD responses. With other techniques available (Bajo et al., 2010; Leach et al., 2013), including optogenetics tools (Boyd et al., 2005; Lee et al., 2010), future studies may further investigate how the interactions between different neuron populations (e.g. excitatory and inhibitory) in different cortical fields or layers exactly contribute to the corticofugal effects revealed by this imaging study on a finer spatiotemporal scale, both within and across sensory modalities.

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AC</td>
<td>auditory cortex</td>
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<tr>
<td>IC</td>
<td>inferior colliculus</td>
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<td>LL</td>
<td>lateral lemniscus</td>
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<td>VC</td>
<td>visual cortex</td>
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<td>CNIC</td>
<td>central nucleus of the IC</td>
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<td>DCIC</td>
<td>dorsal cortex of the IC</td>
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<td>ECIC</td>
<td>external cortex of the IC</td>
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<tr>
<td>DNL</td>
<td>dorsal nucleus of the LL</td>
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<td>ACA</td>
<td>auditory cortex ablation</td>
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<tr>
<td>VCA</td>
<td>visual cortex ablation</td>
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<tr>
<td>NM</td>
<td>normal</td>
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<td>BOLD</td>
<td>blood-oxygen-level-dependent</td>
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<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
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<td>RARE</td>
<td>rapid acquisition with refocused echoes</td>
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<td>bSSFP</td>
<td>balanced steady-state-free-precession</td>
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<tr>
<td>GE-EPI</td>
<td>gradient-echo echo-planar-imaging</td>
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<tr>
<td>SPL</td>
<td>sound pressure level</td>
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<td>CC</td>
<td>correlation coefficient</td>
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<td>GLM</td>
<td>general linear model</td>
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<tr>
<td>ROI</td>
<td>region of interest</td>
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**Author contributions**

This study was conceived and designed by E. X. W. and D. H. S. The animal surgery was conducted by S.-J. F. The auditory fMRI experiments were performed by P. P. G. and J. W. Z. Data were analyzed by P. P. G., J. W. Z., and E. X. W. The results were discussed by all authors. The


