

Research paper

Development of functional organization of the pallid bat auditory cortex

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Abstract

The primary auditory cortex is characterized by a tonotopic map and a clustered organization of binaural properties. The factors involved in the development of overlain representation of these two properties are unclear. We addressed this issue in the auditory cortex of the pallid bat. The adult pallid bat cortex contains a systematic relationship between best frequency (BF) and binaural properties. Most neurons with BF <30 kHz are binaurally inhibited (EO/I), while most neurons with BF >30 kHz are monaural (EO). As in other species, binaural properties are clustered. The EO/I cluster contains a systematic map of interaural intensity difference (IID) sensitivity. We asked if these properties are present at the time the bat acquires its full audible range (postnatal day [P] 15). Tonotopy, relationship between BF and binaural properties, and the map of IID sensitivity are adult-like at P15. However, binaural facilitation is only observed in pups older than P25. Frequency selectivity shows a BF-dependent sharpening during development. Thus, overlain representation of binaural properties and tonotopy in the pallid bat cortex is remarkably adult-like at an age when the full audible range is first present, suggesting an experience-independent development of overlapping feature maps.

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1. Introduction

Both experience-dependent and -independent factors guide the development of topographic maps and response properties in the central nervous system. The relative contributions of these factors have been extensively studied in the visual and somatosensory systems (Fox, 1996; Katz and Shatz, 1996; Naeyele et al., 1988). The general picture emerging from these studies is that experience-independent factors, such as gradients of chemicals and/or correlated

spontaneous activity, guide the initial gross topography, while sensory-driven activity is involved in the subsequent refinement of maps. Similarly, response properties such as receptive field diameters (Carrasco et al., 2005), ocular dominance (Crowley and Katz, 2002) and orientation selectivity (Chapman et al., 1999) may also be initially shaped by experience-independent factors, and require visual experience for further refinement or maintenance.

Studies on the development of primary auditory cortex (A1) have focused on excitatory frequency selectivity and tonotopy. While the time course of maturation of tonotopy shows species-specific differences, the initial formation of tonotopy appears to be driven by experience-independent factors (Bonham et al., 2004; Eggermont, 1996; Pienkowski and Harrison, 2005; Zhang et al., 2001). Experience with sound is, however, important for either the refinement or maintenance of tonotopy, as evidenced by the effects of partial lesions of the cochlea or noise-rearing on tonotopy in A1 (Harrison et al., 1993; Zhang et al., 2001). Frequency

Abbreviations: A1, primary auditory cortex; BF, best frequency; CL, contralateral; EE, generic term for binaurally excited neuron; EI or EO/I, term for binaurally inhibited neuron; EO or EO/O, monaural neuron; EO/F, binaurally facilitated neuron; EO/FI, binaurally facilitated and inhibited neuron; FM, frequency modulated; IID, interaural intensity difference; IL, ipsilateral; IT, inhibitory threshold measured in IID values; P, postnatal day

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selectivity shows a protracted period of sharpening or broadening following hearing onset, and may depend on sensory-driven activity (Bonham et al., 2004; Zhang et al., 2001). Thus, A1 appears to be similar to other sensory cortices in the development of topographical representation of the receptor surface.

Besides tonotopy, A1 is characterized by non-random distributions of other response properties (reviewed in Brugge, 1985; Ehret, 1997; Read et al., 2002). The best studied of these is binaural selectivity. A consistent finding is that cortical neurons with similar binaural properties occur in clusters (Imig and Adrian, 1977; Middlebrooks et al., 1980; Reale and Kettner, 1986; Nakamoto et al., 2004; Kelly and Sally, 1988; Kelly and Judge, 1994; Liu and Suga, 1997; Shen et al., 1997; Recanzone et al., 1999; Rutkowski et al., 2000; Velenovsky et al., 2003). In cats and ferrets, clusters of neurons with different binaural properties such as binaural inhibition (EI) and excitation (EE) are represented orthogonal to the isofrequency contours (Imig and Adrian, 1977; Kelly and Judge, 1994). This form of organization has given rise to the idea that isofrequency contours form functional subunits in A1 (Read et al., 2002). Binaural cues change as the head and ear morphology change during development. It has been shown that binaural selectivity of individual A1 neurons is adult-like before ear and head morphology become adult-like (Brugge et al., 1988). That is, binaural properties are mature while frequency selectivity is still undergoing developmental changes. However, the maturation of the clustered representation of binaural selectivity and its relationship to tonotopy has not been studied. The main objective of this study was to address this issue in the auditory cortex of the pallid bat.

The pallid bat is well suited to address this developmental issue for three reasons. First, it is an altricial species in which hearing development has been well characterized (Brown, 1976). Second, the cortical representation of response properties has been studied in the adult. Binaural selectivity shows a frequency-dependent distribution in adult cortex (Razak and Fuzessery, 2002). Most neurons with best frequencies (BF) <30 kHz are binaurally inhibited (EO/I type), while most neurons with BF >30 kHz are not influenced by binaural stimulation (monaural or EO type). The pallid bat is a gleaning bat that uses passive hearing of prey-generated noise (5–35 kHz) for prey localization, while reserving echolocation (downward frequency-modulated sweeps, 60–30 kHz) for obstacle avoidance. In the adult cortex, most neurons with BF <35 kHz are selective for noise, while most neurons with BF >35 kHz are downward frequency-modulated (FM) sweep-selective. Because of the relationship between BF and noise- or FM sweep-selectivity, most noise-selective neurons are binaurally inhibited, while most FM sweep-selective neurons are monaural. Thus, binaural sensitivities are largely different in the two regions representing noise and downward FM sweeps, two sounds used in two different behaviors. In this study, we examined the development

of frequency selectivity, binaural properties, and the systematic spatial relationship between these properties.

A second reason for studying the development of cortical binaural topography in the pallid bat is because it is the only species in which a systematic representation of interaural intensity difference (IID) sensitivity has been reported (Razak and Fuzessery, 2002). Within the noise-selective region, the cluster of EO/I neurons has a systematic representation of IID sensitivity (Razak and Fuzessery, 2000, 2002). This study examined the development of this IID map. We report that adult-like tonotopy, clustered organization of binaural properties, the relationship between BF and binaural type, and the systematic map of IID selectivity are present in the youngest pups examined. However, binaural facilitation is not present until P25 and frequency selectivity is not adult-like up to P40.

2. Materials and methods

Recordings were obtained from the auditory cortex of both cortical hemispheres in pallid bat pups that were born and raised in the University of Wyoming animal facility. Pregnant bats were netted in New Mexico and transported to the animal care facility at the University of Wyoming. The bats were maintained in cages and fed mealworms raised on ground Purina rat chow. Their room was heated, and maintained on a reversed 12:12 h light:dark cycle. When pups were born, the mom and her pups were moved to another cage to keep track of the pups' ages. Pups born within three days of each other were housed together. Therefore, the ages reported in this paper are accurate to within three days. The pups and moms remained in their cages until the day of recording from the pups.

2.1. Surgical procedures

The procedures used in pups were identical to those used in adults, and reported previously (Razak and Fuzessery, 2002). Recordings were obtained from bats that were anesthetized with Metofane (methoxyflurane) inhalation, followed by an intraperitoneal injection of pentobarbital sodium (30 µg/g body wt) and acepromazine (2 µg/g body wt). To expose the auditory cortex, the head was held in a bite bar, a midline incision was made in the scalp, and the muscles over the dorsal surface of the skull were reflected to the sides. The front of the skull was scraped clean and a layer of glass microbeads applied, followed by a layer of dental cement. The bat was then placed in a Plexiglas restraining device. A cylindrical aluminum head pin was inserted through a cross bar over the bat's head and cemented to the previously prepared region of the skull. This pin served to hold the bat's head secure during the recording session. The location of the auditory cortex was determined relative to the rostrocaudal extent of the midsagittal sinus, the distance laterally from the midsagittal sinus, and the location of a prominent lateral blood ves-

sel that travels parallel to the midsagittal sinus. The size of the exposure was usually $\sim 2 \text{ mm}^2$. Exposed muscle was covered with petroleum jelly (Vaseline), and exposed brain surface was covered with paraffin oil to prevent desiccation.

2.2. Recording procedures

Experiments were conducted in a heated ($\sim 30^\circ\text{C}$), sound-proofed chamber lined with anechoic foam. Bats were kept lightly anesthetized throughout the course of the experiments with sedation maintained by inhalation of Metofane and additional pentobarbital sodium (1/3 of pre-surgical dose) injections. Stimuli were generated using Modular Instruments and Tucker Davis Technologies digital hardware, and custom-written software (Fuzessery et al., 1991). The waveforms were amplified with a stereo amplifier and presented as closed-field stimuli through Infinity emit-K ribbon tweeters fitted with funnels that were inserted into the pinnae and sealed there with petroleum jelly. This procedure attenuated speaker intensity level at the opposite ear by $\geq 30 \text{ dB}$ and permitted presentation of the $\pm 25 \text{ dB}$ IIDs that the bat normally experiences (Fuzessery, 1996) without significant acoustic interactions between speaker outputs. The speaker-funnel frequency response curve showed a gradual increase of 20 dB from 6 to 70 kHz , as measured with a Bruel and Kjaer $1/8 \text{ in.}$ microphone placed at the tip of the funnel. Bandpass filtering of noise stimuli was performed using a Krohn Hite (model 3550) filter (24 dB/octave attenuation slope).

Using glass microelectrodes (1 M NaCl , $3\text{--}10 \text{ M}\Omega$ impedance), recordings were obtained at depths between 200 and $600 \mu\text{m}$, which corresponds to layers $3\text{--}5$ in the adult cortex. In the youngest pups (P15), these depths correspond to layers $3\text{--}6$. By P20, the cortical thickness and layers are adult-like. Penetrations were made orthogonal to the surface of the cortex. Electrode penetrations were placed as uniformly as possible. Response magnitudes and post-stimulus time histograms were acquired and stored with the use of a Modular Instruments high-speed clock controlled by custom software. Responses magnitudes are shown in graphs as the total number of spikes elicited by 20 stimulus presentations.

Results are based on both single-unit and multiunit recordings. Single units were identified based on waveform shape, duration and stability and isolated based on amplitude using a window discriminator (BAK Electronics, MD). The response properties of the majority of cortical sites were quantified with multiunit cluster recording due to the need to rapidly survey as many sites as possible. In these cases, measures of selectivity for relevant complex sounds were therefore influenced by the least selective neurons in the cluster. Nonetheless, as seen in adults, when a single unit was isolated from a multiunit recording, the selectivity was similar in both the neuron and multiunit cluster, suggesting that neurons in close anatomical prox-

imity had similar response properties. In this paper, ‘site’ is used to refer to multiunit recording and ‘neuron’ is used to refer to single unit recording.

2.3. Data acquisition

Pure tones ($5\text{--}70 \text{ kHz}$, $5\text{--}10 \text{ ms}$ duration, 1 ms rise/fall times, 1 Hz repetition rate) were used to determine BFs and frequency tuning. BF was defined as the frequency that elicited a response to at least five successive stimulus repetitions at the lowest intensity. The intensity was then increased in 5 or 10 dB steps to record tuning characteristics.

The choice of test stimuli in this study was based on the knowledge of the bat’s auditory behavior and on previous studies of the selectivity present in the adult cortex (Bell, 1982; Razak and Fuzessery, 2002). The pallid bat attends to noise transients to detect and locate prey. Its echolocation pulse is a short, $2\text{--}6 \text{ ms}$ duration, $60\text{--}30 \text{ kHz}$ downward FM sweep (Brown, 1976; Fuzessery et al., 1993). Therefore, the following short-duration ($\leq 10 \text{ ms}$) sounds were used as probes to test for functional specialization: pure tones at BF, broadband noise ($1\text{--}100 \text{ kHz}$) and upward and downward FM sweeps of identical duration (5 ms) and spectrum ($30\text{--}50$ or $30\text{--}60 \text{ kHz}$). Rate-level functions were obtained for each stimulus, typically from 0 to 40 dB above response threshold. Neurons termed selective for one of these stimuli exhibited a response threshold for that stimulus that was at least 10 dB lower than the others, or elicited responses that were at least 30% greater than the maximum response to other stimuli.

IID sensitivity was determined with either noise or downward FM sweeps ($60\text{--}30 \text{ kHz}$) depending on which of these two stimuli produced maximum response. IID sensitivity was studied by fixing the contralateral (CL) intensity, at $10\text{--}40 \text{ dB}$ above threshold, while the intensity at the ipsilateral (IL) ear was varied from 30 dB below to 30 dB above the CL intensity, in 5 dB steps. IID sensitivity was determined at more than one CL intensity. IID is defined as the CL intensity minus the IL intensity. Neurons were considered binaurally inhibited (EO/I) if the response to binaural stimulation at any IID within the test range was $\leq 30\%$ of the maximum response to contralateral stimulation alone (Fig. 1a). Neurons whose maximum responses to binaural and monaural CL stimulation were within 30% of each other were classified as EO (Fig. 1b). A neuron was considered binaurally facilitated (EO/F) if the maximum binaural response was $\geq 30\%$ of the maximum response to contralateral stimulation, and if the response did not decline below the monaural CL response as the IL intensity was increased (Fig. 1c). Neurons with mixed binaural interactions (EO/FI) exhibited a mixture of facilitation and inhibition for binaural stimuli compared with monaural CL stimuli (Fig. 1d). The EO/FI neurons were similar to the predominantly binaural neurons described in the literature (e.g. Nakamoto et al., 2004).

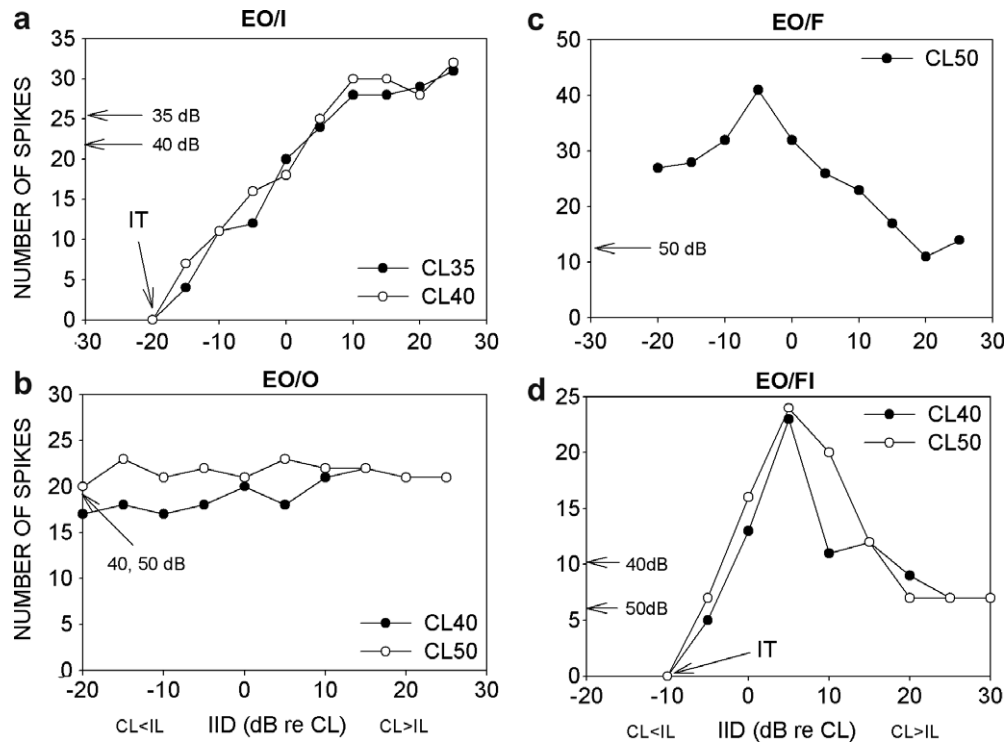


Fig. 1. Types of binaural responses in the pallid bat auditory cortex. Binaural selectivity was determined by presenting sound stimulus at a fixed intensity in the contralateral (CL) ear, and increasing the ipsilateral (IL) intensity from 20 to 30 dB below to 20–30 dB above the CL intensity. In most neurons, interaural intensity difference (IID) functions were obtained for at least two different CL intensities. The CL intensity at which each IID function was obtained is shown in the binaural plot legend; positive values indicate that the CL intensity is louder. The arrows in each panel denote the response to monaural CL stimulation at the intensity at which binaural functions were obtained. (a) EO/I – binaural inhibition. For binaural stimuli favoring the CL ear, the neuron's response was similar to the monaural response. As the intensity in the IL ear was increased, the response was suppressed below the monaural response. (b) EO/O – monaural neuron. The response of the neuron at all IIDs tested was within 30% of monaural response. (c) EO/F – binaural facilitation. Binaural response was at least 30% greater than monaural response. In addition, the response of the neuron did not decline below monaural response for increasing IL intensities. (d) EO/FI – neuron with mixed binaural interactions. The response of the neuron to binaural stimuli with near equal intensities at the two ears was at least 30% greater than its response to monaural stimuli or stimuli favoring either ear. IID sensitivity was quantified in EO/I and EO/FI neurons by measuring the inhibitory threshold (IT). IT (arrows in a, d) was determined in EO/I and EO/FI neurons as the IID at which the neuron was maximally inhibited. Arrows pointing to the y-axis indicate monaural response.

IID sensitivity was quantified by measuring the inhibitory threshold (IT), defined as the IID at which a neuron was maximally inhibited (arrows in Fig. 1a and d). IID sensitivity was determined at more than one CL intensity and averaged to provide an IT value for each site (Razak and Fuzessery, 2002). All procedures followed the animal welfare guidelines required by the National Institutes of Health and the Institutional Animal Care and Use Committee.

3. Results

Hearing onset in the pallid bat occurs at P8 (Brown et al., 1978). Low-frequency (<30 kHz) sensitivity is mature before P10 and the full audible range (5–80 kHz) is present at P15 (Brown et al., 1978). Pups start to emit orientation pulses resembling those used in echolocation around P9 (Brown, 1976). Pallid bats begin flying and echolocating between P30–P35 (Brown, 1976). Electrophysiological recordings were obtained from P15–P40 pups, when the complete audible range is first present to the time of flight onset, to delineate tonotopy and maps of binaural proper-

ties. Fourteen bat pups were used in this study. The focus was on the development of two specialized regions studied extensively in the adult pallid bat cortex, namely the FM sweep-selective region and the noise-selective region (Razak and Fuzessery, 2002, 2006; Razak et al., 2006).

3.1. Development of tonotopy

The adult pallid bat auditory cortex exhibits three features in terms of responses to pure tones (Razak and Fuzessery, 2002; Razak et al., 2006). First, BF increases from 7 kHz to >50 kHz in a caudolateral to rostromedial direction. Second, the BF distribution is not uniform. BFs between 10 and 20 kHz and 35–45 kHz are over-represented, while BFs between 20 and 30 kHz are under-represented. This results in abrupt transitions in the tonotopic map between closely spaced recording sites across the cortex. The most frequently observed jumps are those from below 20 kHz to 30–40 kHz. Third, single neurons in these transition regions exhibit multi-peaked frequency tuning curves (Razak et al., 1999). These neurons respond to two different bands of frequencies, one typically centered

between 10 and 20 kHz and the other between 30 and 45 kHz. These bands are discrete with no intervening frequencies eliciting any response. The centers of these bands correspond to the frequencies used in passive localization of prey and echolocation, respectively.

Responses to pure tones were recorded in pallid bat pups to determine the time course of development of these three features of adult cortex. Fig. 2a shows the BF map in the youngest pup (P15) tested. BF increased from 8 kHz to >40 kHz in a caudolateral to rostromedial direction. BFs between 20 and 30 kHz were under-represented in this cortex. Sudden transitions in BF from <18 kHz to >40 kHz were observed in the rostrocaudal direction. Three sites in the transition region (shown in boxes) exhibited multi-peaked tuning curves. The most medial of these three sites exhibited three peaks in its tuning curve, while the other two sites showed two peaks. The individual variability in tonotopy and the direction of sudden transitions in BF can be seen from the additional examples shown in

Fig. 2b–e. Despite the variability, the three features of frequency representation in adult cortex were present in all these maps.

Frequency tuning curves of neurons with multiple peaks are shown in Fig. 3. The neuron in Fig. 3a responded to two separate bands of frequencies with similar thresholds, while the neuron shown in Fig. 3b exhibited a higher threshold for lower frequencies. As in adults, the peaks were discrete and separated by a range of frequencies that elicited no response. The neuron shown in Fig. 3c exhibited three peaks in its frequency tuning. Four sites with three peaks in their frequency tuning curves were observed in pups. Neurons with three peaks in their tuning curve were not observed in adults (Razak et al., 1999).

Differences between pups and adults were also observed in the spatial location of neurons with multi-peaked tuning. In the example shown in Fig. 2b, BF increased from 7 kHz to 59 kHz in a caudolateral to rostromedial direction. The transition in BF from <20 kHz to >40 kHz occurred in a

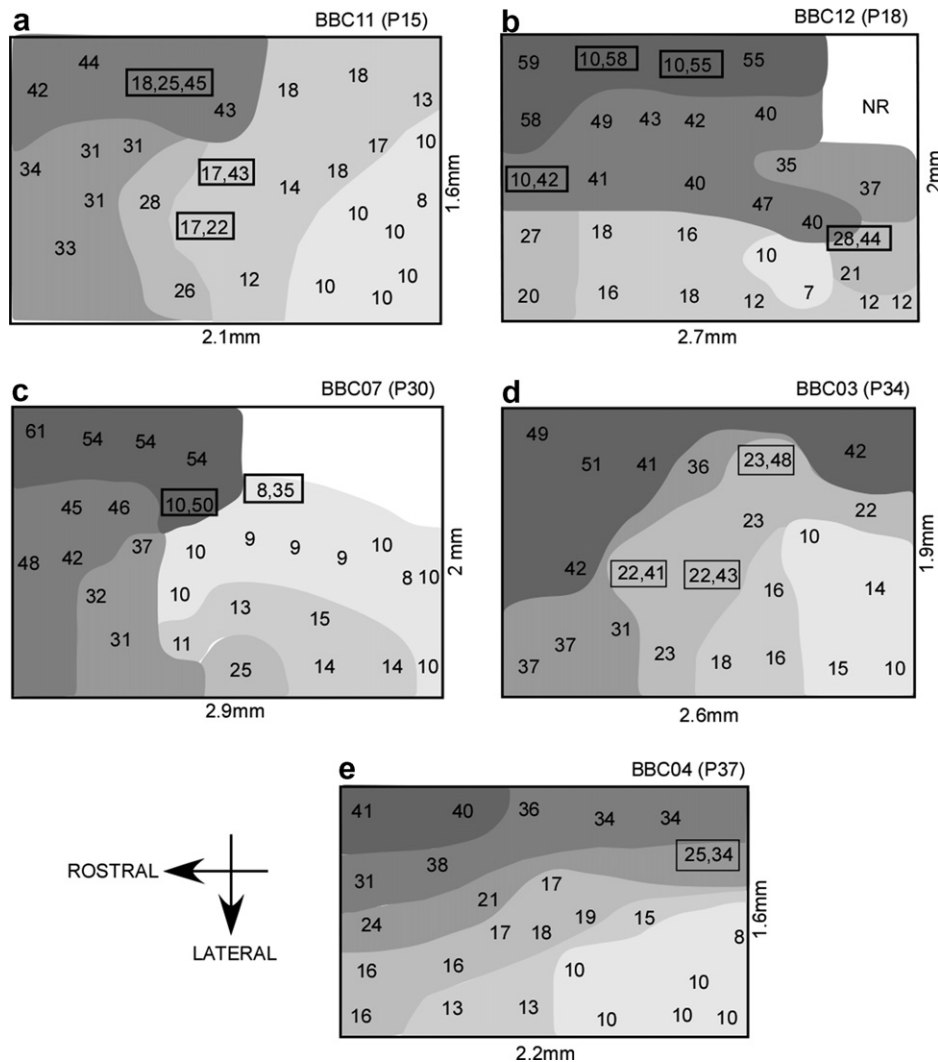


Fig. 2. Development of tonotopy in the pallid bat auditory cortex. The best frequency representation is shown in P15 (a), P18 (b) and P30 (c), P34 (d) and P37 (e) pup cortices. Various shades of gray delineate bands of frequencies. Sites shown in boxes exhibited multiple peaks in their tuning curves. A general increase in the caudolateral to rostromedial direction can be seen. NR – no response to tones. Areas in white were either not mapped, or did not exhibit response to tones.

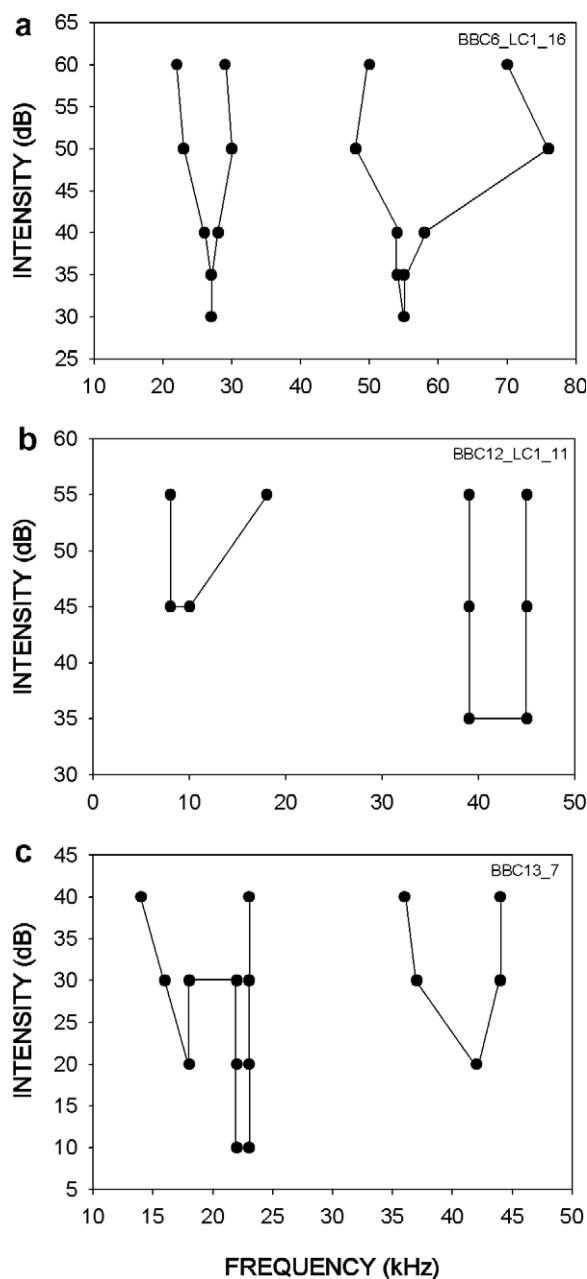


Fig. 3. Examples of neurons with multi-peaked tuning curves recorded from pup cortex. The neurons shown in (a) and (b) had showed two peaks in their tuning curves. The neuron in (c) exhibited three peaks in its tuning curve.

lateromedial direction. Four sites (shown in boxes) exhibited multi-peaked frequency tuning curves. In this cortex, two sites (10, 58 kHz and 10, 55 kHz) with two peaks of frequency tuning were recorded away from the transition region in the most medial locations of the map. The other two sites with multi-peaked tuning curves were recorded in the transition region. This is in contrast to adult maps wherein multi-peaked frequency tuning is typically found only in the region of transition (Razak et al., 1999; Razak and Fuzessery, 2002).

The spatial locations of neurons with multi-peaked tuning curves can also be inferred from the frequencies at which the peak occurs. In adults, 30/31 (97%) neurons with multi-peaked tuning curves exhibited high-frequency tuning centered between 30 and 44 kHz (Table 1), which is the frequency range of neurons in the transition region. In pups, 59% of multi-peaked neurons had high-frequency tuning centered at frequencies 30–44 kHz. The remaining 41% had high-frequency tuning centered at frequencies >45 kHz (Table 1, e.g. Fig. 3a). This shows that more multi-peaked neurons are present further from the transition region in pups than in adults.

Similar to adults (Razak and Fuzessery, 2002), the distribution of BF was not uniform in pup cortex (Fig. 4). In both the young (15–25 days, Fig. 4a) and the older (26–40 days, Fig. 4b) pups, BF distribution peaked at <20 kHz and around 40 kHz, with frequencies between 20 and 30 kHz being under-represented. Taken together, these data show that while there were individual differences in the representation of frequencies, the three features described in adult cortex were found from P15 onwards. However, multi-peaked tuning curves were found further away from the transition region in pups than in adults.

3.2. Development of frequency selectivity and minimum thresholds

Excitatory frequency tuning curves showed a frequency-dependent sharpening with age. The bandwidth of excitatory frequency tuning curves at 10, 20 and 30 dB above minimum threshold (MT) was used to quantify frequency selectivity in the auditory cortex of adults and during development (Fig. 5a–c, Table 2). In neurons with BF <30 kHz, bandwidth was not significantly different between pups and adults at any of the three intensities tested ($p > 0.05$, one-way ANOVA with Tukey post hoc pairwise comparison). However, for BF >30 kHz, developmental change was observed. At 10 and 20 dB above MT, the bandwidth in pups was broader than in adults (Fig. 5a and b, $*p < 0.05$, one-way ANOVA with Tukey post hoc pairwise comparison). At 30 dB above MT, the bandwidth was broader in the youngest pups (15–25 days old) compared to older pups (26–40 days old) and adults. There was no significant difference between older pups and adults. These data show that frequency selectivity sharpens with age in neurons that are involved in echolocation behavior. The average MT of neurons with BF <20 kHz, 20–30 kHz

Table 1

The number in each cell indicates the percentage of bimodal neurons with low- (LF) and high-frequency (HF) tuning within a certain range

Frequency range (kHz)	Pups (%)	Adults (%)
LF range 8–19	65	52
LF range 20–30	35	48
HF range 30–44	59	97
HF range 45–60	41	3

A higher percentage of pup multimodal neurons exhibited a high-frequency peak centered at frequencies >45 kHz.

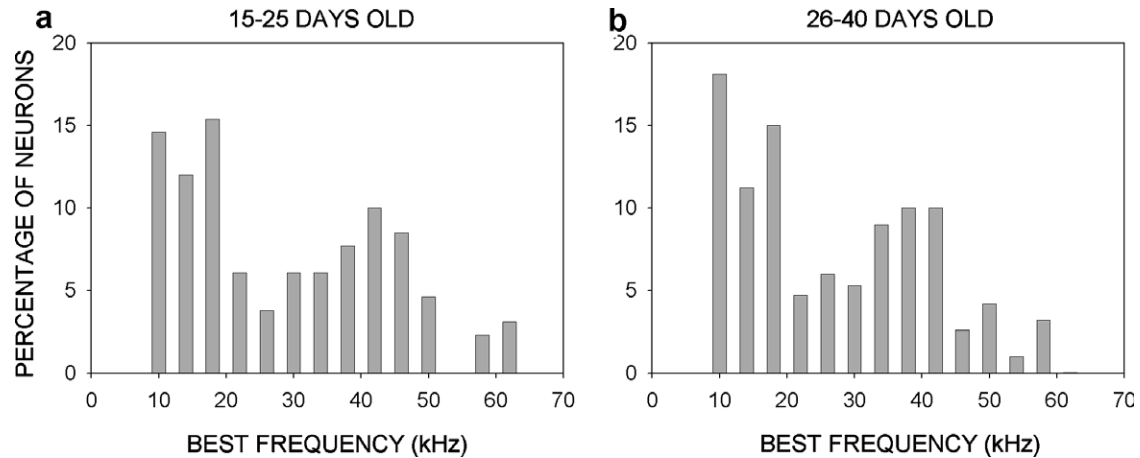


Fig. 4. Distribution of BF in the pallid bat auditory cortex during development. BF had a bimodal distribution with peaks <20 kHz and between 35–45 kHz in the young (a) and older (b) pup groups. Each bin in the histogram encompasses a 4 kHz range of BF.

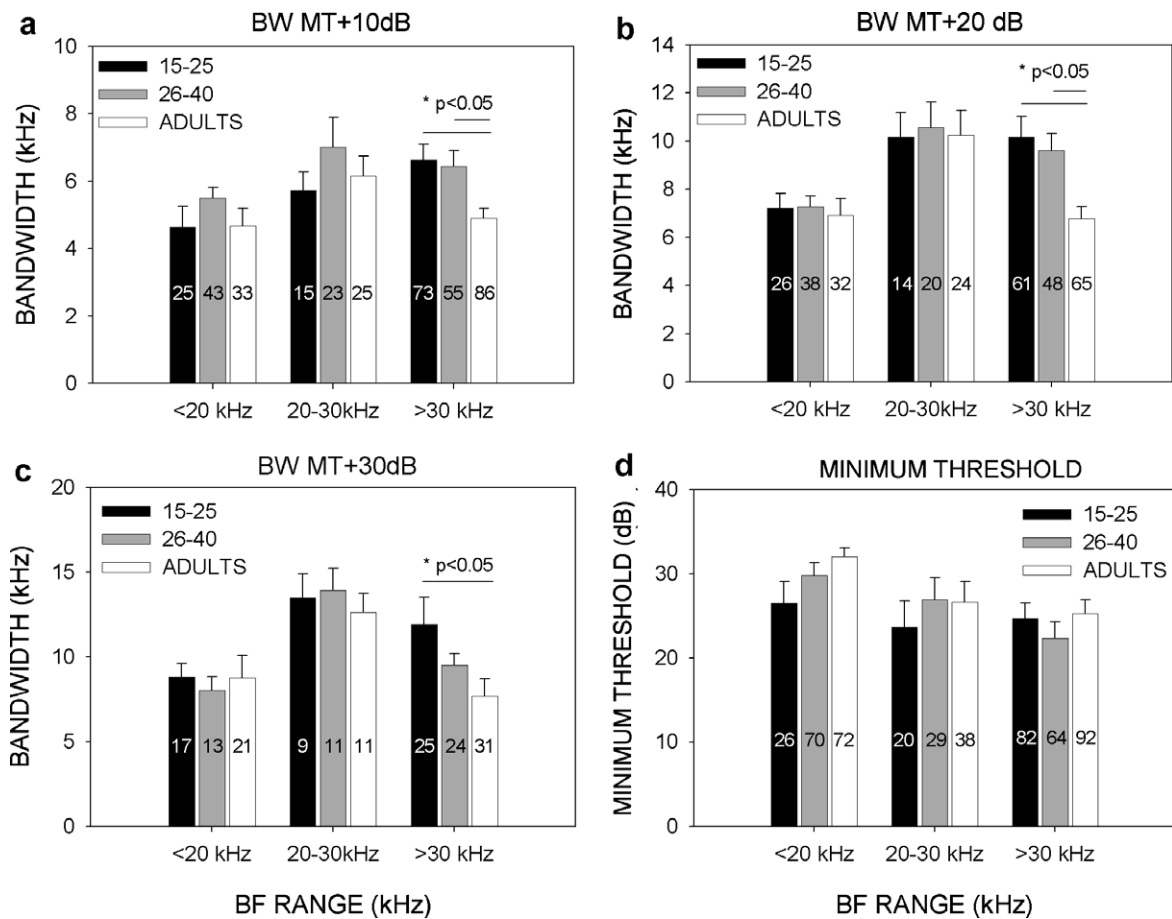


Fig. 5. Development of frequency selectivity and minimum threshold (MT). Sharpness of frequency selectivity was quantified using the bandwidth (BW) at 10 (a), 20 (b) and 30 (c) dB above MT. At all three intensities, neurons with BF <30 kHz exhibited similar bandwidths in pups and adults. Neurons with BF >30 kHz showed a developmental reduction in bandwidths. The number in each bar indicates the number of neurons. (d) MTs of neurons with BF <20 kHz, 20–30 kHz and >30 kHz were not significantly different across the different age groups. Numbers with legends indicate age in days.

and >30 kHz was not significantly different across the three age groups (one-way ANOVA, Tukey post hoc test for pairwise comparisons, $p > 0.05$). This suggests that the

observed difference in frequency selectivity was not due to different intensities relative to MT at which tuning bandwidths were measured at different ages.

Table 2
Bandwidth (BW) of excitatory tuning curves determined at 10, 20, 30 dB above minimum threshold in pup and adult cortices

BF range	15–25 days old			26–40 days old			Adults		
	BW10	BW20	BW30	BW10	BW20	BW30	BW10	BW20	BW30
<20 kHz	4.6 ± 0.6	7.2 ± 0.6	8.8 ± 0.8	5.5 ± 0.3	7.3 ± 0.4	8 ± 0.8	4.7 ± 0.5	6.9 ± 0.7	8.7 ± 1.3
20–30 kHz	5.7 ± 0.5	10.1 ± 1	13.4 ± 1.4	7 ± 0.9	10.5 ± 1.1	14 ± 1.3	6.2 ± 0.6	10.3 ± 1	12.6 ± 1.0
>30 kHz	6.7 ± 0.5	10 ± 0.9	11.5 ± 1.5	6.3 ± 0.5	9.4 ± 0.7	9.5 ± 0.7	5.0 ± 0.3	6.9 ± 0.5	8.2 ± 0.9

Each cell shows the mean (± s.e.) BW (in kHz) for sites with BF in a certain range.

3.3. Development of binaural properties

The adult pallid bat auditory cortex exhibits a clustered organization of binaural properties (Razak and Fuzessery, 2000, 2002). The cortical region with BF <30 kHz is dominated by a large cluster of EO/I neurons. The cortical region with BF >30 kHz contains a large cluster of EO neurons and smaller clusters of EO/F and EO/I neurons. A cluster of neurons with EO/FI sensitivity straddles both

regions, and is found rostralateral to the low-frequency EO/I cluster and lateral to the high-frequency EO cluster. Therefore, binaural properties show a BF-dependent distribution. Nearly 70% of neurons with BF <30 kHz are EO/I, while 60% of neurons with BF >30 kHz are EO or EO/F. Nearly 90% of the neurons with BF >50 kHz are EO or EO/F. The sudden transition in BF from <20 kHz to >35 kHz is accompanied by a concomitant change in binaural type from EO/I to EO.

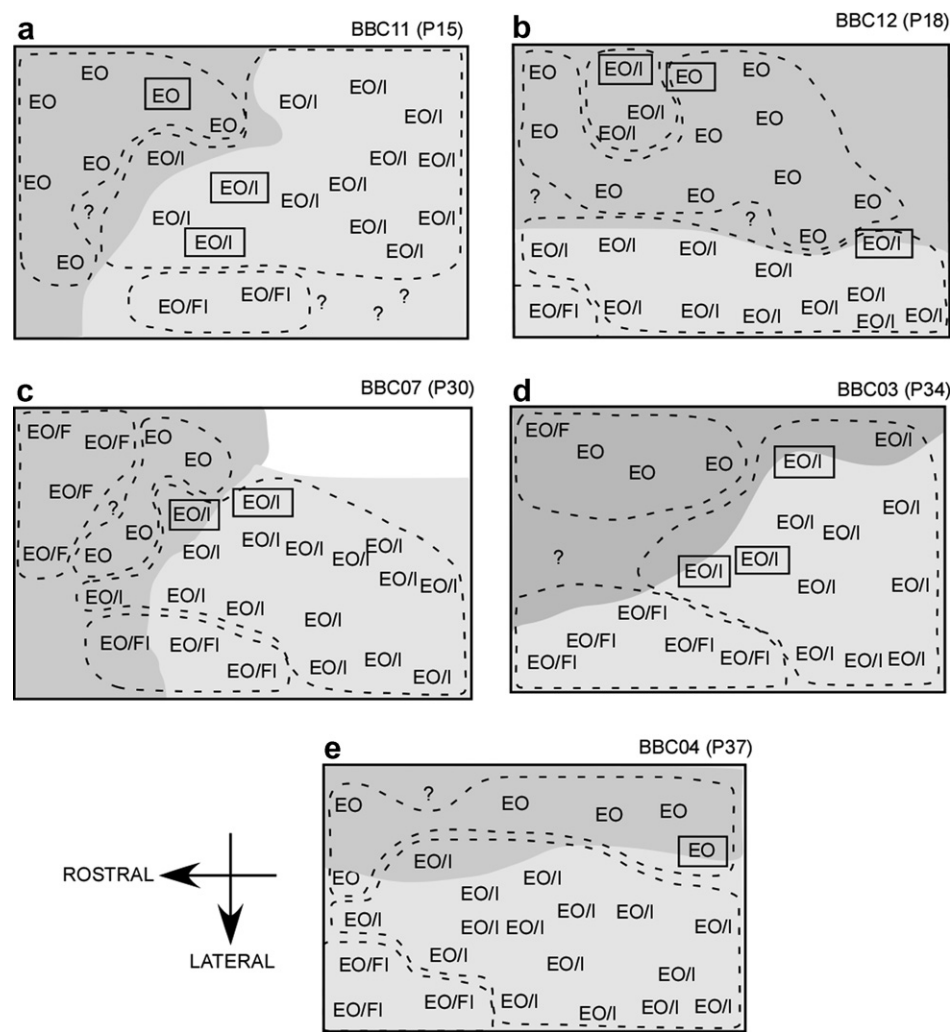


Fig. 6. Spatial overlap between best frequency (BF) and binaural properties. (a, b) Maps of binaural properties in the same P15 and P18 cortices shown in Fig. 1a,b. The dashed contours delineate different clusters of binaural types. The two shades of gray distinguish sites with BF <30 kHz from sites with BF >30 kHz. The question mark indicates sites from which binaural properties were not recorded. Sites in boxes exhibited multi-peaked tuning. It can be seen that as BF changes from <30 kHz to >30 kHz, a concomitant change occurs in binaural type from binaural inhibition (EO/I) to monaural (EO). EO/FI – neurons with mixed facilitation and inhibition interactions.

The clustered organization and the distribution of binaural sensitivity across BF in pups were similar to that seen in adults. Maps of binaural sensitivity in the youngest pups recorded from are shown in Fig. 6a and b. The corresponding frequency maps in these cortices were presented in Fig. 2a and b. In order to visualize the relationship between BF and binaural properties in each cortex, sites with BFs above and below 30 kHz are distinguished by two shades of gray in the binaural maps. It can be seen that the transition from BF <30 kHz to BF >30 kHz is accompanied by a concomitant shift in binaural properties from EO/I to EO neurons (Fig. 6a and b). The locations of the different clusters were also similar to adults. Additional examples of binaural maps in pups are shown in Fig. 6c–e. Pups older than P25 exhibited EO/F type selectivity that was not seen in younger pups (e.g. Fig. 6c and d). The relationship between BF and binaural properties holds even though considerable individual variability exists in the direction of BF change from <30 kHz to >30 kHz.

Fig. 7 shows the distribution of binaural types across BF. In both young and older pups, the percentage of EO/I neurons decreased, and the percentage of EO neurons increased, with BF. Binaurally facilitated (EO/F) neurons were only seen in the older pups, suggesting some binaural interactions may arise later in development. Taken together, these data show that the frequency-dependent distribution of binaural selectivity is present early in development.

3.4. Systematic representation of binaural sensitivity

The cluster of EO/I neurons within the noise-selective region of the pallid bat adult cortex contains a systematic map of IID sensitivity. These neurons respond well to monaural stimulation of the CL ear. For binaural stimuli, as the intensity in the IL ear increases, responses decrease, with the great majority showing complete inhibition. The IID at which the response was maximally inhibited (termed

inhibitory threshold, IT) changed systematically within the cluster. Neurons with positive ITs (maximal inhibition when IL < CL), are partially surrounded by neurons with progressively more negative ITs.

A similar arrangement of IID sensitivity was observed in all pup cortices examined, including the P15 pup. Fig. 8 shows IID sensitivity maps obtained at different ages. Contour lines delineate different ranges of IT values. In every map, a systematic organization can be seen with neurons with positive ITs (solid contours) being partially surrounded by neurons with progressively more negative ITs (dashed contours). The systematic changes in IID sensitivity were present in all maps despite considerable individual variability seen in the range of ITs recorded.

The distribution of IT was similar between pups and adults (Fig. 9a). There was also no difference in the mean IT between pups and adults. These data suggest that the range of IIDs to which neurons respond are similar in pups and adults. When IID sensitivity was tested at multiple fixed CL intensities, IT values were stable across CL intensities in the vast majority of neurons. Using the method of Irvine and Gago (1990), the IT/CL ratio was determined to quantify the effect of absolute CL intensity on IID sensitivity in binaurally inhibited neurons. If the IT of a neuron was 3, 4 and 5 dB at CL intensities of 30, 35 and 50 dB, respectively, then the IT/CL ratio was 0.1 [i.e. $(5-3)/(50-30) = 2/20 = 0.1$]. The distribution of this ratio (Fig. 9b) shows that nearly 75% of the neurons had a ratio <0.2. These data show that the IT of the majority of neurons is relatively stable with changes in absolute intensity levels.

4. Discussion

This study examined the development of tonotopy, frequency selectivity, binaural properties and the topographic relationships between these properties in the pallid bat auditory cortex. Adult-like tonotopy, clustered organiza-

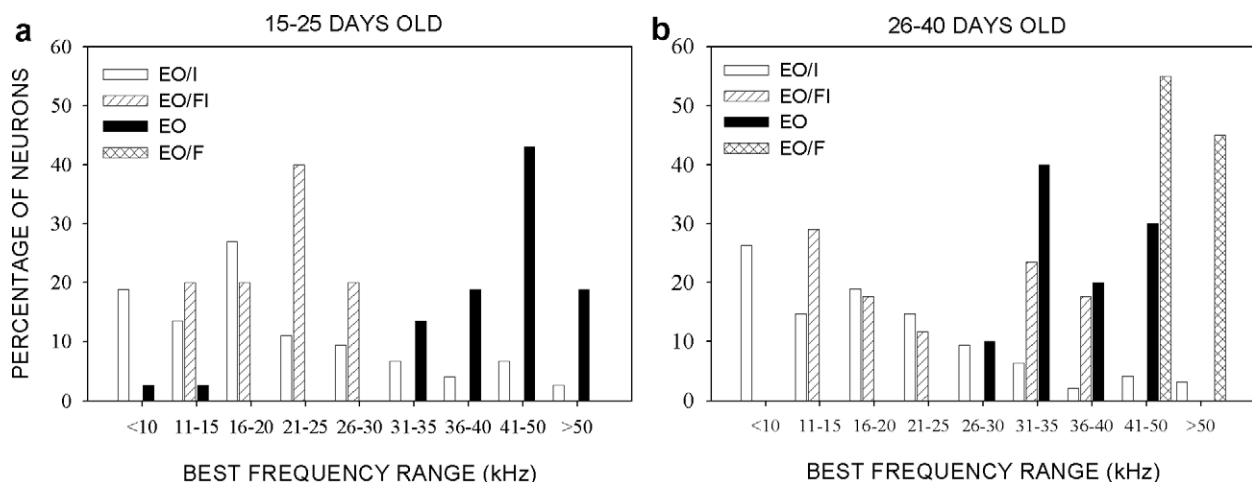


Fig. 7. Best frequency-dependent distribution of binaural types in pallid bat pup auditory cortex. In both young (a) and older (b) pups, most low-frequency neurons were binaurally inhibited (EO/I) or had mixed binaural interactions (EO/FI), while most high-frequency neurons were either monaural (EO) or binaurally facilitated (EO/F).

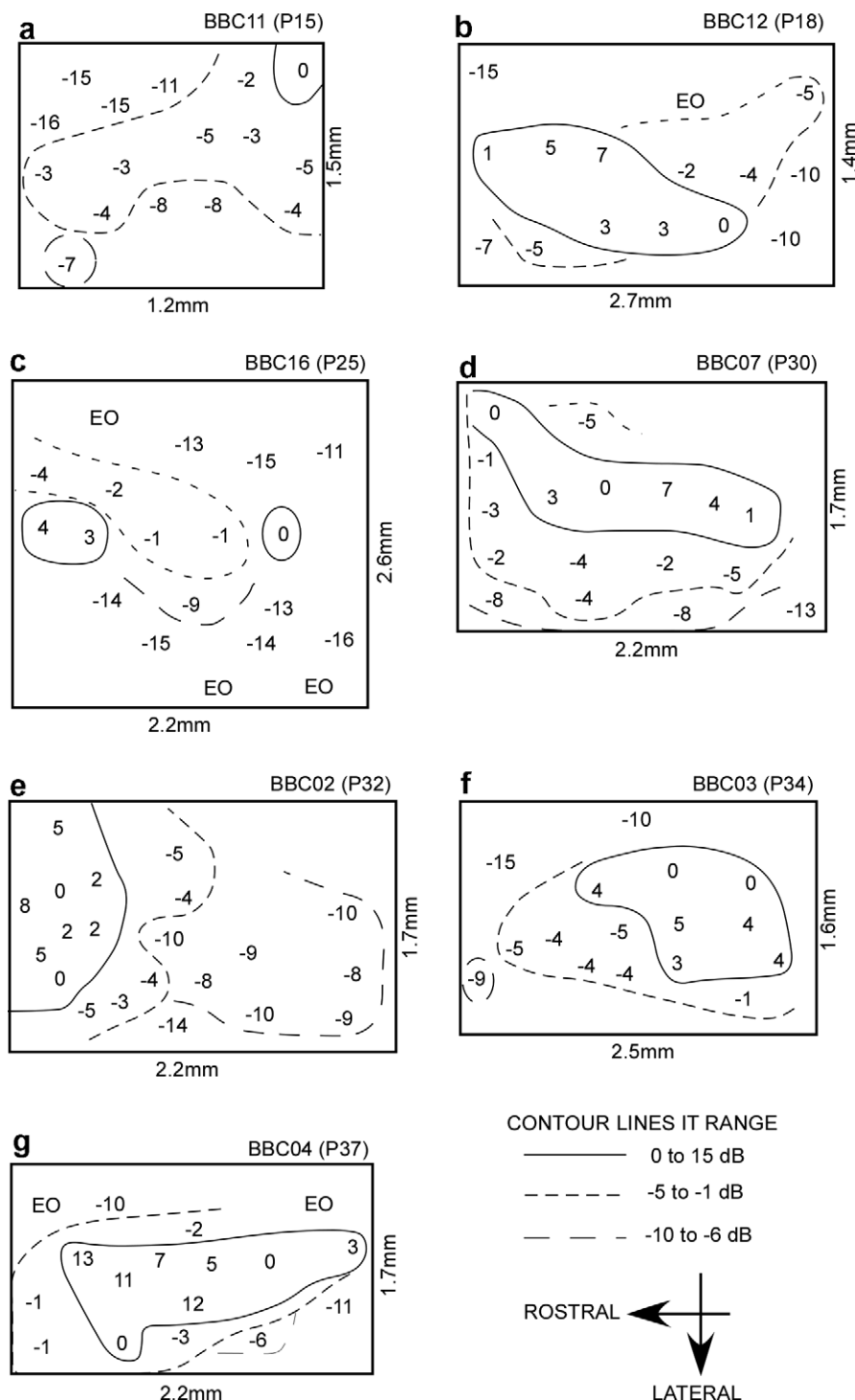


Fig. 8. A systematic representation of interaural intensity difference sensitivity is present throughout development in the pallid bat auditory cortex. (a–g) Maps of inhibitory thresholds (IT) within the noise-selective region recorded in P15–P37 pup cortices. All these neurons had best frequencies between 7 and 35 kHz. Inhibitory thresholds were arranged systematically such that a positive cluster (solid contour) was partially surrounded by increasingly more negative values (dashed contours).

tion of binaural properties, the relationship between BF and binaural type, and the systematic map of IID selectivity were present in the youngest pups from which we recorded. Three differences were observed between pups and adults. First, bandwidths of excitatory frequency tun-

ing curves showed a frequency-dependent narrowing during development. Second, more neurons with multi-peaked tuning curves were present further away from the transition region in pups than in adults. Third, neurons with binaural facilitation were observed only after P25.

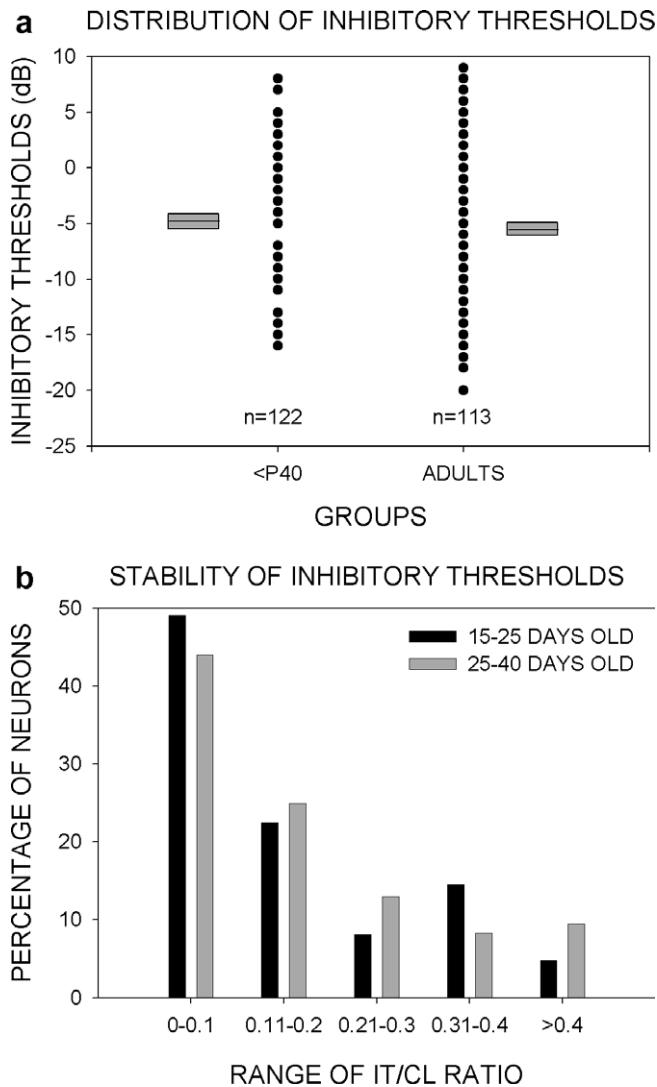


Fig. 9. (a) Distribution of inhibitory thresholds (IT) in pups and adults. No difference was observed in the range of inhibitory thresholds and the mean. (b) Effect of absolute intensity on interaural intensity difference sensitivity in pup cortical neurons. The IT/CL ratio was <0.2 in more than 70% of low-frequency neurons. This suggests that the values of IT are relatively stable across intensity.

4.1. Development of tonotopy and frequency selectivity in auditory cortex

Development of tonotopy and frequency selectivity in the auditory cortex has been studied in the rat, cat and the chinchilla. Each of these three species exhibits a tonotopic map in the youngest animals recorded from (Zhang et al., 2001; Bonham et al., 2004; Pienkowski and Harrison, 2005), but shows species-specific differences in the direction and time course of maturation of frequency tuning. In the rat and the cat, frequency selectivity undergoes significant changes during the initial weeks of hearing. In rats, bandwidths decrease during development. In the cat, frequency tuning becomes broader. The broadening is more pronounced in the dorsal and ventral parts of an isofrequency contour. This selective broadening appears to establish the

adult topographic distribution of bandwidths within an isofrequency contour, wherein central parts of isofrequency contours are narrowly tuned, and dorsal and ventral parts are broadly tuned and more likely to exhibit multiple peaks in their tuning curve. In the chinchilla, frequency selectivity appears to be mature at hearing onset. The receptive fields become spectrotemporally more complex during development (Pienkowski and Harrison, 2005). More neurons in adults exhibit multiple peaks in the frequency tuning curve and show different temporal properties for different frequencies. Thus, the auditory cortex is similar to the visual cortex in that the initial development of topographic representation of the receptor surface is shaped by experience-independent factors. Receptive field sizes develop over a prolonged time frame, and may be affected by experience and/or may be dependent on the maturation of inhibitory properties (Chang et al., 2005). Notably, considerable species differences exist in the direction of change of excitatory receptive field sizes, with both expansion and narrowing being observed.

The pallid bat cortex exhibits tonotopy at the youngest age tested (P15). The under-representation of BF between 20 and 30 kHz was also present in pups, suggesting that experience-independent factors shape tonotopy. Two features of frequency representation, however, were not adult-like. First, a BF-specific narrowing of tuning curves was observed with age. Low-frequency neurons were adult-like in their bandwidths at P15, but high-frequency neurons became more sharply tuned during development. Low-frequency neurons may mature earlier because low-frequency hearing develops earlier than high-frequency hearing (Brown, 1976). Hearing onset occurs around P8 and is limited to frequencies <30 kHz. Representation of the full audible range is present at P15. Therefore, at P15 the pups have already been exposed to low-frequency sounds for a week, while they are just beginning to hear high-frequency sounds. The difference in the time of exposure to low and high frequencies may underlie the BF-specific difference in the maturation of frequency tuning.

Second, neurons with multi-peaked tuning were found in areas further from the transition region from BF <30 kHz to 30–44 kHz. In adults, such neurons are found only in the transition region. Unlike adults, pup neurons also exhibited tuning curves with three peaks. In pups, high-frequency peaks of nearly 60% of neurons with multi-peaked tuning curves were centered at frequencies between 30 and 44 kHz. The remaining peaks were centered at frequencies between 45 and 60 kHz suggesting that multi-peaked tuning is initially present across the FM sweep-selective region, but is more restricted in adults. Thus, the pallid bat is similar to the other species examined in that tonotopy is in place early in development, while frequency tuning matures at a slower pace.

The presence of neurons with multi-peaked tuning across a more widespread extent of the pup cortex may be indicative of a larger overlap of two parallel inputs representing two different behaviors in the pallid bat. The pal-

lid bat is a gleaner bat that listens passively to low-frequency (<35 kHz) noise to localize prey. It uses echolocation (30–60 kHz) for obstacle avoidance. Frequencies between 10 and 20 kHz and between 35 and 45 kHz correspond, respectively, to centers of spectral bands used in passive prey localization and echolocation. We have previously suggested that the pallid bat represents prey-generated noise and echolocation information in parallel pathways (Fuzessery, 1994; Razak and Fuzessery, 2002; Razak et al., 2006). Physiological recordings in the adult inferior colliculus show that neurons selective for noise and FM sweeps are segregated. Similarly, in cortex, the two representations are mostly segregated, with the region of transition containing multi-peaked neurons and neurons that respond similarly to noise and FM sweeps. Thalamic inputs to the cortical noise- and FM sweep-selective regions arise from different thalamic divisions (Razak et al., 2006). Noise-selective neurons receive most of their inputs from the ventral division of the medial geniculate body. The FM sweep-selective neurons receive the majority of their inputs from the supragenulate body of the dorsal division. During development, these two inputs may overlap to a greater extent, as indicated by the presence of neurons with three peaks in their tuning curve and the observation that multi-peaked neurons are found further away from the transition zone in the pup. This physiological observation is supported by preliminary thalamocortical pathway tracing studies in pups which show an overlap of early projections between the pathways that is not seen in adults. These pathways may later segregate during development in an experience-dependent fashion.

4.2. Development of binaural properties

Besides tonotopy, the adult auditory cortex contains systematic representations of other response properties such as bandwidth of frequency tuning curves, binaural properties and FM sweep properties (reviewed in Ehret, 1997; Read et al., 2002). Studies of the development of binaural selectivity have shown that adult-like selectivity is present before morphological development of external structures is complete. In the cat, most neurons in the cortex and the inferior colliculus are influenced by binaural stimuli even at P8 (Moore and Irvine, 1980; Brugge et al., 1988; Blatchley and Brugge, 1990). Classes of binaural interactions such as EE, EO/I and predominantly binaural (EO/FI) are found in kittens and adults in similar percentages. Interestingly, significant IIDs are created only for frequencies >7 kHz in a P35 kitten (Moore and Irvine, 1979). Neurons with adult-like IID sensitivity are found in P8 kittens for BF <5 kHz. In addition, kittens first exhibit head-orienting to sounds between P21 and P28 (Clements and Kelly, 1978). These data suggest that the excitatory-inhibitory interactions creating IID sensitivity in adults are present well before experience with sound-localization behavior, and before the appropriate IIDs can be created by external structures. However, this does not mean that

free-field spatial selectivity is adult-like, as spatial receptive fields of auditory neurons are governed by ear directionality and excitatory and inhibitory frequency tuning, in addition to binaural properties. Indeed, a study of spatial selectivity in A1 has shown that the development of external structures dictates the development of spatial tuning, even though binaural and monaural properties that influence sound localization are adult-like in young animals (Mrsic-Flogel et al., 2003).

We compared four features of binaural representation between pup and adult cortex: clustering of binaural properties, BF-dependent distribution of binaural properties, the systematic map of IID sensitivity and the stability of IID sensitivity over absolute intensity. All four features were adult-like from P15. As in adults, at least three clusters of binaural properties (EO/I, EO and EO/FI) were found in pups. Most neurons with BF <30 kHz were EO/I, while most neurons with BF >30 kHz were EO. A systematic distribution of IID sensitivity was found in the cortices of P15 pups. The systematic representation of IID sensitivity and IT stability at different CL intensities were similar in pups and adults. Head and ear size are still in the process of maturation at P15, and reach adult dimensions after P35. While the IIDs produced at different frequencies during development in the pallid bat are not known, they are unlikely to be adult-like at P15. Taken together, data from cats, ferrets and pallid bats suggest that binaural properties and their topographical representation are mostly mature well before binaural cues become available.

One difference between pups and adults was that binaural facilitation was not observed in pups younger than 25 days. Thus, unlike cats, the full complement of binaural types develops slowly in pallid bats. Studies using the GABA-A receptor antagonists bicuculline and gabazine show that binaural facilitation in the pallid bat cortex is shaped by intracortical GABA (Razak and Fuzessery, *SFN abstracts* 2006). Binaural inhibition appears to be primarily inherited from subcortical structures. The delayed development of binaural facilitation may be a reflection of delayed development of cortical inhibition (Chang et al., 2005).

We have previously suggested that the EO neurons with BF >30 kHz show spatial sensitivity for the area directly in front of the bat (Fuzessery, 1996; Razak and Fuzessery, 2002). As most neurons with BF >30 kHz are selective for the downward FM sweep used in echolocation, this spatial selectivity for the region in front of the bat will facilitate localization of echoes along the flight path. Most neurons with BF <30 kHz are selective for noise, and are EO/I. The presence of a systematic IID map in the EO/I cluster in the youngest pups suggests that an adult-like substrate for sound localization is present well before the pallid bat takes flight and captures terrestrial prey. Thus, the overall cortical functional organization is remarkably adult-like at an early age in the pallid bat, with differences being observed in some aspects of frequency and binaural representation.

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