Experience is required for the maintenance and refinement of FM sweep selectivity in the developing auditory cortex

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Edited by Eric I. Knudsen, Stanford University School of Medicine, Stanford, CA, and approved January 22, 2008 (received for review October 8, 2007)

Frequency modulated (FM) sweeps are common components of vocalizations, including human speech. How developmental experience shapes neuronal selectivity for these important signals is not well understood. Here, we show that altered developmental experience with FM sweeps used in echolocation by the pallid bat leads to either a loss of sideband inhibition or millisecond delays in the timing of inhibitory inputs, both of which lead to a reduction in rate and direction selectivity in auditory cortex. FM rate selectivity develops in an experience-independent manner, but requires experience for subsequent maintenance. Direction selectivity depends on experience for both development and maintenance. Rate and direction selectivity are affected by experience over different time periods during development. Altered inhibition may be a general mechanism of experience-dependent plasticity of selectivity for vocalizations.

development | response selectivity

Neural selectivity for species-specific vocalizations is present in several vertebrate groups (1–8). In songbirds, experience shapes the development of vocalization selectivity (9–11). How experience modifies the receptive fields (RFs) underlying such selectivity has not been characterized. This report focuses on the mechanisms through which developmental experience shapes selectivity of auditory cortex neurons for the rate and direction of FM sweeps present in the echolocation calls of the pallid bat.

The pallid bat brings several advantages to the study of experience-dependent plasticity of vocalization selectivity. First, the ontogeny of hearing and vocalization (echolocation) behavior is known (12). Second, nearly 70% of adult auditory cortical neurons tuned in the echolocation range are selective for the sweep rates and the downward sweep direction used in echolocation (13, 14), providing a model system for examining the development of this selectivity. Third, the mechanisms that shape FM sweep selectivity in the adult cortex and the developmental time course of these mechanisms are known (14, 15). FM rate selectivity and the underlying RF properties are adult-like from postnatal day (P) 14, at the onset of adult-like hearing in the echolocation range. This suggests that rate selectivity develops in an experience-independent manner. Direction selectivity and the underlying RF properties become adult-like between P60 and P90, well after the bat has begun to use echolocation, suggesting that experience plays a role in its development. The primary goal of this study was to determine the effects of altering experience with the echolocation call on the development of mechanisms that shape FM rate and direction selectivity.

Results

We compared FM rate and direction selectivity in four groups of bats: experimental (EXP), control (CTRL), normal, and adults. The EXP pups were isolated from other bats and received laryngeal muscle Botox injections ($n = 7$ pups) or laryngeal muscle lesions ($n = 3$ pups). The muscle manipulation was done before the onset of high-frequency hearing (between P11 and P13). Pups were kept isolated until the day of electrophysiological recording. The purpose of this group was to determine whether the absence of normal experience with echolocation calls affects cortical FM rate and direction selectivity. The CTRL pups were isolated from P13 without manipulation of the larynx ($n = 6$ pups). The CTRL pups served to determine whether developmental isolation alone has any effects and whether the pup's own vocalizations are sufficient for normal development of sweep selectivity. Electrophysiological recordings from both EXP and CTRL groups were obtained at P30 or P90. Data from these two groups were compared with the normal pup (15) and adult (14) data. The normal pups (in ref. 15) developed in the presence of other bats (adults and pups).

Effects of Laryngeal Manipulations on Echolocation Call Development. Laryngeal manipulations altered but did not eliminate echolocation calls. The normal adult echolocation call is a downward FM sweep from 60 to 30 kHz [≈2-ms duration) [supporting information (SI) *Appendix*, Fig. 1]. During development, the highest frequency (Fig. 1*a*), the rate of change of frequencies (FM rate) (Fig. 1*b*), and the lowest frequency of the call (data not shown) become adult-like ≈P20 in the normal and CTRL groups. Echolocation calls of P30 normal and CTRL pups are similar to the adult calls (*SI Appendix*, Fig. 1; one-way ANOVA, $P > 0.05$). In the EXP group, however, the highest frequency (Fig. 1*a*) and the FM rate (Fig. 1*b*) were low compared with normal and CTRL pups after P20 (one-way ANOVA, Tukey post hoc test for pairwise comparisons, $P < 0.05$). An example sequence of calls from an EXP pup at P30 (*SI Appendix*, Fig. 1) shows that the highest frequency was ≈40 kHz, a call character typical of P15 pups. There was no difference in the lowest frequency of the calls (data not shown, one-way ANOVA, $P > 0.05$). There was no difference in call properties between CTRL and normal pups (one-way ANOVA, $P > 0.05$), showing that pups raised in isolation produce normal calls.

Botox effects are reversible (16). However, pups with lesions in the laryngeal muscles do not produce adult like calls up to P90. The data in Fig. 1 *a* and *b* for ages >P40 are for pups with laryngeal muscle lesions. The data for ages <P40 are from both lesion and Botox groups. The highest frequency and FM rate were significantly lower in the lesion group compared with the Botox group at ≈P20 (one-way ANOVA, $P > 0.05$). Vocalization data from both groups are pooled together for ages <P40,

Author contributions: Z.M.F. designed research; K.A.R. and M.D.R. performed research; K.A.R. analyzed data; and K.A.R. and Z.M.F. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0709504105/DC1.

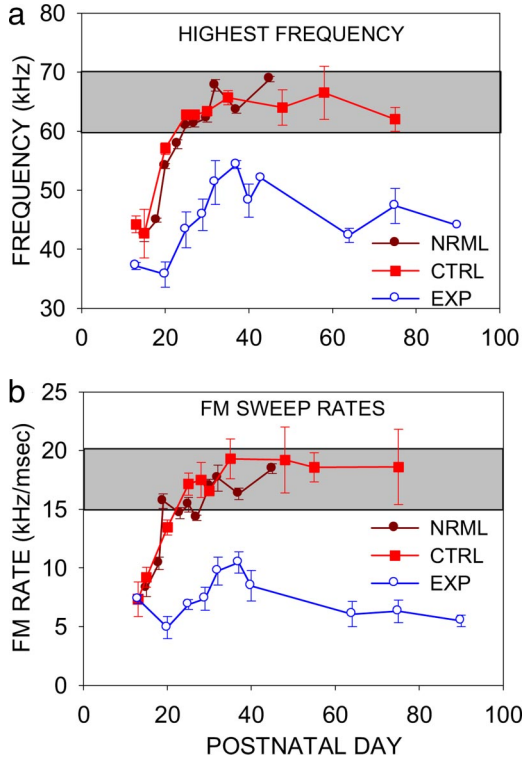

Fig. 1. EXP pups produced echolocation calls with reduced high-frequency energy and slower FM rates. (*a*) Development of the highest frequency of echolocation calls. The gray band shows the typical adult range of highest frequencies. The EXP pups lacked highest frequencies in the adult range at any age tested. Normal and CTRL groups started to produce highest frequencies in the adult range between P20 and P25. (*b*) Development of FM rates used in the echolocation call. The gray band shows the adult range. The FM sweep rate was below adult range in the EXP group at all ages tested, whereas the normal and CTRL groups produced calls with adult-like sweep rates from approximately P20.

because both lesion and Botox pups showed significantly lower FM rate and highest frequency compared with age-matched CTRL and normal pups. Thus, compared with normal and CTRL pups, EXP pups were deprived of normal experience with echolocation calls until the day of electrophysiological recordings. In terms of response properties described below, it must be noted that Botox pups were studied at P30, and lesion pups were studied at P90.

Effects of Laryngeal Manipulations on Best-Frequency Distribution. The FM-selective region of the pallid bat cortex contains neurons with best frequencies (BF) between 20 and 70 kHz, with an over-representation of 35–45 kHz (13). A similar BF distribution (χ^2 test, $P > 0.05$) was observed in all three pup groups at P30 (*SI Appendix*, Fig. 2*a*) and P90 (*SI Appendix*, Fig. 2*b*) indicating that the BF distribution is experience-independent. Importantly, this allowed a comparison of FM rate and direction selectivity without any sampling bias in terms of BF across the three pup groups.

Normal Experience Is Required for the Maintenance of FM Rate Selectivity. In normal pups, the percentage of rate-selective (RS) neurons and the rates for which these neurons are selective are adult-like at P14, the age when the adult audible range is acquired (12). This early maturation suggests an experience-independent development of FM sweep rate selectivity. Therefore, our hypothesis was that, unless experience was required for the maintenance of selectivity, the three groups would not show differences in rate selectivity at either P30 or P90.

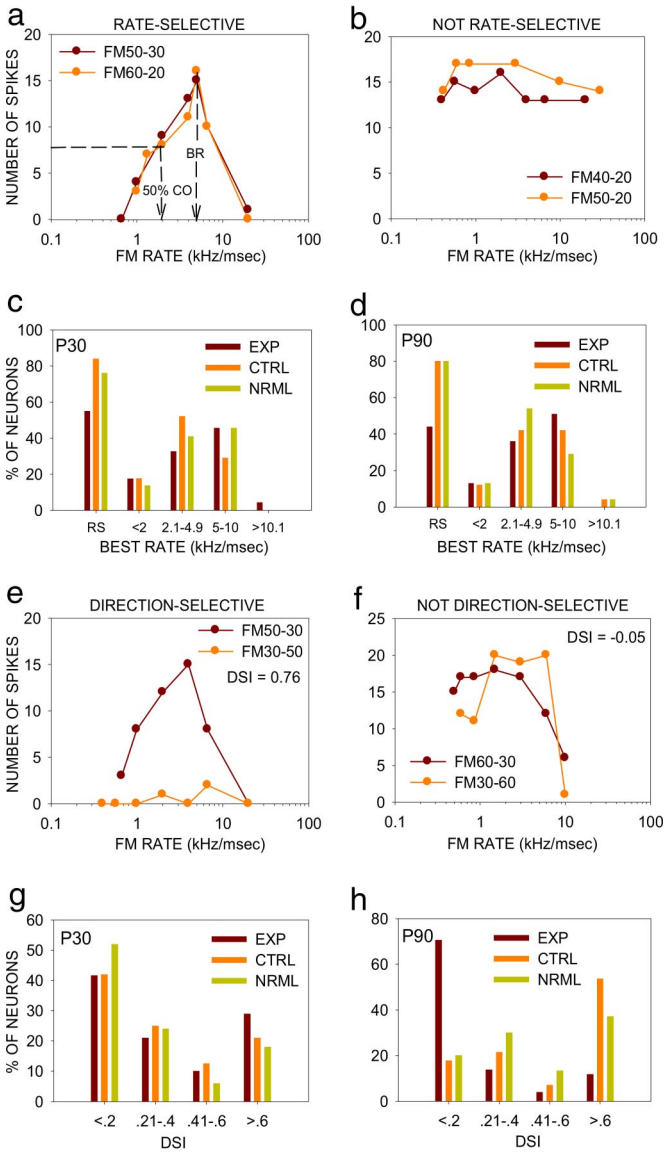

Fig. 2. Experience-dependent development of FM rate and direction selectivity. (*a*) An example RS neuron. (*b*) A typical nonselective neuron. (*c*) At P30, the percentage of RS (first set of bars) neurons is significantly lower in the EXP group ($n = 71$ neurons) compared with age-matched normal ($n = 30$) and CTRL ($n = 48$) pups. The remainder of the graph shows the distribution of BR of neurons across the groups. (*d*) This graph illustrates the same type of data as in *c*, but it is recorded at P90 (sample sizes: EXP, $n = 53$; CTRL, $n = 31$; normal, $n = 32$). (*e*) A direction-selective neuron (DSI = 0.76). (*f*) A neuron with no direction selectivity (DSI≈0). (*g*) At P30, the distribution of DSI values was similar across the three groups (EXP, $n = 48$; CTRL, $n = 24$; normal, $n = 31$). (*h*) At P90, there was a significant increase in the percentage of neurons with DSI <0.2 in the EXP group ($n = 49$; CTRL, $n = 29$; normal, $n = 30$) compared with age-matched CTRL and normal pups. BR, best rate; CO, cut-off.

Fig. 2 shows examples of neurons that were classified as RS (Fig. 2*a*) and nonselective (Fig. 2*b*). Comparison of rate selectivity across groups shows that both P30 (Fig. 2*c*) and P90 (Fig. 2*d*) EXP pups exhibit a significantly lower percentage of RS neurons compared with age matched normal and control groups, P14 normal and adults (χ^2 test, $P < 0.05$). This suggests that maintenance of rate-selectivity requires normal experience. There was no difference between the normal and CTRL groups

Table 1. Comparison of FM rate selectivity and average DSI across the three pup groups

Postnatal day *n*	Pups	Best rate	50% cut-off rate	DSI
30	EXP	5.0 ± 0.5	1.7 ± 0.24	0.38 ± 0.05
	CTRL	3.8 ± 0.4	1.2 ± 0.13	0.37 ± 0.06
	NRML	5.3 ± 0.7	1.2 ± 0.1	0.3 ± 0.05
90	EXP	5.4 ± 0.5	1.7 ± 0.2	0.14 ± 0.04
	CTRL	4.9 ± 0.6	1.5 ± 0.2	0.5 ± 0.05
	NRML	4.1 ± 0.6	1.3 ± 0.2	0.5 ± 0.05

at either P30 or P90 (χ^2 test, $P > 0.05$). This shows that echolocation calls produced by the developing CTRL pups were sufficient to maintain rate selectivity in this group. Despite the decline in the percentage of RS neurons in EXP pups, there was no difference across groups in the distribution of best rates in neurons that continued to exhibit rate selectivity (χ^2 test, $P > 0.05$, Fig. 2 *c* and *d*). The average best rates and 50% cut-off (CO) rates were similar across groups at both P30 and P90 (Table 1, one-way ANOVA and Tukey post hoc test for pairwise comparisons, $P > 0.05$). Together, these data suggest that normal experience between P14 and P30 is required not for the initial development of rate selectivity but for its subsequent maintenance. Exposure to echolocation calls with reduced rates causes a loss of rate selectivity in a subgroup of neurons, whereas others are unaffected.

Experience Is Required for Development and Maintenance of Direction Selectivity. The majority of neurons in normal P14 and P30 pups exhibit a direction selectivity index (DSI) <0.2 (weak direction selectivity) compared with a DSI >0.6 (strong selectivity for downward sweeps) in adults (15). The average DSI increases from ≈0.3 at P14 and P30 to ≈0.6 in adults. This increase in selectivity for downward sweeps may require experience with echolocation calls after P30. If this is the case, then the three groups should have similar DSI at P30. At P90, DSI in the EXP pups should not increase, and if experience is required for maintenance, it may decrease. Fig. 2 *e* and *f* shows examples of a downward direction-selective neuron and a nonselective neuron, respectively. At P30, there was no difference in the distribution of DSI values across the three pup groups (Fig. 2*g*), with the majority of neurons showing DSI <0.2 (χ^2 test, $P > 0.05$). The average DSI values were not different across the three pup groups at P30 (Table 1, one-way ANOVA, $P > 0.05$). These data show that direction selectivity was slow to mature in all pup groups and that experience did not affect direction selectivity before P30.

A dramatic reduction in direction selectivity was observed in the EXP group at P90 compared with age-matched CTRL and normal pups (Fig. 2*h*, χ^2 test, $P < 0.05$). The vast majority of neurons in the P90 EXP animals exhibited DSI <0.2, whereas normal and CTRL P90 pups were similar to adults. The average DSI in the P90 EXP group was significantly lower than in CTRL and normal pups (Table 1, one-way ANOVA, $P < 0.05$). In addition, the percentage of neurons with DSI >0.6 was lower in the P90 EXP pups compared with the P30 EXP pups (χ^2 test, $P < 0.05$). The average DSI was also lower in the P90 EXP compared with P30 EXP pups (Table 1, one-way ANOVA, $P < 0.05$). With the caveat that the P30 and P90 EXP pups received different laryngeal manipulations, this suggests that normal experience between P30 and P90 is required for both the increase and maintenance of direction selectivity. As with rate selectivity, self-vocalization in the CTRL group was sufficient for normal development of direction selectivity.

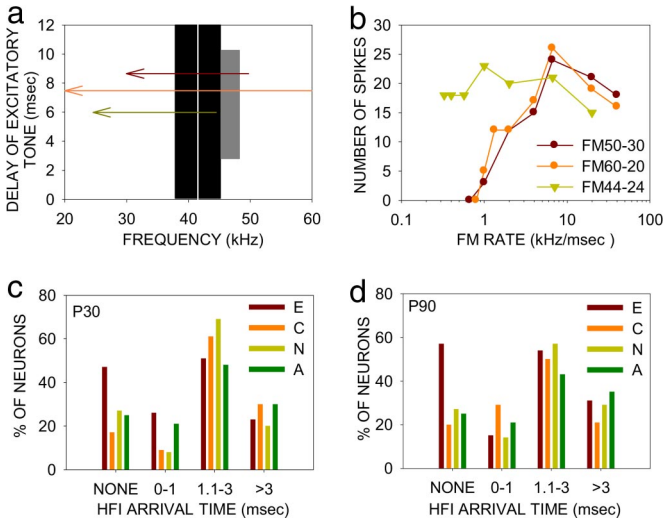

Fig. 3. Loss of HFI underlies reduced rate selectivity in the EXP pups. (*a*) Delayed HFI shapes rate selectivity for downward FM sweeps. An example TTI plot from a neuron with excitatory tuning (black) between 38 and 45 kHz, and a delayed HFI (gray) centered at 46.5 kHz. HFI was delayed by 3 ms. The BF is indicated by the white line. Arrows indicate different downward FM sweeps used to determine rate selectivity shown in *b*. (*b*) Downward sweeps that included HFI (orange and dark red) elicited RS responses from this neuron. The neuron was not RS for the sweep that excluded HFI (green), showing that HFI was required for rate selectivity in this neuron. (*c* and *d*) At both P30 (*c*) and P90 (*d*), a significantly higher percentage of neurons in the EXP group exhibited no HFI (NONE-first set of bars) compared with the CTRL and normal groups (sample sizes for P30: EXP, $n = 55$; CTRL, $n = 36$; normal, $n = 28$; for P90: EXP, $n = 43$; CTRL, $n = 25$; normal, $n = 26$). The remainder of the graph shows the distribution of HFI arrival times in neurons with HFI.

Experience Is Required for the Maintenance of High Frequency Inhibition. Delayed high frequency inhibition (HFI) shapes rate selectivity for downward sweeps in adult and normal pup cortex (14, 15). Therefore, the observed reduction in rate selectivity in the EXP group may be due to changes in HFI. Fig. 3*a* shows the two tone inhibition over time (TTI) plot from a P30 EXP pup neuron with delayed HFI centered at 46.5 kHz. The HFI arrived 3 ms after excitation. The neuron was RS for sweeps that included the HFI, but not if the downward sweep started inside the tuning curve and therefore excluded the HFI (Fig. 3 *a* and *b*). Exclusion of HFI from a downward sweep caused a loss or reduction of rate selectivity in all EXP ($n = 69$; P30 and P90 combined) and CTRL ($n = 51$; P30 and P90 combined) group neurons tested. In addition, all EXP and CTRL pup neurons lacking HFI were not RS. These data show that, as in normal pups, delayed HFI shapes rate selectivity for downward sweeps in the EXP group.

At both P30 and P90, HFI was absent in a significantly higher percentage of neurons in the EXP group than in age-matched normal and CTRL groups (χ^2 test, $P < 0.05$, NONE in Fig. 3*c* and *d*). Because all neurons without HFI were not RS, this indicates that loss of HFI underlies the reduced percentage of rate selective neurons in the EXP group. In neurons with HFI, the distribution of HFI arrival times was similar across all pup groups and adults (χ^2 test, $P > 0.05$, Fig. 3*d*). This is consistent with the lack of effects on best rate and 50% cut-off rate in the EXP pups and suggests that a subgroup of neurons is affected by abnormal experience in terms of both HFI and rate selectivity.

Experience Is Required for Normal Development of Low Frequency Inhibition. In both adult and normal pup cortex (14, 15), early long frequency inhibition (LFI) shapes selectivity for the downward sweep direction. A neuron from an EXP pup shown in Fig.

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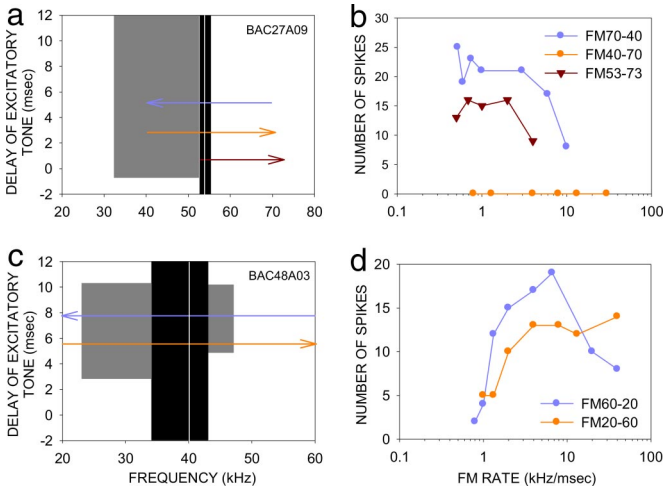

Fig. 4. Early LFI shapes FM direction selectivity. (*a*) This direction-selective neuron exhibited a broad band of LFI centered at 42 kHz. LFI was observed even if the LFI tones were delayed by 0.5 ms, showing that LFI arrived early. The arrows indicate the different sweeps used to determine direction selectivity in *b*. (*b*) The neuron did not respond to the upward sweep (orange) that included the LFI, whereas a downward sweep (blue) with the same frequencies elicited robust responses. The neuron responded to an upward sweep that excluded the LFI (brown), showing that direction selectivity was shaped by the early LFI. (*c* and *d*) A neuron with delayed LFI is not direction-selective. (*c*) This neuron had LFI centered at 30 kHz and delayed by 3 ms. (*d*) The maximum responses to the two sweep directions were similar even if the upward sweep included the LFI (orange).

4*a* had a broad (32- to 52-kHz) band of LFI that arrived 0.5 ms earlier than excitation. This neuron was selective for the downward sweep direction, responding to a downward 70- to 40-kHz but not an upward 40- to 70-kHz sweep (Fig. 4*b*). However, if the LFI was excluded from an upward sweep (53–73 kHz, Fig. 4 *a* and *b*), the neuron responded, demonstrating that LFI shaped its direction selectivity. Across the population, exclusion of LFI from upward sweeps eliminated or reduced direction selectivity in 95% of CTRL ($n = 47$; P30 and P90 combined) and 97% of EXP ($n = 63$; P30 and P90 combined) group neurons. Thus, early LFI is required for direction selectivity in the vast majority of neurons.

The early arrival time of LFI is critical for direction selectivity. In the example neuron shown in Fig. 4 *c* and *d*, LFI centered at 30 kHz arrived 3 ms late. The neuron had a DSI of 0.18. There is a correlation between the arrival time of LFI and the DSI of the neuron in all pup groups (*SI Appendix*, Fig. 3), as shown for adults (14). Most neurons with delayed LFI exhibit poor direction selectivity.

The low direction selectivity in the EXP group at P90 may therefore be either due to an absence of LFI or a delay in its arrival time. Evidence for both mechanisms was found. At P30, the percentage of neurons without LFI (NONE in Fig. 5*a*) was similar across all pup groups and adults (χ^2 test, $P > 0.05$). Among neurons with LFI, the distribution of LFI arrival times was similar across the three pup groups, with nearly 50% of neurons showing early LFI (χ^2 test, $P > 0.05$, Fig. 5*a*). The P30 pups were different from the adults in that nearly 80% of adult neurons had early LFI (χ^2 test, $P < 0.05$). This is consistent with the lower average DSI across all three pup groups at P30.

However, at P90, the percentage of neurons without LFI was significantly higher in the EXP group compared with normal and CTRL pups (χ^2 test, $P < 0.05$, NONE in Fig. 5*b*). Among neurons with LFI, a significantly higher percentage exhibited delayed LFI in the EXP group (χ^2 test, $P < 0.05$). In the normal and CTRL groups, the percentage of neurons with early LFI increased between P30 and P90. In the EXP group, the percentage of neurons with early LFI decreased during this time period. In the normal and CTRL groups, the average LFI arrival time decreased (one-way ANOVA, $P < 0.05$, Fig. 5*c*) between P30 and P90 and is indistinguishable from adult values at P90 (one-way ANOVA, $P > 0.05$). In the EXP group, average LFI arrival time increased between P30 to P90 (Fig. 5*c*). Thus, normal echolocation experience is required for the refinement of arrival time of LFI between P30 and P90. This is in contrast to HFI in EXP pups, which was not affected in neurons that continued to exhibit rate selectivity (Fig. 3*d*). Thus, there are differences in how experience influences the two inhibitory sidebands.

Experience Is Not Required for Normal Development of Response Latency and Tuning Curve Bandwidth. Because the arrival times of inhibition were referenced against excitatory response latencies, the timing of inhibition may appear to be altered by abnormal experience because of a change in the arrival times of excitatory inputs. However, this was not the case. Average first spike latency in response to the BF tone presented at 5–10 dB above minimum threshold was not different between the CTRL and the EXP groups at P30 (CTRL: 22.36 ± 0.6 ms; EXP: 21.05 ± 0.6 ms; $P > 0.05$, *t* test) and P90 (CTRL: 23.80 ± 0.7 ms; EXP: 22.12 ± 0.92 ms; $P > 0.05$, *t* test). Moreover, these response latencies were not different from the normal group (15).

Altered auditory experience affects excitatory bandwidths in rat auditory cortex (17). The bandwidth (BW) of excitatory tuning curves at 10 dB above threshold was not different between the CTRL and EXP pallid bat pups at both P30 (CTRL: 6.03 ±

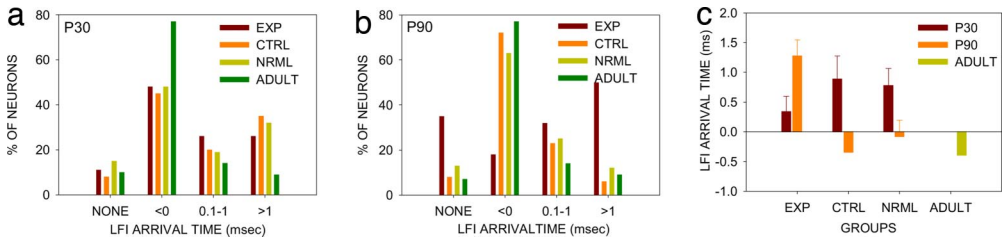

Fig. 5. Loss of direction selectivity is due to either a loss of LFI or a delay in the arrival time of LFI. (*a*) At P30, there was no difference in the percentage of neurons without LFI across the pup groups and adults (NONE, first set of bars; sample sizes: EXP, $n = 45$; CTRL, $n = 40$; normal, $n = 30$). The remainder of the graph shows the distribution of LFI arrival times in neurons with LFI. The pup groups did not differ from each other in the distribution of LFI arrival times, but they did show a significantly lower percentage of neurons with early (<0 msec) arrival time compared with adults. (*b*) At P90, a higher percentage of EXP neurons exhibited no LFI (NONE, first set of bars; sample sizes: EXP, $n = 43$; CTRL, $n = 25$; normal, $n = 27$). In neurons with LFI, the distribution of arrival times was significantly biased toward slower arrival times (>1 msec) in the EXP group. (*c*) The average 50% arrival time of LFI was similar across pup groups at P30 and was delayed compared with adults. At P90, the normal and CTRL pups showed early LFI similar to adults. At P90, however, LFI was delayed in the EXP group compared with CTRL, normal, and EPX pups and adults.

0.6 kHz; EXP: 6.39 ± 0.60 kHz; $P > 0.05$, t test) and P90 (CTRL: 5.50 ± 0.51 kHz; EXP: 6.08 ± 0.53 kHz; $P > 0.05$, t test). Similarly, there was no difference in the BW at 20 dB above threshold at P30 (CTRL: 9.5 ± 1.2 kHz; EXP: 9.7 ± 1.3 kHz; $P > 0.05$, t test) or P90 (CTRL: 8.05 ± 0.8 kHz; EXP: 9.6 ± 0.96 kHz; $P > 0.05$, t test). Moreover, the bandwidths were not different from the normal group at corresponding ages (15). These data suggest that excitatory properties are not affected in the EXP group.

Discussion

This study shows that plasticity of sideband inhibition is a mechanism through which experience influences development and maintenance of cortical FM sweep selectivity. The mechanisms that shape selectivity for FM sweep rate and direction are affected by experience over different time courses. Rate selectivity and underlying HFI are largely adult-like before echolocation experience, whereas direction selectivity and LFI require normal experience. If deprived of experience, the percentage of RS neurons decreases over a 2-week period because of the loss of HFI. There is a reduction in the number of RS neurons rather than a shift in selectivity toward the slower rates present in the abnormal calls of the EXP pups, suggesting that experience is not playing an instructive role. It appears that normal experience is required to maintain the innately specified rate selectivity.

The experience-dependent development of direction selectivity follows a slower time course. Direction selectivity increases between P30 and P90 in normal and CTRL pups. This refinement is prevented in the EXP pups because of either a loss of LFI or millisecond delays in its relative arrival time.

A significant question is whether these changes are occurring at the cortical level or at lower levels of the system. Essentially identical forms of rate and direction selectivity are already present at the level of the inferior colliculus (18), suggesting that the auditory cortex may inherit some of its selectivity. However, the blockade of intracortical GABA-A receptors in adult auditory cortex results in a reduction of response selectivity in the majority of cortical neurons,[‡] suggesting that intracortical inhibition also plays a role in shaping and maintaining selectivity.

The role of experience in the maintenance of innately specified neural circuits may be common across sensory systems. In the ferret lateral geniculate nucleus, blocking retinal activity after the experience-independent establishment of eye-specific layers results in a loss of this specificity (20). In the hamster superior colliculus, visual RFs become narrow during development even in the absence of light (21). However, a continued absence of visual input causes RFs to broaden again. These studies suggest that visual experience is required for maintenance of response properties.

Experience-Dependent Development of Neuronal Selectivity for Vocalizations. The response properties of auditory neurons are known to be influenced by vocalizations both during developmental critical periods (9–11, 22) and in adults (23). The most thoroughly studied of such systems is the development of selectivity for the bird's own song in the avian forebrain, which has a time course paralleling the maturation of the bird's vocalizations. Although it has been established that forebrain neurons in the songbird develop remarkably selective spectrotemporal filters for their vocalizations (9, 10), the nature of the RF changes that cause this maturation is not well understood, in part because of the complexity of the vocalizations. An important contribution of the present study is the description of the spectrotemporal changes in the inhibitory inputs underlying selectivity for a comparatively simple vocalization, a downward FM sweep. Given the ubiquity of FM sweeps in vocalizations, it is likely that similar inhibitory mechanisms operate in other species.

Experience with vocal production is essential for normal development of response selectivity in the songbird forebrain. It is likely that experience with echolocation is essential for the development of response selectivity in the pallid bat auditory cortex. Although we have focused on the role of sensory experience, motor feedback may play a role (22–24). Further studies will need to examine whether purely sensory experience with externally generated echolocation pulses is sufficient to support the development of response selectivity, or whether the combined sensorimotor experience of echolocating is required.

Conclusions

This study makes two significant contributions. First, the mechanisms that shape spectrotemporal filters for different parameters of vocalizations do not necessarily mature over the same time course and may differ in the extent to which their maturation is experience-dependent. Second, sampling the maturation process from the earliest postnatal date possible is necessary for distinguishing the effects of sensory deprivation on refinement and maintenance. In the present study, early sampling revealed that experience is not required for the expression of adult-like rate selectivity, but it is required for its maintenance.

Materials and Methods

Single-unit recordings were obtained from auditory cortex of pallid bats that were born in the University of Wyoming animal facility. All procedures followed the National Institutes of Health and Institutional Animal Care and Use Committee animal welfare guidelines.

Laryngeal Manipulations and Vocalization Recordings. Laryngeal muscles were paralyzed with botulinum toxin A (Botox; Metabiologics). Botox was diluted to 60 LD_{50} per ml, using gel phosphate buffer (pH 6.3). To expose the laryngeal muscles, bat pups (P11–P13) were anesthetized with methoxyflurane inhalation followed by an i.p. injection of pentobarbital sodium (30 μg per gram of body weight). A midline incision was made on the skin ventral to the trachea and larynx. Superficial muscle layers were parted along the midline to expose the trachea and larynx. An injection of Botox (volume 0.3–0.4 μl) was made into the muscles surrounding the larynx. In three pups, the laryngeal muscles were lesioned bilaterally with electrocautery.

Vocalizations were recorded by using the Petterrson D980 ultrasound detector. Bats held upside down start to orient with echolocation calls. The reported properties are based on calls made under such conditions for all bats and do not provide any indication about call intensity or how frequently calls were produced in the holding cages. The highest frequency, lowest frequency, and duration of each call were measured from the spectrograms. The FM rates of calls were determined by dividing the difference between the highest and lowest frequencies by the duration. Between 6 and 12 calls were analyzed from each bat (*SI Appendix*, Fig. 1). Three adults and 10 EXP (7 Botox, 3 lesion), 6 CTRL, and 2 normal pups were used for recording vocalizations.

Procedures for Electrophysiology. The surgical and recording procedures were identical to those reported (13–15). Recordings were obtained from bats that were anesthetized with pentobarbital sodium (30 μg per gram of body weight) and acepromazine (2 μg per gram of body weight). Using glass microelectrodes (1 M NaCl, 2–5 MΩ impedance), single unit recordings were obtained at depths between 200 and 600 μm.

Data Acquisition. Once a neuron was isolated within the FM-selective region of the cortex (13), we recorded as many of the following response properties as possible.

Excitatory frequency tuning curve. Pure tones (25–75 kHz, 5-ms duration, 1-ms rise/fall times) were used to determine the best frequency and excitatory frequency tuning curve.

FM rate and direction selectivity. Rate selectivity was determined by first recording responses to downward sweeps of at least two different BWs presented with durations of 0.5–70 ms. The sweeps were centered approximately at the BF of each neuron. The sweep duration functions were then converted to rate selectivity functions by dividing the sweep BW by the sweep duration (kHz/ms). Neurons were classified as RS if the responses at the slowest rates tested

[‡]Razak KA, Fuzessery ZM, 29th Annual Midwinter Research Meeting of the Association for Research in Otolaryngology, February 5–9, 2006, Baltimore, MD, abstr 229.

declined <25% of maximum response (e.g., Figs. 2*a* and 3*b*). A neuron was categorized as not RS if the response did not decline <75% of maximum for slow rates (e.g., Fig. 2*b*). In all RS neurons, the 50% CO rate was the rate at which response declines to 50% of maximum for slower rates (e.g., Fig. 2*a*, 50% CO = 2 kHz/ms). In neurons that also showed a decline in response at fast rates (e.g., Fig. 2*a*), the best rate (BR) was determined in RS neurons as the geometric center of the range of rates that produced >80% of maximum response (Fig. 2*a*, BR = 5 kHz/ms).

To test for direction selectivity, the response to upward FM sweeps with the same range of duration and BW as the downward sweeps was recorded. A DSI was calculated to quantify direction selectivity (19). The formula used was DSI = $(D - U)/(D + U)$. D and U are the maximum response magnitudes for downward and upward sweeps, respectively. More positive DSI values indicate higher selectivity for downward sweeps.

Two Tone Inhibition Over Time (TTI) Tuning Curves. To determine the arrival time and BW of sideband inhibition, two tones, one excitatory and the other inhibitory, were presented with different delays between them (*SI Appendix*, Fig. 4) (14–15, 19). The frequency of the excitatory tone was set at the BF, and was presented at an intensity 10–20 dB above threshold with a duration of either 3 or 5 ms. The inhibitory tone was presented at the same intensity and a longer duration (10 ms). Its frequency was varied between 5 and 70 kHz in 1-kHz steps. The delay-frequency combinations that resulted in inhibition of response to the excitatory tone served to map out the spectrum and arrival times of inhibitory frequencies. Inhibition that occurred only when the excitatory tone was delayed indicated delayed inhibition (forward masking). Inhibition that occurred even when the excitatory tone was advanced demonstrated an early inhibition that arrived before excitation (backward masking). To obtain more accurate quantification of the arrival times of inhibitory input, the TTI procedure was repeated with the best inhibitory frequency (center frequency of the inhibitory tuning curve) and the BF tone to determine the delay at which response magnitude decreased to 50% of the excitatory tone alone (control) response. This value is referred to as the 50% arrival time of inhibition (e.g., *SI Appendix*, Fig. 4).

Data Representation and Analysis. Pups between 30 and 45 days old are referred to as one-month-old (P30), and pups between 75 and 100 days are referred to as three-month-old (P90). The response magnitudes reported were the number of action potentials in response to 20 stimulus repetitions at 1 Hz repetition rate.

ACKNOWLEDGMENTS. We thank T. Zumsteg for valuable comments on the manuscript and G. McLellan for programming the software required for this study. This work was supported by National Institutes on Deafness and Other Communication Disorders Grant DC005202.

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