Development of spectral and temporal response selectivity in the auditory cortex

Edward F. Chang*, Shaowen Bao, Kazuo Imaizumi, Christoph E. Schreiner, and Michael M. Merzenich†

Coleman Memorial Laboratory, Department of Otolaryngology, W. M. Keck Center for Integrative Neuroscience, University of California, San Francisco, CA 94143-0444

Contributed by Michael M. Merzenich, September 21, 2005

The mechanisms by which hearing selectivity is elaborated and refined in early development are very incompletely determined. In this study, we documented contributions of progressively maturing inhibitory influences on the refinement of spectral and temporal response properties in the primary auditory cortex. Inhibitory receptive fields (IRFs) of infant rat auditory cortical neurons were spectrally far broader and had extended over far longer duration than did those of adults. The selective refinement of IRFs was delayed relative to that of excitatory receptive fields by an ≈2-week period that corresponded to the critical period for plasticity. Local application of a $GABA_A$ receptor antagonist revealed that intracortical inhibition contributes to this progressive receptive field maturation for response selectivity in frequency. Conversely, it had no effect on the duration of IRFs or successive-signal cortical response recovery times. The importance of exposure to patterned acoustic inputs was suggested when both spectral and temporal IRF maturation were disrupted in rat pups reared in continuous, moderate-intensity noise. They were subsequently renormalized when animals were returned to standard housing conditions as adults.

Psychophysical testing of human infants has revealed significant limitations in spectral and temporal sound resolution compared with adults (1). Immaturity of the developing sound processing apparatus is reflected in early speech perception, such as infant preferences for slow cadences and exaggerated pitch contours (2). In speech, important information-carrying features include the relative loudness, duration, separation, and order of individual segments, as well as dynamic combinations of all these elemental sound stimulus parameters.

Studies of hearing development in animal models have begun to characterize the cortical factors that shape sound processing during early life. The spectral receptive field size and temporal modulation response properties ("envelope sampling rates") of auditory cortical neurons change systematically between the onset of hearing and adulthood (3, 4). In rats, sound frequencies become represented in a compact, well-organized topographic primary auditory cortex (A1) marked by spectrally selective (narrowly tuned) neurons by the end of the first month of life (5). Mechanisms accounting for these improvements in sensory processing and topographic organization during development remain unclear.

A possible contribution to the refinement of excitatory auditory response properties may come from subcortical and/or cortical inhibition that is weak at early ages and then progressively strengthens. Alternatively, as growing evidence suggests, the inhibitory circuitry may be functionally robust during early development but may increase its specificity under environmental influence. Inhibition has been convincingly shown to enable experience-dependent plasticity. In the developing visual cortex, for example, the reduction of inhibition can indefinitely extend the period during which monocular deprivation-induced alterations can occur (6). Absolute sensory deprivation has similar effects on prolonging the timing of developmental cortical plasticity, as shown by experiments involving dark-rearing and congenital deafness (7, 8). We recently showed that the absence of patterned acoustic experience had similar developmental effects as no sensory input at all (9).

These findings raise fundamental questions about how developmentally regulated inhibitory patterns in sensory processing contribute to cortical maturation and plasticity. In the present study, we examined the postnatal ontogeny of functional inhibition in the rat auditory cortex by using two-tone stimulation to characterize spectral and temporal inhibitory receptive fields (IRFs).

Materials and Methods

All experiments were carried out by using protocols approved by the Institutional Animal Care and Use Committee at the University of California, San Francisco. See *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site, for a complete description of methods and stimuli used.

Electrophysiology. We investigated the development of inhibition by documenting auditory cortical response properties in rats within six postnatal age groups extending from postnatal day (P) 15 to P90 (n = 3–7 per age group; units recorded at 67–105 sites per animal). At each A1 sampled location, a conventional pure tone "tuning curve" (or excitatory receptive field; ERF) was obtained from extracellular neural responses (Fig. 1*a*). The modulatory effect of tones from outside of the ERF on responses to excitatory tones, however, imply that single-tone tuning curves do not adequately characterize the spectral and temporal input of neurons (10–12).

Therefore, IRFs also were derived by using a two-tone stimulus paradigm, in which a varying "masker" tone suppressed the cortical response to a constant "probe" tone (Fig. 1*b*; see Fig. 7, which is published as supporting information on the PNAS web site, for schematic of stimulus presentations) (13–15). The probe tone frequency that was used in these studies was the ERF characteristic frequency (CF), delivered 10–15 dB above response threshold. By varying the frequency and intensity of the pure-tone masker, the range of sounds that suppressed responses to the probe was determined. The two tones were either presented simultaneously (Fig. 1*b*) or sequentially at variable delays ("stimulus onset asynchronies"; SOAs) in steps of 20 ms (Fig. 1*c*).

It should be noted that the masker-induced reduction of the response to the probe is here called "suppression" or "inhibition." In using those terms, we do not mean to imply that either the specific source of that response reduction or the auditory system level(s) at which it arises is determined.

To further characterize temporal aspects of developmental auditory processing, repetition rate transfer functions (RRTFs) were derived from responses to trains of six short noise bursts presented at repetition rates ranging from 2 to 20 Hz. The noise burst trains were delivered four times at each of 10 repetition rates (2, 4, 5, 7,

Conflict of interest statement: No conflicts declared.

Abbreviations: IRF, inhibitory receptive field; ERF, excitatory receptive field; A1, primary auditory cortex; CF, characteristic frequency; BWn, bandwidth measured n dB above threshold; Pn, postnatal day n; RRTF, repetition rate transfer function; SOA, stimulus onset asynchronies; VS, vector strength; CNR, continuous-noise reared.

*To whom correspondence may be addressed at: University of California, 513 Parnassus Avenue, Box 0444, San Francisco, CA 94143-0444. E-mail: echang@itsa.ucsf.edu.

†To whom correspondence may be addressed. E-mail: merz@phy.ucsf.edu.

www.pnas.org/cgi/doi/10.1073/pnas.0508239102

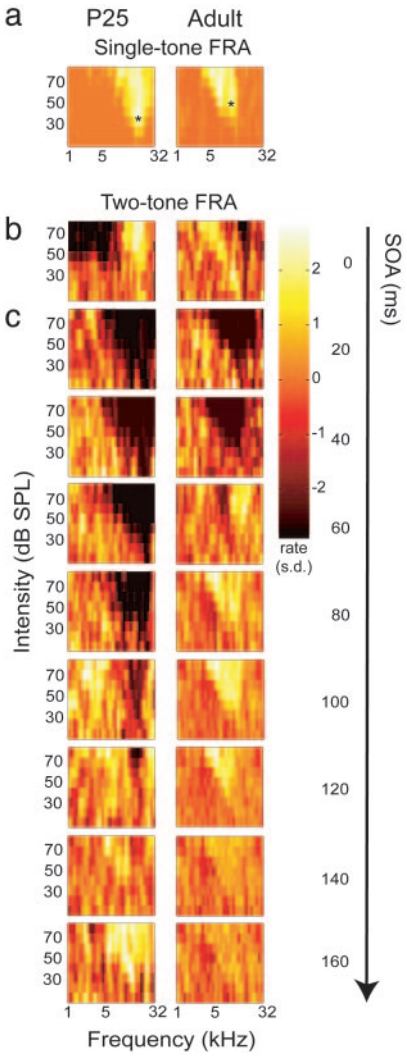

Fig. 1. Excitatory and inhibitory responses in infant and adult rat auditory cortical neurons. (*a*) Typical ERFs (yellow) from one-tone stimuli for P25 infant and adult rats. Normalized response magnitude is plotted as standard deviation (s.d.) from background firing rate (orange). Stimulus used for probe tone in two-tone presentations was chosen at 10–15 dB above threshold, shown by asterisk. (*b* and *c*) IRFs (black) generated from responses to simultaneous (SOA = 0 ms) and asynchronous (SOA ≥ 20 ms) two-tone stimuli.

10, 12.5, 15, 17.5, and 20 pulses per s) in a randomly interleaved sequence. The normalized cortical response at each repetition rate was calculated as the average response magnitude to the last five noise pulses divided by the response magnitude to the first noise pulse. The RRTF is the normalized cortical response as a function of temporal rate (16, 17). In addition, cortical ability for processing high-rate stimuli was estimated with the highest temporal rate at which the RRTF was at least half of its maximum, referred to as $f^{1/2}$. Vector strength (VS) and Rayleigh statistic were used to quantify how well spikes were time-locked to pulses (18).

Pharmacology. For a subset of recordings both from adult and infant rats ($n = 61$), we used microiontophoresis to study the effect of $GABA_A$ antagonist. Recordings were made by using four-barrel assemblies (Kation Scientific, Minneapolis), with an etched carbon fiber in the central barrel. One barrel was filled with 0.9% NaCl (pH 3.0) and used for current balancing and to test for current and pH artifacts. The other barrels were filled with either 10 mM bicuculline methiodide (Sigma) in 0.9% NaCl (pH 3.0) or 500 mM L-glutamate

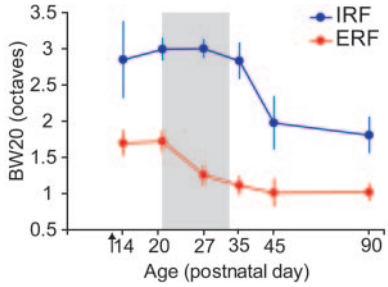

Fig. 2. Developmental changes in spectral excitatory and inhibitory BW20. Blue and red correspond to IRF and ERF, respectively. Onset of hearing is denoted by arrow; critical period of plasticity is in gray-shaded zone. Each point represents mean ± SD.

(Sigma) in 0.9% NaCl (pH 8) adjusted by 30 mM Hepes. Iontophoretic ejection and retaining currents were generated by using a microiontophoresis system (Fintronics, Foster City, CA). Retaining currents of −15 nA were used for all drugs to prevent spontaneous drug diffusion from the tips. Ejection currents were usually in the range of 5–30 nA. Auditory stimuli were presented before, during, and after drug application. In general, 2–3 min after drug application, neural activity (spike rate at CF and spontaneous activity) recovered to baseline.

Noise Rearing. Litters of rat pups were housed in a sound-attenuation chamber and exposed to continuous white noise starting at P7 until age P50 (9). The noise signal was produced by a white noise generator and amplified gradually to 65–70 dB sound pressure level over the first 3 days (for 24 h/day). Rats were given access to food and water *ad libitum* under a 12/12-h light–dark cycle. The weights of all pups and mothers were continuously monitored. No weight loss was recorded in exposed rats compared with control rats. All rats exhibited normal active behavior. A separate set of rats was reared under these conditions and then returned to standard housing with normal acoustic environment.

Results

Spectral and Temporal Inhibition. The development of inhibition was investigated by documenting auditory cortical response properties in rats within six successive postnatal age groups extending from P14 to P90. Three-dimensional plots of response magnitude vs. intensity and frequency were constructed for units recorded at each A1 location. Response magnitudes were normalized to the SD of the background firing rate and plotted in color scale. In Fig. 1, normalized excitation and inhibition (response suppression) are represented in incremental shades of white–yellow to black, respectively. Background firing rate is represented in orange.

Spectral selectivity of infant cortical neurons was poorly differentiated. Excitatory tuning in the auditory cortex of infant rats was correspondingly broad compared with adults, as evidenced by the ERF bandwidth measured 20 dB above threshold (BW20) (Figs. 1*a* and 2, ERF BW20 for P20 infants, 1.76 ± 0.05 octaves, and for adults, 0.94 ± 0.04 octaves; $P < 0.001$, *t* test). The average BW20 gradually narrowed with age, stabilizing to adult values between ages P20 and P27.

We initially hypothesized that ERF narrowing might be accounted for by increasing efficacy of sideband inhibition. Instead, however, we found that IRF bandwidth decreased with increasing age. IRFs generated from simultaneous two-tone stimuli (SOA = 0) in infant rats were, on average, 1.5–2 times as broad as those in adults, as measured 20 dB above the lowest inhibitory threshold (Figs. 1*b* and 2, IRF BW20 for P20 infant, 2.99 ± 0.08 octaves, and P90 adult, 1.81 ± 0.05 octaves; $P < 0.001$, *t* test). In many units, IRFs in infant rats included almost all frequencies outside of the excitatory tuning curve (see example in Fig. 1*a* for P25 infant). Adult A1

neurons typically exhibited narrower IRFs, often only flanking the ERF on one side (Fig. 1*b* for adult auditory cortical neuron).

At all age points, the bandwidth of sideband inhibition was significantly broader than that of excitation (Fig. 2, $P < 0.01$, ANOVA). The BW20 for IRFs stabilized between P35 and P45: Thus, the spectral selectivity of inhibition underwent similar changes as excitatory selectivity, but on a slower maturational course. The developmental changes in sideband IRFs ended ≈2 weeks after ERF changes, resulting in a peak ratio of inhibitory to excitatory bandwidth between P27 and P35.

During simultaneous two-tone presentation, ≈43–56% of sampled neurons in each animal had a clearly demarcated sideband IRF that were used for further analysis. It is possible that the extents of IRFs are underestimated because it was not feasible to study each unit with all possible combinations of two-tone frequencies and intensities. Nonetheless, we found that the presence of a sideband IRF(s) was associated with narrower ERF BW20 in infant and adult animals ($P < 0.05$, logistic regression). The precise IRF BW20 for those neurons, however, did not appear to correlate with ERF BW20 ($P = 0.13$, linear regression). Many neurons with similar excitatory tuning (i.e., CF and tuning bandwidth) differed in the frequency extent of inhibition. For example, within an individual animal, some neurons demonstrated narrow IRFs, whereas others with the same CF showed very broad IRFs. Thus, excitatory tuning was not a direct function of the bandwidth of sideband inhibition.

Small systematic changes in the probe frequency did not change the IRF structure as long as the probe tone could reliably drive the unit to fire. When probe tone intensity was increased far above threshold at the CF, IRF BW20s were reduced in size (for adults, BW20 with probe tone at 10 dB above threshold = 1.81 ± 0.04 octaves; at 30 dB above threshold = 0.23 ± 0.3 octaves; $P < 0.05$, *t* test).

Acoustic sequences are fundamental features in sound recognition. We used asynchronous two-tone presentation, or forward masking, to characterize progressive developmental changes in the temporal aspects of cortical response suppression. The forward masking paradigm has revealed important details regarding spectrotemporal interactions in complex sounds in the adult cortex, but it has not been explored in the developing cortex. Forward masking effects were evaluated by introducing various SOAs between the masker and probe stimuli.

Beginning with a SOA of 20 ms, the forward masking IRF was broadly tuned and centered over the ERF (Fig. 1*c*), in agreement with previous adult cortical studies (14, 15). At shorter SOAs (20–60 ms), masker tones near but outside the border of the ERF also often suppressed responses to the probe stimulus. As the SOA interval increased, the masker frequency range of suppressing probe tones was gradually narrowed. In other words, recovery of the probe tone response from suppression was fastest for maskers at the low- and high-frequency borders of the receptive field and slowest (longer SOAs) at frequencies nearer the CF (Fig. 8*a*, which is published as supporting information on the PNAS web site; $P < 0.001$, multivariate ANOVA). The CF of the IRF was strongly correlated with that of the ERF (Fig. 8*b*; for infants, $r^2 = 0.89$, $P < 0.001$; for adults, $r^2 = 0.92$, $P < 0.001$; linear regression). After the epoch of suppression, 10–20 ms of "rebound" facilitation above the response to the probe tone alone followed (Fig. 1*c*).

Forward masking was significantly longer in infant rat auditory cortical neurons than in adults (Fig. 8*c*) (maximum duration of forward inhibition for P25, 137 ± 13 ms, and for adults, 66.8 ± 11 ms; $P < 0.0001$, *t* test). Thus, the ability of cortical neurons to respond successively with excitatory activity, i.e., the energy envelope sampling rate of its A1 processing channels, depended on the stimulus context. At any age, neurons could follow faster stimulus sequences when they differed substantially in their spectral content. Successive spectrally similar stimuli effectively engaged A1 neurons only after significant response recovery times (14). The extent of spectral interference domains and the duration of forward masking narrowed with age, resulting in substantial modifications of sound integration constants for acoustic processing during development.

These forward masking results were closely related to repetition rate transfer functions derived from responses to longer sets of identical, multiple successive stimuli (3). The RRTF reflects the sequences of excitatory and inhibitory cortical circuit events in temporally modulated or repetitive sounds (19), in which the neural discharge rate is expressed as a function of the stimulus repetition

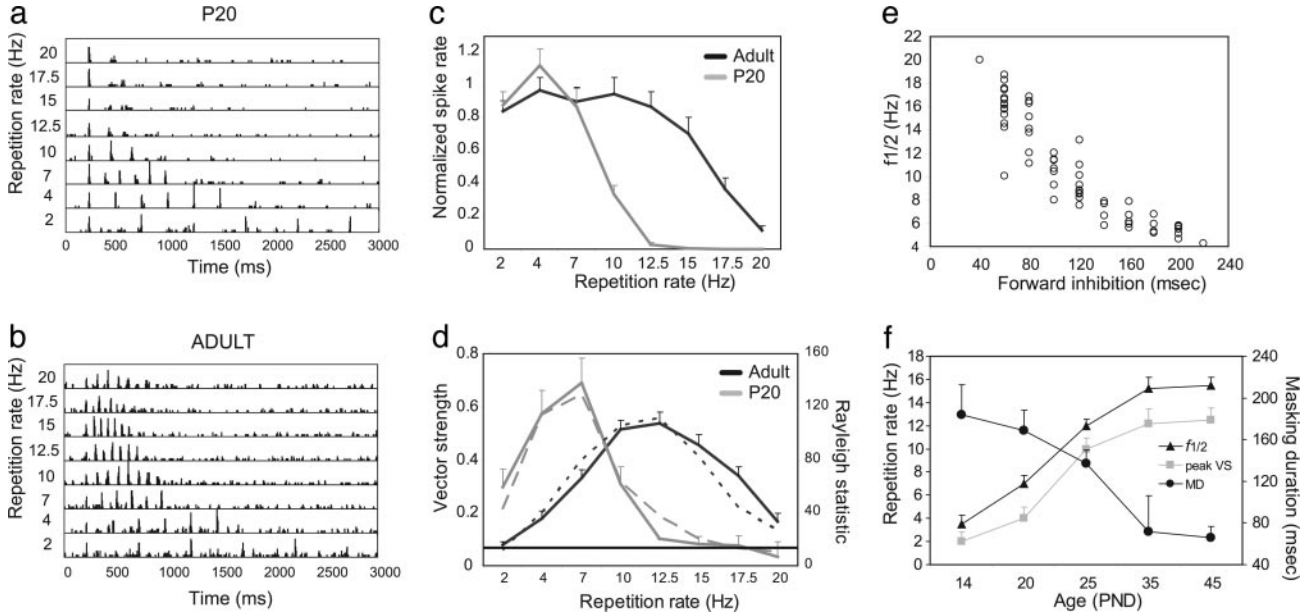

Fig. 3. Quantification of temporal response properties during auditory cortical development. (*a* and *b*) Poststimulus time histogram for the responses to six noise bursts at various repetition rates. (*c*) The normalized RRTF for infant and adult rats. (*d*) VS (continuous line) and Rayleigh statistic (dotted line) for infant and adult rats. A Rayleigh statistic value of >13.8 indicates $P < 0.001$, indicated by the horizontal thick black line. (*e*) Time course of temporal response properties throughout normal development. ($f^{1/2}$, repetition rate at half-maximal RRTF; MD, duration of forward masking). (*f*) Summary of temporal response selectivity: comparing following rate derived from noise burst train ($f^{1/2}$) and masking duration from two-tone forward masking across all age groups.

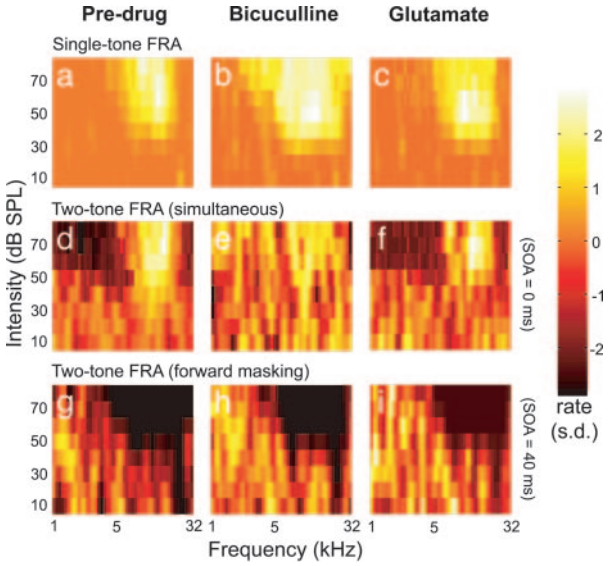

Fig. 4. Microiontophoresis under predrug, bicuculline, and glutamate conditions from a P20 infant rat auditory cortical neuron. Stimuli included one-tone (*a–c*), simultaneous two-tones (SOA = 0 ms; *d–f*), and forward masking two-tones (SOA = 40 ms; *g–i*).

rate. RRTFs were derived from responses to trains of six short noise bursts presented at repetition rates ranging from 2 to 20 Hz at 65 dB sound pressure level. The RRTF here is the average number of spikes for each of the last five bursts of the six-burst train plotted as a function of repetition rate.

In adult rats, at repetition rates up to ≈12.5 Hz, each noise burst evoked approximately the same number of spikes from A1 neurons as did the first burst in the train (Fig. 3*b*). At repetition rates from 15–17.5 Hz the number of spikes per noise burst fell off rapidly, and only a few neurons responded at all to rates of >17.5 Hz. In infant P20 rats, by contrast, no neurons could follow rates of >7–10 Hz (Fig. 3*a*). For each recording site, the mean response to the last five noise bursts was normalized by the response to the first noise burst. In general, at low rates, a high-amplitude response was maintained throughout the pulse train. For a small percentage of neurons, the response at lower rates was facilitated. Overall, however, neurons had predominately low-pass RRTF characteristics. Cortical processing of temporal rate stimuli, as measured by the estimated rate at which RRTF was half of its maximum ($f^{1/2}$), was significantly greater in adult animals compared with infants (Fig. 3*c*; $P < 0.0001$, Kruskal–Willis, post hoc Bonferroni *t* test). Importantly, the RRTF was inversely correlated with the duration of suppression defined in the forward masking paradigm across all age groups (Fig. 3*e*; $r^2 = -0.81$).

The temporal fidelity of successive-signal responses was quantified by VS and statistically assessed with a Rayleigh test. VS reflects the precision with which a response locks its firing to the same phase of the stimulus period, with higher values signifying more precise spike timing per cycle. Temporal modulation transfer functions reconstructed from VS revealed band-pass characteristics with highest temporal fidelities in the range of 10–15 Hz for adults. By contrast, VS peaked at ≈7 Hz for P25 infant rats (Fig. 3*d*). These measures of temporal processing (forward masking, RRTF, and VS) all demonstrate progressive temporal response refinements with maturation up to approximately P35 (Fig. 3*f*; $P < 0.01$, multivariate ANOVA).

Cortical GABA-ergic Contributions to Two-Tone Suppression. Because simultaneous two-tone suppression can be observed as peripherally as the cochlea, bicuculline (a $GABA_A$ antagonist) was applied microiontophoretically to A1 during a subset of recordings in infant (P20, $n = 7$) and adult (P90, $n = 7$; 5–6 units per animal) groups to evaluate the possible contributions of local GABA-ergic cortical circuitry to observed response suppression effects. Glutamate also was applied through an adjacent micropipette to delineate whether any bicuculline-induced receptive field changes could be attributed to a selective decrease in inhibition as opposed to a relative increase in excitation. The predrug two-tone suppression at each chosen application site was strong (BW20 > 1 octave).

ERFs were consistently broadened with bicuculline application. In Fig. 4 *a–c*, a representative recording series from a P20 animal shows that bicuculline administration expanded the ERF on both the low- and high-frequency sides of the tuning curve. Response thresholds also were significantly decreased by ≈5.2 dB ($P < 0.05$, before vs. during bicuculline, *t* test). Glutamate application, by contrast, did not significantly change the ERF. Iontophoresis in adults exhibited similar effects (Fig. 5*a*; percent change in BW20 from bicuculline, +46.3 ± 12.1% for P50 adults and +38.1 ± 10.2% for P20 infants; $P = 0.13$, between age groups, unpaired *t* test; $P < 0.001$, *t* test), consistent with previous reports (20, 21).

The contribution of local cortical inhibition to suppression patterns generated by simultaneous two-tone stimuli is not well understood. We found that IRFs were narrowed but not eliminated by the administration of bicuculline. A significantly greater effect was recorded in infant rats compared with adults when measuring the percentage change in BW20 from predrug conditions (Figs. 4 *d–f* and 5*a*; percent change in BW20, −73.8 ± 9.4% for P20 rats, $P < 0.001$, *t* test; −43.2 ± 7.0% for adult rats, $P < 0.01$, *t* test; difference between P20 vs. adult, $P < 0.05$, *t* test). In Fig. 5, these findings are summarized with infant data in light shading and adult data in dark shading and with ERF changes in red and IRF changes in blue. Complete and partial elimination of the IRF was observed in 37% and 44% of recordings, respectively. No effect was observed in 19%. Glutamate application did not significantly affect IRF or ERF bandwidths (percent change: IRF, −6.3 ± 19%; ERF, −7.2 ±

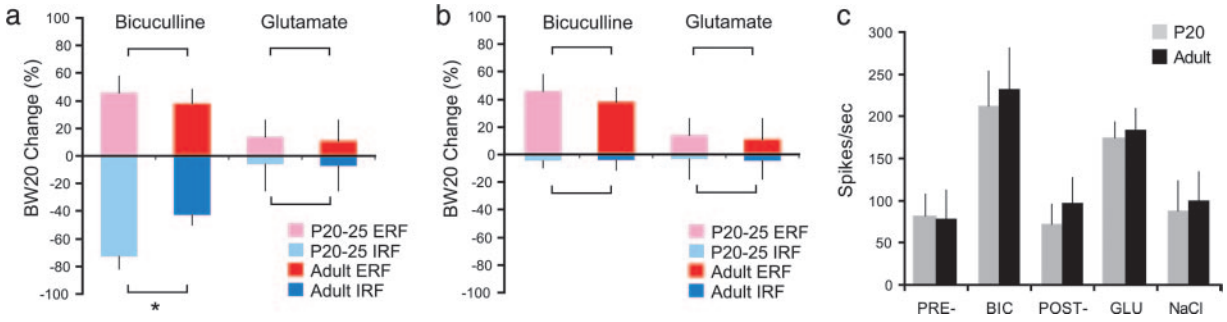

Fig. 5. Changes in the ERF and IRF bandwidth during local pharmacological manipulation of excitation and inhibition. Percent change for simultaneous two-tone (*a*) and forward masking two-tone stimulation (*b*) is shown. *, $P < 0.05$. (*c*) Evoked spike rates during iontophoresis of bicuculline, glutamate, and pH-controlled NaCl (pH 3), and pre- and post-bicuculline conditions.

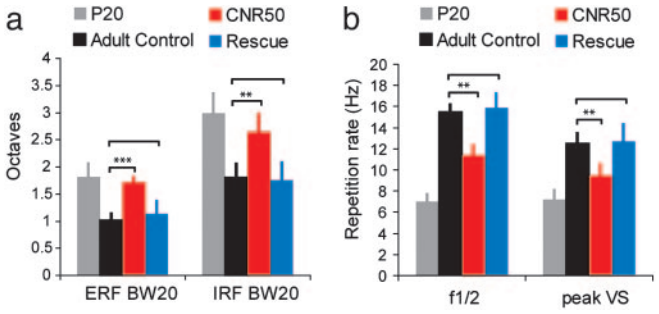

Fig. 6. Effects of continuous-white-noise rearing on development of spectral and temporal selectivity. (*a*) Comparison of ERF and IRF bandwidths for infant (P20, gray), control adult (black), CNR adult rats (CNR50; red; noise reared to age P50), and rescued adult (rescue, blue; noise reared to age P50, then housed in normal acoustic environment for 2 months). ⋆, $P < 0.05$. (*b*) $f^{1/2}$ and peak VS comparisons.

17%; $P = 0.13$, *t* test). In some neurons, bicuculline application resulted in the near complete loss of IRFs, far beyond the amount observed for ERFs (see Fig. 4 *b* and *e*). Therefore, sideband inhibitory patterns derived from simultaneous two-tone stimuli are not fully predicted by ERF expansion during inhibitory blockade. These IRF patterns represent the specific contribution of inhibitory cortical integration of two tones rather than solely shaping a neuron's response to single tones.

Forward masking patterns generated from asynchronous two-tone stimuli were not significantly altered by bicuculline application. As shown in Fig. 4 *g–i*, IRF BW20s at SOA of 40 ms were not statistically different among the predrug, bicuculline, and glutamate conditions (Fig. 5*b*). This finding is consistent with previous reports on temporal processing in somatosensory cortex and is thought to be related to short-term depression at the thalamocortical synapse (22).

The evoked spike rate at CF was equivalently increased for both infant and adult animals after either bicuculline or glutamate administration, compared with the predrug, postdrug, and pH-controlled NaCl conditions (Fig. 5*c*; percent change in spikes per s under each condition were bicuculline, +260% for P20 rats and +297% for adults, $P = 0.43$; glutamate = +215% for P20 rats and +235% for adults, $P = 0.58$; *t* test). Although glutamate application increased the spike rate, it did not induce significant changes of ERF or IRF BW20s. Thus, bicuculline-induced changes in receptive field properties did not result from a generalized increase in excitability to tones at CF but, rather, from a specific block of cortical, tone-evoked inhibition. A significant component, but not all, of simultaneous two-tone suppression was therefore demonstrated to be inhibitory processing of cortical origin.

Effects of Noise Rearing on Inhibitory Development. The above findings reveal that cortical inhibition undergoes predictable maturation with physical age. Whether experience affects IRF maturation is unclear. One method of exploring the role of experience is to deprive rats of saliently patterned sound inputs during development by rearing pups in continuous noise. This method has been shown previously to delay maturation of excitatory cortical receptive field structure and topographic A1 organization (9). To determine whether maturation of inhibitory/suppressive components of the receptive field was affected by experience, rat pups ($n = 4$; 54–113 units per rat) were reared in the presence of moderate-intensity (70 dB sound pressure level) continuous white noise from age P7 to P50 and then studied electrophysiologically.

Continuous-noise-reared (CNR) rats had significantly broader ERF and IRF BW20s than age-matched controls (Fig. 6*a*) (ERF control P50, 1.02 ± 0.04 octaves; CNR, 1.72 ± 0.03 octaves; $P < 0.005$, *t* test; IRF control P50, 1.81 ± 0.27 octaves; CNR, 2.65 ± 0.32 octaves; $P < 0.01$, *t* test). CNR rats also had reduced RRTFs (Fig. 6*b*; $f^{1/2}$ for CNR = 11.4 ± 0.95 Hz and for P50 controls = 15.6 ± 0.79 Hz; $P < 0.01$, *t* test), and lower peak VSs compared with adult controls (peak repetition rate for CNR rats = 9.5 ± 1.02 Hz and for naïve P50 adults = 12.5 ± 0.96 Hz; $P < 0.01$, *t* test). These results indicate that in addition to delays in ERF bandwidth narrowing and topographical maturation, the IRFs contributing to spectral and temporal response selectivity also are greatly altered by noise rearing.

To show that noise rearing delays, but does not suppress, the refinement of IRFs and/or ERFs, a "rescue" experiment was performed. A separate set of rats ($n = 4$; 57–92 units per rat) were reared in continuous white noise to age P50 and then returned to normal housing conditions for 2 months. In general, these CNR rats mapped at P120, long after noise cessation, exhibited receptive field properties that were substantially like those of control adult rats.

ERF and IRF bandwidths from simultaneous two-tone stimulation were not different from control naïve animals (IRF rescue, 1.73 ± 0.32 octaves; ERF rescue, 1.18 ± 0.10 octaves; see above for control P50 values; $P = 0.38$, $P = 0.21$, *t* test). Rescued rats had similar RRTF (Fig. 6*b*; $f^{1/2} = 15.9 \pm 1.30$ Hz; $P < 0.01$, *t* test) and similar peak VS compared with adult controls (peak VS = 12.7 ± 1.62 Hz, $P < 0.01$, *t* test). These results suggest that moderate-intensity noise rearing during early development does not necessarily permanently impair auditory cortical processing into adulthood. Once the source of noise was eliminated, the still-open critical period window permitted the emergence of relatively normal spectral and temporal receptive field properties.

Discussion

Our findings demonstrate that intracortical inhibition undergoes substantial postnatal maturation in rat A1. The stimulus selectivity of excitatory responses of A1 neurons is progressively sharpened with age. Contrary to our initial predictions that these refinements were due to increasing inhibition, we found that inhibitory response patterns were already robust in both the spectral and temporal domains soon after the onset of hearing. IRFs underwent maturational changes that at least in some aspects paralleled those of excitatory response patterns. In infant rats, IRFs were spectrally broad and temporally extended. In adults, IRFs were more spectrally and temporally refined.

Two basic experiments were performed to explore the mechanisms underlying these progressive changes in cortical acoustic responses. First, bicuculline microiontophoresis revealed $GABA_A$ intracortical contributions to the simultaneous two-tone inhibition. Second, rearing animals in the presence of continuous moderate-level noise led to a delay of normal IRF maturation. After allowing noise-reared rats to recover in a normal acoustic environment, both IRF and ERF properties progressively "matured," indicating that exposure to patterned sound inputs is crucial to inhibitory as well as excitatory dimensions of A1 response maturation.

Using two-tone stimuli to probe cortical receptive field properties provides a coarse estimation of complex sound representation. Responses to two-tone stimuli predict, for example, responses evoked by frequency sweeps and ripple spectra (23, 24). From our data, it appears that the infant cortex is better suited for spectral and temporal integration across a very broad range of acoustic inputs. The capacity for more refined discrimination undergoes continuous improvement until adulthood.

The effects of bicuculline on broadening ERF and narrowing IRF bandwidth provided direct evidence that the local inhibitory circuitry contributes to cortical spectral selectivity. This result was more pronounced in infant than in adult A1 neurons. At the same time, bicuculline did not entirely eliminate IRFs or broaden ERFs by more than several octaves, implying that cortical response patterns also were constrained by subcortical factors, such as the suppressive sidebands generated in the subcortical auditory system.

Auditory cortical IRFs were far more heterogeneous and irregular than those described for the auditory nerve, which shows the opposite trend of bandwidth changes with age (25). Moreover, the auditory nerve and brainstem mature very early in the course of development compared with what we observed in the cortex (25–29).

The maturation of auditory receptive fields probably reflects anatomical and/or synaptic properties that change over development. Density of GABA-stained neurons in the auditory cortex peaks in late development and actually diminishes across the progression into adulthood (30). Inputs from pyramidal neurons that project long-range horizontal connections across auditory and visual cortices (31–33) and exuberance of cortical connections can perhaps account for the broad spectral integration observed at young ages.

These arguments support the theory that intracortical inhibition has a more complex role than just helping to sharpen pure-tone evoked excitation. First, although the presence of an IRF was associated with narrowed ERF tuning, the actual size of IRF bandwidth was not. Second, both IRF and ERF bandwidth narrowed during maturation, which is opposite our initial prediction that narrow ERF tuning resulted from increasing inhibition. Third, although both underwent roughly parallel changes with age, the maturation of inhibition was delayed by $\approx$2 weeks compared with excitation. This offset coincided with the "critical period" for tonotopic plasticity at the end of first month of life (5, 9).

We propose that this early powerful expression of cortical inhibition in auditory sensory processing bears important implications for plasticity mechanisms. Broad spectral and long temporal IRFs in infant rats elicited by two-tone stimuli reveal an effective intrinsic mechanism for decorrelating neuronal activity by "sorting" temporally coincident or nearly coincident sensory inputs (as in simultaneous and asynchronous stimuli, respectively). Sorting sound inputs to the developing auditory cortex may be important given the large spectral range of converging and diverging inputs from subcortical auditory nuclei, as confirmed by extremely broad subthreshold responses in intracellular studies (34). The inputs to infant cortical neurons are almost certainly more numerous and unrefined given their characteristically broader receptive field properties.

An important shaping role of temporally sorted competitive acoustic inputs on the establishment of structural organization and neural processing capabilities has been indicated by previous studies. Rearing mice in an acoustic environment with broadband click stimuli resulted in reduced frequency tuning of neurons documented at the level of inferior colliculus (35). Introduction of synchronous inputs into the auditory pathway achieved by exposing rat pups to pulsed noise resulted in a disrupted tonotopicity and degraded frequency-response selectivity for neurons in the adult A1 and actually prematurely advanced the timing of critical period of plasticity (36). In each of these cases, plasticity mechanisms induced the functional differentiation of auditory cortex according to early stimulus properties.

For continuous-noise rearing, in contrast, cortical maturation and plasticity are delayed by the deprivation of patterned inputs. Because sound energy in white noise is diffused across spectral and temporal domains, acoustic inputs in this exposure are essentially unsortable. We found that the inhibitory network also remains in a prolonged state of immaturity, essentially until normal acoustic inputs are restored. At this point, cortical inhibition may participate in the rapid establishment of adult A1. Therefore, normal cortical development may be contingent on the presence of both intrinsic and extrinsic factors: functional cortical inhibition and patterned sound exposure.

Clearly, our data cannot address all of these points, but they raise important unresolved questions of whether the end of the critical period can crucially enable subsequent inhibitory maturation. The mechanisms by which sensory exposure to patterned sounds engages inhibitory maturation need to be delineated.

There are several caveats in the interpretation of our results. Successive signal suppression is thought to be partially generated in the cortex because neurons in the medial geniculate nucleus can follow faster stimuli (37). The differential effects of bicuculline on spectral vs. temporal inhibition suggest that spectral inhibition from simultaneous two-tone stimuli has a potentially different mechanistic origin than temporal inhibition in forward masking. Short-term synaptic suppression (e.g., at the thalamocortical synapse) or $GABA_B$ are candidate mechanisms for the probable cortical-level contributions to these effects. Furthermore, alternative mechanisms related to the relative strengths (as opposed to the temporal and spectral extent) of excitation and inhibition might better explain how ERFs are refined over development. In the two-tone paradigm, it is difficult to assess this contribution given that suppression was complete in most cases for both infant and adult animals.

The association of inhibition and experience-dependent plasticity in the developing auditory cortex must have consequences for understanding language learning and its disorders. Studies demonstrate that children with specific language impairment exhibit deficits in auditory discrimination of both spectral and temporal cues (38). It is possible that inhibitory circuitry may be impaired in such individuals. Remediation of some language impairments is possible through intensive training with exposure to modified speech sounds that emphasize salient features in rapidly changing acoustic elements.

We thank I. Meng and H. Fields for use of iontophoresis equipment and members of the M.M.M. and C.E.S. laboratories for helpful comments on the manuscript. This work was supported by National Institutes of Health Grants NS-10414, DG-02260, and NS-34835; The Coleman Fund; The Sandler Fund; and the Mental Insight Foundation.

1. Werner, L. A. & Rubel, E. W. (1992) *Developmental Psychoacoustics* (Am. Psychol. Assoc., Washington, DC).
2. Kuhl, P. K., Andruski, J. E., Chistovich, I. A., Chistovich, L. A., Kozhevnikova, E. V., Ryskina, V. L., Stolyarova, E. I., Sundberg, U. & Lacerda, F. (1997) *Science* **277,** 684–686.
3. Eggermont, J. J. (1996) *Auditory Neurosci.* **2,** 309–327.
4. Bonham, B. H., Cheung, S. W., Godey, B. & Schreiner, C. E. (2004) *J. Neurophysiol.* **91,** 841–854.
5. Zhang, L. I., Bao, S. & Merzenich, M. M. (2001) *Nat. Neurosci.* **4,** 1123–1130.
6. Hensch, T. K., Fagiolini, M., Mataga, N., Stryker, M. P., Baekkeskov, S. & Kash, S. F. (1998) *Science* **282,** 1504–1508.
7. Cynader, M. & Mitchell, D. E. (1980) *J. Neurophysiol.* **43,** 1026–1040.
8. Kral, A., Hartmann, R., Tillein, J., Heid, S. & Klinke, R. (2001) *Audiol. Neurootol.* **6,** 346–362.
9. Chang, E. F. & Merzenich, M. M. (2003) *Science* **300,** 498–502.
10. Nelken, I., Prut, Y., Vaddia, E. & Abeles, M. (1994) *Hearing Res.* **72,** 223–236.
11. Suga, N. & Tsuzuki, K. (1985) *J. Neurophysiol.* **53,** 1109–1145.
12. Kadia, S. C. & Wang, X. (2003) *J. Neurophysiol.* **89,** 1603–1622.
13. Sutter, M. L., Schreiner, C. E., McLean, M., O'Connor K, N. & Loftus, W. C. (1999) *J. Neurophysiol.* **82,** 2358–2371.
14. Brosch, M. & Schreiner, C. E. (1997) *J. Neurophysiol.* **77,** 923–943.
15. Calford, M. B. & Semple, M. N. (1995) *J. Neurophysiol.* **73,** 1876–1891.
16. Kilgard, M. P. & Merzenich, M. M. (1998) *Nat. Neurosci.* **1,** 727–731.
17. Bao, S., Chang, E. F., Woods, J. & Merzenich, M. M. (2004) *Nat. Neurosci.* **7,** 974–981.
18. Mardia, K. V. & Jupp, P. E. (2000) *Directional Statistics* (Wiley, New York).
19. deRibaupierre, F., Goldstein, M. H. & Yeni-komshian, G. (1972) *Brain Res.* **48,** 205–225.
20. Wang, J., McFadden, S. L., Caspary, D. & Salvi, R. (2002) *Brain Res.* **944,** 219–231.
21. Muller, C. M. & Scheich, H. (1988) *J. Neurophysiol.* **59,** 1673–1689.
22. Chung, S., Li, X. & Nelson, S. B. (2002) *Neuron* **34,** 437–446.
23. Zhang, L. I., Tan, A. Y., Schreiner, C. E. & Merzenich, M. M. (2003) *Nature* **424,** 201–205.
24. Schreiner, C. E. & Calhoun, B. M. (1994) *Auditory Neurosci.* **1,** 39–59.
25. Fitzakerley, J. L., McGee, J. & Walsh, E. J. (1994) *Hearing Res.* **77,** 135–149.
26. Geal-Dor, M., Freeman, S., Li, G. & Sohmer, H. (1993) *Hearing Res.* **69,** 236–242.
27. Sanes, D. H. & Rubel, E. W. (1988) *J. Neurosci.* **8,** 682–700.
28. Kim, G. & Kandler, K. (2003) *Nat. Neurosci.* **6,** 282–290.
29. Brugge, J. F., Blatchley, B. & Kudoh, M. (1993) *Brain Res.* **615,** 199–217.
30. Gao, W. J., Newman, D. E., Wormington, A. B. & Pallas, S. L. (1999) *J. Comp. Neurol.* **409,** 261–273.
31. Ojima, H., Honda, C. N. & Jones, E. G. (1991) *Cereb. Cortex* **1,** 80–94.
32. Bringuier, V., Chavane, F., Glaeser, L. & Fregnac, Y. (1999) *Science* **283,** 695–699.
33. Kaur, S., Lazar, R. & Metherate, R. (2004) *J. Neurophysiol.* **91,** 2551–2567.
34. Ojima, H. & Murakami, K. (2002) *Cereb. Cortex* **12,** 1079–1091.
35. Sanes, D. H. & Constantine-Paton, M. (1985) *J. Neurosci.* **5,** 1152–1166.
36. Zhang, L. I., Bao, S. & Merzenich, M. M. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 2309–2314.
37. Creutzfeldt, O., Hellweg, F. C. & Schreiner, C. (1980) *Exp. Brain Res.* **39,** 87–104.
38. Wright, B. A., Lombardino, L. J., King, W. M., Puranik, C. S., Leonard, C. M. & Merzenich, M. M. (1997) *Nature* **387,** 176–178.