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Manipulating critical period closure across different sectors of the primary auditory cortex

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Abstract

During early brain development and through ‘adult’ experience-dependent plasticity, neural circuits are shaped to represent the external world with high fidelity. When raised in a quiet environment, the rat primary auditory cortex (A1) has a well-defined ‘critical period’, lasting several days, for its representation of sound frequency. The addition of environmental noise extends the critical period duration as a variable function of noise level. It remains unclear whether critical period closure should be regarded as a unified, externally gated event that applies for all of A1 or if it is controlled by progressive, local, activity-driven changes in this cortical area. We found that rearing rats in the presence of a spectrally limited noise band resulted in the closure of the critical period for A1 sectors representing the noise-free spectral bands, whereas the critical period appeared to remain open in noise-exposed sectors, where the cortex was still functionally and physically immature.

The critical period is an early postnatal epoch of plasticity during which large-scale changes in response selectivity can be induced by exposure to environmental stimuli. It is distinguished from the ensuing life-long epoch of ‘adult plasticity’, where representational plasticity is modulated as a function of alertness, attention and behavioral outcome¹. One of the most powerful products of critical period plasticity in humans is the universal acquisition of whatever ‘native’ language a young child is exposed to, regardless of their genetic background². A potential downside of this powerful, early environmental exposure-driven representational malleability is that distorted sensory inputs during this early developmental phase can have life-long repercussions on neurological performance abilities and may lead to substantial delays or impairments in learning^{1,3}.

During the critical period, cortical circuits and the systems that feed them initially bias (specialize) their selective neuronal responses. In the normal primary somatosensory cortex of the rat, the critical period is only a few days long and ends at postnatal day 5 (P5)^{4,5}. Critical period in the rat auditory system, as documented in A1, extends from about P11 to P13 when a rat pup is raised in a quiet environment⁶. The critical period for ocular dominance in the rat’s

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AUTHOR CONTRIBUTIONS

E.d.V.-S. contributed to the experimental design, electrophysiological data collection, analysis and writing of the manuscript. K.L.S., Y.-F.L. and R.C.S.L. performed the histological analysis and edited the text. M.M.M. participated in experimental design and writing.

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primary visual cortex (V1) begins in the third postnatal week and extends over an epoch of about 2 weeks⁷.

It should be noted that the timing of the onsets and the duration (offsets) of these epochs can be manipulated by altering early sensory experiences. In the rat A1 and V1, critical period onset coincides with the establishment of peripheral sensory organ acuity and an absence of coherent or patterned sensory input prevents normal cortical columnar organization^{6,8–12}. For example, exposure to continuous broadband noise delays or indefinitely prevents critical period closure in A1, whereas pulsed noise promotes its early closure^{12,13}. Dark rearing similarly delays or indefinitely prevents ocular dominance–assessed critical period closure in V1 (ref. 7), whereas rearing animals in the presence of stroboscopic light can result in an earlier than normal critical period closure¹⁴.

It has been recently argued that there may be separate, but overlapping, critical period epochs that apply for different levels of complexity of the processing of sensory inputs in V1 (ref. 15), suggesting the presence of complex and necessarily local mechanisms for its regulation.

Although the effects of early sensory experience on critical period timing and regulation are undeniable, there is a large and growing body of data that confirms the importance of molecular and cellular events that contribute to both the maintenance and the offset of the critical period¹⁵. Maturation of the cortical inhibitory circuitry, and particularly of parvalbumin-positive fast-spiking interneurons, leads to stable GABA-mediated connections being established, dendritic spines being eliminated and unused representations becoming suppressed^{4,16}. However, the complex interplay between experiential, developmental and cellular events is still obscure. Is critical period closure an all-or-none event that is triggered outside of the cortex when a set of conditions are met or is it induced by activity-dependent signals arising locally in the cortex itself? Furthermore, how do sensory input statistics relate to the regulation of critical period timing and the establishment of stable sensory representation in A1?

To answer these important questions, we exposed developing rat pups to spectrally limited acoustic noise before the onset of hearing at P7 and up to 1 week after the normal critical period closure for spectral tuning. We found that exposure of rat pups to such stimuli during auditory system development distorted the frequency representations in A1 to strongly favor frequencies that were just outside the noise band. We also found that A1 sectors that had been exposed to noisy, incoherent inputs were held in a functionally and histologically immature state and retained a capacity for experience-dependent plasticity, whereas the remainder of A1 did not. These results indicate that the control of the duration and closure of the critical period are dependent on the local state of cortical (or limited-sector system) maturation.

RESULTS

Effect of spectrally-limited noise on A1 frequency tuning

To examine the impact of continuous spectrally restricted random noise on A1 frequency tuning during the critical period, we continuously exposed rat pups to band-limited noise (BLN) or notched noise at an intensity of 70 dB SPL between P7 (before the onset of rat hearing) and P20 (7 d past the normal critical period closure for quiet-reared rat pups, at P13; Fig. 1)⁶. The noiseless notch extended from 5 to 10 kHz and the BLN noise extended from 5 to 20 kHz. Both notched noise ($n = 4$) and BLN ($n = 4$) exposures resulted in a marked over-representation in A1 of cortical sites with characteristic frequencies outside of the noise band compared with controls ($n = 8$; Fig. 1a). Notched noise exposure doubled the area of A1 tuned to frequencies in the noiseless notch between 5 and 10 kHz compared with controls ($13.4 \pm 1.3\%$ of A1 for controls and $33.1 \pm 3.3\%$ of A1 for notched noise exposed; $P = 0.0004$, t test). Concurrently,

in the notched noise group, the area of A1 tuned to frequencies below 5 kHz was decreased ($26.1 \pm 2.0\%$ of A1 for controls and $17.5 \pm 1.5\%$ of A1 for notched noise; $P = 0.006$, t test), as was the area tuned to frequencies above 10 kHz ($60.3 \pm 2.7\%$ of A1 for controls and $49.4 \pm 3.1\%$ of A1 for notched noise; $P = 0.02$, t test).

BLN exposure resulted in a significant over-representation of cortical sites with characteristic frequencies lower than 5 kHz ($23.1 \pm 1.1\%$ of A1 for controls and $31.1 \pm 2.1\%$ for BLN; $P = 0.012$, t test) or higher than 20 kHz ($38.4 \pm 1.4\%$ of A1 for controls and $47.2 \pm 2.6\%$ for BLN; $P = 0.02$, t test). The area of A1 tuned to frequencies between 5 and 20 kHz was consequently decreased in the BLN group ($38.5 \pm 3.1\%$ of A1 for controls and $21.9 \pm 3.1\%$ for BLN; $P = 0.004$, t test). When frequency representation in A1 was separated by characteristic frequencies, notched noise and BLN exposures not only resulted in an expansion of sites tuned to frequencies outside of the noise bands, but also reduced the representation of frequencies in the noisy portion of the auditory spectrum (Fig. 1c). Noise exposures had no substantial impact on total A1 size.

Notched noise and BLN exposures also resulted in a distortion of the A1 tonotopic gradient. We plotted the average location of A1 iso-frequency boundaries with respect to the normalized tonotopic axis of A1 (see Methods) for controls and for both exposed groups (Fig. 1d). In notched noise-exposed rats, the region of A1 tuned to frequencies between 5 and 10 kHz was almost twice that of controls (0.13 normalized units for controls and 0.24 normalized units for notched noise; $P = 0.0016$, t test), and in BLN exposed rats, the A1 sector tuned to 5–20 kHz was 35% smaller than in controls (0.32 normalized units for controls and 0.21 normalized units for BLN; $P = 0.0032$, t test).

We measured response bandwidths at 10 dB above threshold (BW10) for litters exposed to BLN ($n = 4$, cortical sites = 188) and to notched noise ($n = 4$, cortical sites = 175) between P7 and P20. BW10 measures were also recorded for controls ($n = 5$, cortical sites = 243) and broadband noise-exposed rats (BBN, $n = 4$, cortical sites = 201). In these BBN controls, rats were exposed to random noise that completely spanned their hearing range. In all cases, noise exposure resulted in significantly broadened receptive fields (see below) only for cortical sites with characteristic frequencies falling in the noise band spectra (Fig. 1e and Supplementary Fig. 1 online). After BLN exposure, only sites with a characteristic frequency in the 5–20 kHz sector had broader tuning (BW10; 5–10 kHz: 0.95 ± 0.06 octave for controls and 1.35 ± 0.18 octaves for BLN, $P = 0.012$; 10–20 kHz: 1.01 ± 0.05 octave for controls and 1.44 ± 0.12 octaves for BLN, $P < 0.001$, t test). Similarly, in the group exposed to notched noise, only A1 neurons tuned to frequencies outside of the 5–10-kHz band had significantly increased BW10s (1–5 kHz: 0.86 ± 0.05 octave for controls and 1.50 ± 0.11 octaves for notched noise, $P = 0.0002$; 10–20 kHz: 1.01 ± 0.05 octave for controls and 1.72 ± 0.09 octaves for notched noise, $P = 0.00002$; 20–30 kHz: 0.65 ± 0.04 octave for controls and 0.94 ± 0.06 octaves for notched noise, $P = 0.019$, t test). With BBN exposure, BW10s were significantly increased for all cortical sites (1–5 kHz: 0.86 ± 0.05 octave for controls and 1.31 ± 0.08 octaves for BBN, $P = 0.00003$; 5–10 kHz: 0.95 ± 0.06 octave for controls and 1.41 ± 0.06 octaves for BBN, $P = 0.0003$; 10–20 kHz: 1.01 ± 0.05 octave for controls and 1.61 ± 0.09 octaves for BBN, $P = 0.0009$; 20–30 kHz: 0.65 ± 0.04 octave for controls and 1.86 ± 0.07 octaves for BBN, $P = 0.013$, t test). Response thresholds in A1 were not affected by any of the noise exposures that we used (Supplementary Fig. 1).

Note: Supplementary information is available on the Nature Neuroscience website.

Sector-specific differences in BLN-reared rats

The ability of A1 cortical neurons to respond to temporally modulated stimuli has previously been employed as an index of the maturation of successive-signal cortical inhibition¹⁷. We recorded cortical responses to trains of eight noise bursts that were randomly presented at variable rates in BLN-exposed rats and in age-matched controls (representative response patterns to noise burst for both groups are shown in Fig. 2a). We obtained normalized repetition rate transfer functions (RRTFs) for every recorded cortical site by dividing the average responses to the last seven noise bursts by the average response to the first noise burst. The average RRTF for cortical sites with a characteristic frequency between 5–20-kHz octave (in the middle of the noise band) had poorer responses for higher repetition rates in BLN-exposed rats (number of recorded cortical sites = 90) than in controls (number of recorded cortical sites = 128) (Fig. 2b). We quantified the ability of cortical sites to follow high repetition rates between BLN and controls by identifying the highest repetition rate at which the RRTF was at least half of its maximum ($f_{h1/2}$, see Methods; Fig. 2c). The $f_{h1/2}$ were found to be significantly lower in the BLN-exposed group than in controls, but only for A1 neurons preferring frequencies in the 5–20-kHz noise band (7 kHz: 7.2 ± 0.3 pulses per second (pps) for controls and 5.7 ± 0.4 pps for BLN, $P = 0.018$; 14 kHz: 6.8 ± 0.5 pps. for controls and 5.2 ± 0.2 pps for BLN, $P = 0.03$, t test). We found no significant difference ($P > 0.2$) between sites with a lower (1.75 or 3.5 kHz) or higher (28 kHz) characteristic frequency.

We examined neural synchrony in A1 by simultaneously recording spontaneous neural firing during silent periods from two or more channels in BLN-exposed rats (recorded pairs = 300) and P20 controls (recorded pairs = 288). We considered events to be synchronous when they occurred in both recording channels within 10 ms of each other. Average cross-correlograms normalized for firing rate were computed for both groups for cortical sites with characteristic frequencies of 1–5 kHz and 5–20 kHz (Fig. 3a). The average cross-correlogram functions between –10 and 10 ms lags were significantly different in both groups for 5–20 kHz–tuned sites (0.024 ± 0.003 for controls and 0.038 ± 0.002 for BLN, $P = 0.0002$, t test) but were similar for 1–5 kHz–tuned sites (0.037 ± 0.006 for controls and 0.038 ± 0.002 for BLN, $P > 0.2$, t test).

We then quantified the strength of discharge synchrony between cortical sites that were recorded simultaneously in A1 for various inter-electrode distances and for various positions on A1 tonotopic axis using a z score (see Methods). The z score value is the observed frequency of synchronized events in two neurons corrected for what would be expected by chance alone. We determined the distribution of z scores for controls and BLN-exposed rats for all electrode pairs recorded at 0.5 ± 0.2 units on the normalized tonotopic axis, in the middle of the noise-exposed cortical sector (Fig. 3b). The average z scores for both groups were obtained for pairs of simultaneously recorded cortical sites along the normalized tonotopic axis and for varying inter-electrode separations (Fig. 3c,d). For electrode separations of 200 μm or less, the two groups were only significantly different ($P < 0.05$, t test with Bonferroni correction) between 0.37 and 0.73 on the normalized tonotopic axis, which corresponds approximately to the 5–20 kHz–tuned region of A1 in this group (Fig. 1d). Between 200 and 600 μm of inter-electrode separation, a highly significant difference ($P < 0.001$, t test with Bonferroni correction) was found for all sites located at 0.22 or more on the normalized tonotopic axis, and most pairs recorded in the BLN group had significantly decreased ($P < 0.05$, t test with Bonferroni correction) discharge synchrony for greater electrode separations ranging 700 μm or more.

Parvalbumin-positive cells are important in the termination of the critical period. The number of parvalbumin-positive cells in A1 was quantified in the BLN-exposed (number of hemispheres = 22) and control groups (number of hemispheres = 22). We separated each analyzed A1 equally in ~300- μm caudal, middle and rostral sectors along the length of the tonotopic axis corresponding to the low-(1–5 kHz), middle- (5–20 kHz) and high-frequency (20–30 kHz) domains of A1, respectively, and spanning the whole cortical thickness (Fig. 4).

We found a greater than 25% decrease in the number of parvalbumin-positive cells, restricted to the middle sector of A1, in the BLN group compared with the control group (average number of parvalbumin-positive cells per cortical strip; middle sector: 135 ± 9 for controls and 106 ± 6 for BLN, $P = 0.01$, t test). There was no significant difference between the two groups for the rostral ($P = 0.29$) and caudal ($P = 0.41$) sectors. Parvalbumin-positive cells in the BLN group were not only fewer in number, but were also smaller and had less immunoreactive dendritic processes than the control group (Fig. 4d,e).

Region-specific delay in critical period window closure

To examine whether sound exposure-dependent plasticity was retained in A1 sectors exposed to noise, we transferred rat pups to either normal housing conditions with a neutral auditory environment (BLN neutral, $n = 4$, recorded sites = 192) or to a 3.5-kHz and 14-kHz pure tone-enriched environment (BLN tones, $n = 4$, recorded sites = 182) after a P7–20 exposure to a BLN stimulus covering the low-frequency portion of A1 (0.5 to 6 kHz) (Fig. 5). The pure-tone stimulus used for the BLN tones group was presented for 7 d, 24 h per d, and consisted of regularly alternating 3.5-kHz and 14-kHz pure-tone trains engaging cortical neurons in the previously noise-exposed and noise-free regions of A1, respectively. We then obtained A1 characteristic frequency maps at P27 in both groups and compared them to P27 controls ($n = 5$, recorded sites = 221; representative maps are shown in Fig. 5a and the effect of these exposures on A1 frequency representation is shown for both experimental groups and controls in Fig. 5c–e).

In the BLN neutral and BLN tones groups, the cortical area devoted to 1–6 kHz remained reduced by more than 30% compared with naive controls at 1 week after the end of the BLN exposure (percentage of A1 area tuned to 1–6 kHz: $31.4 \pm 1.8\%$ for controls and $23.2 \pm 2.9\%$ for BLN neutral, $P = 0.01$; $23.4 \pm 2.7\%$ for BLN-tones, $P = 0.02$, t test). In the BLN tones group, the alternating 3.5-kHz and 14-kHz pure-tone enrichment resulted in a sevenfold expansion of 3.5 kHz-tuned area compared with controls (percentage of A1 area tuned to 3.5 kHz: $2.2 \pm 0.2\%$ for controls, $P = 0.0009$; $1.3 \pm 0.4\%$ for BLN neutral, $P = 0.0004$; $16.1 \pm 2.3\%$ for BLN tones, t test), whereas the cortical area tuned to 14 kHz was unaffected (percentage of A1 area tuned to 14 kHz: $12.4 \pm 3.0\%$ for controls and $10.3 \pm 3.1\%$ for BLN neutral, $P = 0.60$; $8.7 \pm 3.6\%$ for BLN tones, $P = 0.75$, t test).

Functional recovery after BLN exposure

We examined spectral and temporal processing in BLN neutral and BLN tones groups 1 week after the release from BLN exposure and compared them with P27 controls. BW10s remained slightly, but significantly, increased for cortical neurons tuned to $3.5 \text{ kHz} \pm 0.3$ octaves in the BLN tones group only (1.04 ± 0.07 octave for controls, recorded sites = 32; 1.25 ± 0.08 octaves for BLN tones, recorded sites = 29, $P = 0.02$; Fig. 6a). BW10s for 3.5 kHz-tuned neurons in the BLN neutral group or for neurons with higher characteristic frequencies in both experimental groups were similar to control values (controls, recorded sites = 268; BLN neutral, recorded sites = 225; BLN tones, recorded sites = 211).

We also examined temporal following limits expressed as $f_{h1/2}$ (see Methods) in these experimental groups and naive P27 controls. The $f_{h1/2}$ values did not differ significantly between both experimental groups and controls for frequencies ranging from 3.5–22 kHz ± 0.5 octaves (Fig. 6b) or if averaged for all neurons tuned to frequencies between 1 and 6 kHz (controls, mean $f_{h1/2} = 8.84 \pm 0.8$, recorded sites = 96; BLN neutral, mean $f_{h1/2} = 8.95 \pm 0.9$, recorded sites = 45, $P > 0.2$; BLN tones, mean $f_{h1/2} = 8.92 \pm 1.2$, recorded sites = 25, $P > 0.2$).

We computed average cross-correlograms normalized for firing rate in controls and both experimental groups. The average cross-correlogram functions between –10-ms and 10-ms

lags were slightly, but significantly, elevated for cortical pairs recorded in the 1–6-kHz sector of A1 in the BLN tones group compared with both controls and BLN neutral group (0.055 ± 0.001 for controls, neuron pairs = 104, $P = 0.0006$; 0.053 ± 0.002 for BLN neutral, neuron pairs = 64, $P = 0.003$, t test; 0.062 ± 0.004 for BLN tones, neuron pairs = 45; Fig. 6c). We did not find significant differences between the average cross-correlogram functions for neuron pairs in the 6–30-kHz zone of A1 (controls, number of sites = 98; BLN neutral, number of sites = 68; BLN tones, number of sites = 43). Note that these neural synchrony measures are higher on average than the values found in 1-week-younger P20 controls (see above).

DISCUSSION

Our results indicate that the progression and ultimate closure of the critical period for spectral tuning in A1 is not a singular event during which frequency selectivity is crystallized simultaneously across the field. To the contrary, bombarding a sector of the cortex with acoustic noise across this developmental epoch sustains just that part of A1 in an immature state, whereas non-engaged sectors appear to mature at the normal rate. The physical and functional immaturity of noise-exposed A1 regions was evidenced by the presence of broad receptive fields, sluggish temporal processing, poor neural synchronization and a low density of proportionally smaller and less elaborate parvalbumin-positive inhibitory interneurons. These response features collectively characterize the immature cortex. Notably, this immature zone was demonstrably still in the critical period at a postnatal age that would normally be far past its termination; the stimulus exposure-driven remodeling that characterized this special plasticity epoch could still be easily induced in this immature zone.

The fact that exposure to notched noise or BLN during the critical period resulted in a marked reduction of cortical sites in A1 tuned to frequencies in the noise band is consistent with several earlier studies that have shown that cortical circuits that are more consistently activated by patterned sensory stimuli during early development are competitively advantaged and preferentially consolidated^{7,8,18,19}. BLN and notched noise exposure results in continuous, stimulus-nonspecific activation of a limited A1 sector. This manifestly confers a competitive disadvantage on this sector, which loses representational dominance over bordering A1 sectors that are more regularly engaged by coordinated spiking activity^{15,20}. The persistent distortion of the A1 frequency maps for more than a week after the end of the BLN exposure also supports the idea that the critical period window had closed for the A1 sectors not engaged by the noise stimulus.

Predominantly slow temporal-modulation rates for modulated noise stimuli also remained functionally immature as a consequence of the noise-exposure protocol that we used in this study. This outcome is consistent with earlier studies that examined the impact of continuous BBN exposure on the progressive development of temporal response selectivity in the rat cortex¹⁷. These studies showed that noise exposure blocked the normal progressive maturation of the processes governing successive-signal inhibition or suppression¹⁷. We found it interesting that similar results were obtained in gerbils that were deafened before the critical period onset²¹. The specific mechanisms accounting for these marked changes in the time constants governing successive signal inhibition and suppression are incompletely understood.

BLN, notched noise and BBN exposures all resulted in a substantial degradation, a broadening and a decrease in response reliability, of A1 receptive fields. Similar receptive field broadening and changes in response reliability were observed in previous studies in which developing rats were exposed to continuous¹² or pulsed broadband noises¹³. Broader tuning bandwidths are hypothesized to be the result of a delay in the progressive selection of more specific thalamocortical inputs from the relatively diffusely distributed thalamocortical inputs present in early development^{22–24}, a delay in the maturation of cortico-cortical cooperativity

contributing to these selectively processes, and/or the immaturity of cortical inhibitory circuitry that is hypothetically instrumental in the sharpening of developing sensory cortical receptive fields^{25,26}. The very substantial decrease in firing synchrony in the noise-exposed A1 sector and the matching grossly immature state of parvalbumin-positive inhibitory neurons support this view.

A decreased firing synchrony in the noise-exposed cortical region hypothetically reflects a delay in the activity-dependent organization of horizontal networks or their myelination during early development^{27,28}. The maturation of parvalbumin-positive neurons has been directly linked to these processes. These neurons increase in their size, dendritic elaborations and numbers in parallel with critical period maturation^{29–31}. They have been shown to drive synchronized activity in the cortex³² and to participate in the termination of the critical period⁴ that is believed to be signaled by that emergent synchronization³³. These special inhibitory interneurons target the axon initial segment and soma^{34,35}, where they can control spike initiation or the backpropagation that enables synaptic plasticity in the dendritic arbors of pyramidal cells. Furthermore, several previous studies have demonstrated a clear link between experience-dependent plasticity and inhibitory interneuron maturation. Whisker trimming starting from P1 reduces the GABAergic cell count by 50% in the rat barrel cortex³⁶ and in the mouse; whisker trimming before P7, but not after P15, results in a substantial reduction of parvalbumin staining in the cortex³⁷.

It should be noted that our results cannot be explained by a noise-induced peripheral hearing loss. All stimuli applied in the exposure regime were delivered at nondamaging intensities and receptive field thresholds in A1 were confirmed to be identical in exposed and control rats across the hearing spectrum. Furthermore, BW10, modulation transfer rates and cortical synchronization measures normalized following the termination of noise exposure and after spending 1 week in a normal auditory environment. The persistent sector-specific elevation in BW10 and augmented local firing synchrony found only in subjects exposed to noise and then to alternating tones suggest that these parameters can be modeled by the nature of stimuli present in the environment during development.

An increasing number of studies suggest that the timing and mechanisms of regulation of cortical plasticity might differ substantially across layers. Although layer 4 is thought to mature earlier than extragranular layers and shows a short and well-defined critical period for sensory plasticity^{10,38,39}, plasticity in layers 2/3 seems to persist well into adulthood^{40,41}. The data presented in this study are derived exclusively from recordings carried out in cortical layers 4/5. It therefore remains a possibility that the effects of BLN on cortical maturation could be different if measured in superficial cortical layers.

The BLN-induced delay in cortical maturation that we observed here is consistent with previous studies in V1 showing a relationship between the degree of sensory input patterning and critical period progression^{42,43}. These results raise the possibility that the rate of functional cortical maturation during early development varies proportionally to sensory input coherence. In addition, the effect of noise exposure on the establishment of more complex sound representations in the auditory cortex is uncertain. Assuming that these representations probably necessitate the pre-existence of stable basic spectral and temporal processing, we would speculate that their consolidation will also be delayed until noise exposure is ended and coherent sensory inputs reach A1. These hypotheses remain to be tested, however.

The critical period has generally been described as a unitary event that applies for an entire cortical field or system. Here, we see that its progression and termination are controlled locally in the cortex; one sector of A1 can be mature, while another sector in the same cortical area can remain immature and still be subject to the powerful exposure-driven plasticity that marks

this early developmental epoch. Our study thereby localizes critical period termination to a limited cortical zone and indicates that the local maturation of the cortex (or a representational auditory system sector) must itself account for the shift from exposure-dependent to learning context-dependent plasticity that marks its closure.

Perhaps more importantly, these studies raise the notion that critical periods across the cortical mantle will directly relate to the local state of cortical maturation; in effect, any small sector of any field will have its own sensitive period of maturation and development. It is reasonable to further hypothesize that the functional cooperative neuronal assemblies in the cortex, its minicolumns and columns, provide the basis for the control of these processes.

METHODS

Mapping the auditory cortex

All procedures were approved under the University of California San Francisco Animal Care Facility protocols. We premedicated 72 female Sprague-Dawley rats, aged P20–P30, with atropine sulfate (0.02 mg per kg of body weight) to minimize bronchial secretions and dexamethasone (0.2 mg per kg) to minimize brain edema. They were then anesthetized intraperitoneally with pentobarbital (35–60 mg per kg). Supplemental doses of diluted pentobarbital were given as required to maintain the rat in an areflexic state while preserving a physiological breathing rate. We drained the cisterna magnum of cerebrospinal fluid to minimize cerebral edema. The skull was secured in a head holder, leaving the ears unobstructed. The right temporalis muscle was reflected, auditory cortex was exposed and the dura was resected. We maintained the cortex under a thin layer of silicone oil to prevent desiccation. Recording sites were marked on a digital image of the cortical surface.

Cortical responses were recorded with tungsten microelectrodes (1–2 Mohm, FHC). Recording sites were chosen to sample evenly from the auditory cortex at inter-electrode distances of 125–175 μm . At every recording site, we lowered the microelectrode orthogonally into the cortex to a depth of 470–600 μm (layers 4/5), where we obtained vigorous stimulus-driven responses. The neural signal was amplified (10,000 $\times$), filtered (0.3–3 kHz) and monitored online. Acoustic stimuli were generated using TDT System III (Tucker-Davis Technology) and delivered to the left ear through a calibrated earphone (STAX54) with a sound tube positioned inside the external auditory meatus. We used a software package (SigGen and Brainware; Tucker-Davis Technology) to generate acoustic stimuli, monitor cortical response properties on-line and store data for off-line analysis. The evoked spikes of a single neuron or a small cluster of neurons were collected at each site.

Frequency-intensity receptive fields were reconstructed by presenting pure tones of 50 frequencies (1–30 kHz, 0.1-octave increments, 25-ms duration, 5-ms ramps) at eight sound intensities (0–70dB SPL in 10-dB increments) to the contralateral ear at a rate of 2 stimuli per s. We obtained RRTFs by presenting trains of broadband noise bursts (25-ms duration, 5-ms ramps) at 70 dB SPL and at various rates (2.5–28.3 pulses per s). Spontaneous activity for synchrony analysis was obtained with simultaneous recording from four electrodes positioned in A1 at variable inter-electrode separations (the RRTFs and neural synchrony measures presented in Figure 2 and Figure 3 were obtained exclusively for the BLN group and not for the notched noise group).

Exposure of rat pups

BLN and notched noise stimuli (Fig. 1b) were created by applying a fast Fourier transform to a random white-noise stimulus, filtering the undesired frequency bands in the frequency domain and then bringing the stimulus back to the time domain with an inverse Fourier

transform. Litters of six P7 rat pups and their mothers were placed in a sound-shielded test chamber where the noise stimulus was presented continuously 24 h per d at a sound level of 70 dB SPL for 13 d (up to P20). The rat pups were then immediately mapped or exposed for a further 7 d to trains of 5 successive 25-ms-long 3.5-kHz pure tones followed by a similar train of 14-kHz tones 24 h per d at a sound level of 70 dB SPL with 500-ms quiet intervals between the tone trains (BLN tones group). For the BLN neutral group, litters were placed back in a normal, quiet housing environment after a week of BLN exposure. No distortion or substantial harmonic signal was found in the chamber when tonal stimuli were delivered. We monitored the weights of all rats continuously. There was no weight loss compared with naive rats, indicating normal lactation. The activities during wakefulness and the sleep behavior of the rat revealed no abnormality (for example, no signs of stress).

Histology

We used 22 rat pups for the histological analysis. At the end of recording sessions, we made electrolytic lesions at the previously functionally defined A1 borders, gave the subjects a high dose of pentobarbital (85mg/kg i.p.) and then perfused them through the heart with saline followed by 3.5% paraformaldehyde (wt/vol) in 0.1 M potassium phosphate buffer in saline at pH 7.2. Brains were removed and placed in the same fixative containing 20% sucrose (wt/vol) for 12–24 h. Fixed material was cut either in the coronal or in the axial plane along the tonotopic axis of A1 on a freezing microtome at 40–80 μ m thickness. Changes in the density of parvalbumin-positive cells were examined by fluorescence immunohistochemistry. Tissue was incubated overnight at 4 °C in either monoclonal or polyclonal antisera (1:1,000–3,000, Chemicon International). After exposure to biotinylated antibody to mouse or rabbit IgG (1:100, Vector, ABC kit), samples were rinsed and treated further with streptavidin-conjugated Cy3 (red) (1:100–300, Jackson ImmunoResearch Lab). Tissues from control and BLN-exposed rats ($n = 11$ sets) were always processed together in pairs during immunostaining procedures to limit variables related to antibody penetration, incubation time and postsectioning age and condition of tissue. A Nikon E800 epifluorescent microscope was used to assess fluorescence in the immunostained material. An imaging system equipped with a Photometrics Coolsnap ES CCD camera (Roper Scientific), and Metamorph imaging software (Molecular Devices Systems) was used to quantify data.

Parvalbumin-positive cell density was evaluated in sections that contained the complete rostral to caudal tonotopic representation of A1 and also spanned the full cortical thickness from layer 1 to the underlying white matter. Cell counts were derived from individual zones of tissue (~300 μ m) that were centered in the caudal, middle and rostral sectors of A1 (corresponding to the low-, intermediate- and high-frequency domains of A1, respectively). Images were acquired from each of two hemispheres per case, keeping exposure times constant for each series of tissue. From these photographs, the numbers of parvalbumin-positive cells in each 300- μ m column were calculated and averaged according to treatment group and A1 sector.

Data analysis

The characteristic frequency of a cortical site was defined as the frequency at the tip of the tuning curve. When a tuning curve had a broad tip or multiple peaks, the median frequency at the threshold intensity was chosen as the characteristic frequency. BW10s were defined for all sites. For multi-peaked tuning curves, the response bandwidth was defined as the range from the lowest to the highest frequency at 10 dB about the most sensitive tips that activated the cortical site, possibly encompassing the frequencies in a trough of the tuning curve that did not activate the cortical neurons. The characteristic frequency, threshold and BW10 were determined by direct visualization of the tuning curve in the MatLab environment (MathWorks) using custom routines.

To generate cortical maps, we created tessellated polygons by Voronoi tessellation (voronoi is a Matlab function; Mathworks), with the electrode penetration sites at their centers. Each polygon was assigned the characteristics (for example, characteristic frequency) of the corresponding penetration site. In that way, every point on the surface of the auditory cortex could be linked to the characteristics that were experimentally derived from the sampled cortical site closest to that point. The boundaries of the primary auditory cortex were functionally determined using the following criteria: (i) primary auditory neurons generally have a continuous, single-peaked, V-shaped receptive field, and (ii) characteristic frequencies of the A1 neurons are tonotopically organized, with high frequencies represented rostral and low frequencies represented caudal⁴⁴. The normalized tonotopic axis of characteristic frequency maps was calculated by rotating the map to make a linear function fit of the penetration coordinates using a least-squares method horizontal. After rotation, penetrations coordinates were vertically collapsed on and normalized to a 0 to 1 range. RRTF data were quantified by determining the number of spikes that arrived in a fixed window (4–39 ms) after tone onset. In this study, the RRTF is the average number of spikes for each of the last 7 tones of the 8-tone train plotted as a function of repetition rate. To allow for comparison across sites, we generated normalized spike rates by dividing the number of spikes evoked by each tone in the tone train by the response to a single tone presented in isolation (first tone)⁴⁵. Normalized spike rates >1 indicate facilitation, whereas rates <1 indicate adaptation of the neural response relative to the response to an isolated tone. The 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 $f_{h\frac{1}{2}}$ (ref. ⁴⁶).

We recorded spontaneous neuronal spikes simultaneously in silence from 2–4 electrodes for 10 10-s periods to assess the degree of synchronization between cortical sites. Cross-correlation functions were computed from each electrode pair by counting the number of spike coincidences for time lags of –50 to 50 ms with a 1-ms bin size and were normalized by dividing each of the bins by the square root of the product of the number of discharges in both spike trains⁴⁷. Neural events occurring within 10 ms of each other in two channels were considered to be synchronous. The degree of synchronization may be correlated with spike rates in a nonlinear manner. For each pair of spike trains, we estimated the number of synchronized events if the two spike trains were not correlated, using $N_A N_B \Delta / T$, where N_A and N_B are the numbers of spikes in the two spike trains, Δ (21 ms) is the bin size and T is the duration of the recording^{44,48}. The strength of the synchrony was then assessed using a z score of the number of synchronous events⁴⁸:

$$z = \frac{(\text{number of sync events} - \frac{N_A N_B \Delta}{T})}{\sqrt{\frac{N_A N_B \Delta}{T}}}$$

For neural synchrony recording, offline spike sorting using TDT OpenSorter (Tucker-Davis Technology) was carried out to include only single units in the analysis. Unless specified otherwise, statistical significance was assessed using unpaired two-tailed t tests. Data are presented as mean $\pm$ s.e.m.

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Figure 1.

Effect of notched noise and BLN exposure on A1 characteristic frequency maps. **(a)** Representative A1 characteristic frequency (CF) map from a naive P20 rat (left), a rat exposed to notched noise (middle) and a rat exposed to BLN (right) between P7 and P20. Hatched polygons represent cortical sites with a characteristic frequency in the noise band of the stimulus. Scale bar represents 0.75 mm. C, caudal; D, dorsal; O, non-A1 cortical site (see Methods); R, rostral; V, ventral; X, unresponsive cortical site. **(b)** Experimental protocol (top) and spectrograms (bottom) of the notched noise (NN), BLN and BBN stimuli. **(c)** Difference in frequency tuning between notched noise–exposed and naive rats (left) and BLN-exposed and naive rats expressed as A1 percentage and separated by characteristic frequency. The gray

bar on the abscissa shows the frequency spectrum of the noise exposure. **(d)** Median characteristic frequencies plotted against position on the normalized tonotopic axis (see Methods) of the corresponding recorded cortical site for controls, notched noise-exposed and BLN-exposed litters (all recorded sites were pooled). The red arrow and lines indicate the span of the 5–20-kHz representation along the normalized tonotopic axis after BLN exposure compared with controls (black arrow and lines). **(e)** Distribution of tuning curve BW10 separated by characteristic frequencies for controls, notched noise-exposed, BLN-exposed and BBN-exposed litters (control: $n = 8$, recorded sites = 382; notched noise: $n = 4$, recorded sites = 175; BLN: $n = 4$, recorded sites = 197; BBN: $n = 4$, recorded sites = 201). Values shown are mean $\pm$ s.e.m. * $P < 0.05$, t test.

Figure 2.

Slowing of temporal processing restricted to noise-exposed neurons. **(a)** Examples of cortical responses to pulsed noise for varying repetition rates. The repetition rates are indicated on the ordinate. The black bars indicate the magnitude of the firing rate with respect to time. The red lines indicate the occurrence and duration of the noise pulse. **(b)** Average RRTF of 5–20 kHz-tuned neurons for controls (C) and BLN-exposed rats (BLN). **(c)** Average highest temporal rate at which cortical responses were at half of their maximum ($f_{h1/2}$) for controls and BLN-exposed rats, separated by characteristic frequency. Error bars are s.e.m. * $P < 0.05$; t -test.

Figure 3.

BLN exposure selectively decreases neural synchrony in A1. **(a)** Mean normalized cross-correlation functions and s.e.m. for A1 neurons in controls (black) and BLN-exposed (red) rats. **(b)** z score of neuronal firing synchrony (see Methods) as a function of distance for site pairs located at 0.5 ± 0.2 on the normalized tonotopic axis for controls (open circles) versus BLN-exposed neurons (closed circles). Most neurons at this position had a characteristic frequency in the BLN 5–20-kHz noise band. **(c)** The average z score of neuronal firing synchrony is plotted for site pairs as a function of position on the normalized tonotopic axis and inter-electrode distance for controls and BLN-exposed rats. **(d)** Statistical test results of z score difference between controls and BLN-exposed neurons for all position–inter-electrode distance combinations (two-tailed t test with Bonferroni correction for multiple comparisons; controls: $n = 5$, number of site pairs = 121; BLN exposed: $n = 6$, number of site pairs = 135).

Figure 4.

BLN exposure reduced the number of parvalbumin-positive cells selectively in the central region of A1. **(a,b)** Monochrome photomicrographs of parvalbumin-immunostained cortical sections that were thresholded with the MetaMorph software program to delineate labeled cells from background activity. The sections were cut parallel to the tonotopic axis of A1 in a representative control **(a)** and BLN-exposed **(b)** rats. Note that the density of the parvalbumin-positive neurons restricted to the middle section of A1 in the BLN-exposed (cell counts for this example, caudal-middle-rostral: 72–54–91) decreased compared with the control (cell counts for this example, caudal-middle-rostral: 98–90–85). Scale bar represents 100 μ m. **(c)** Average parvalbumin-positive cell counts and s.e.m for A1 divided equally in caudal, middle and rostral sectors in control ($n = 22$ hemispheres) and BLN-exposed groups ($n = 22$ hemispheres). ** $P < 0.01$, t test. **(d,e)**, Higher magnification photomicrographs of the same middle portion of A1 for control **(d)** and BLN exposed **(e)** showing the smaller size of parvalbumin-positive cells in the exposed group. Scale bars represents 20 μ m.

Figure 5.

Delaying critical period closure selectively in the low-frequency sector of A1. **(a)** Representative A1 characteristic frequency maps from naive P27 controls, BLN-neutral and BLN-tones rats. Hatched polygons represent cortical sites with a characteristic frequency of 3.5 kHz $\pm$ 0.3 octaves (blue hatching) or 14 kHz $\pm$ 0.3 octaves (orange hatching). **(b)** Schematic representation of the sound-exposure history in BLN-neutral and BLN-tones groups. **(c)** Difference in frequency tuning between BLN-tones and BLN-neutral rats expressed as A1 percentage and separated by characteristic frequencies. Note the increase in 3.5-kHz representation in BLN-tones rats compared with BLN-neutral rats without any change in 14-kHz representation (arrows). The gray bar on the abscissa shows the frequency spectrum of

the BLN exposure in this experiment. **(d)**. Distribution of tuning curve characteristic frequencies plotted against a normalized tonotopic axis (see Methods) for the experimental groups and controls. Blue markers represent 3.5-kHz sites and orange markers represent 14-kHz sites. The gray box represents the spectrum of the noise exposure. **(e)**. Percentage of A1 area with receptive fields between 1 and 6 kHz, at 3.5 kHz and 14 kHz for controls, BLN-neutral and BLN-tones rats. Note the persistent under-representation of 1–6-kHz frequencies at P27 in both BLN-tones and BLN-neutral groups compared with controls. Scale bar represents 0.75mm. P27 controls maps: $n = 5$, recorded sites = 221; BLN-neutral maps: $n = 4$, recorded sites = 192; BLN-tones maps: $n = 4$, recorded sites = 122. Values shown are mean $\pm$ s.e.m. ** $P < 0.01$, t test.

Figure 6.

Recovery of spectral and temporal processing in A1 after BLN exposure. **(a)** BW10s separated by characteristic frequencies are shown for P27 naive controls, BLN-neutral and BLN-tones groups. **(b)** Average highest temporal rate at which cortical responses were at half of their maximum ($f_{h1/2}$) for controls, BLN-neutral and BLN-tones (pooled) rats separated by characteristic frequencies. **(c)** Mean normalized cross-correlation functions and s.e.m. for A1 neurons in controls (black), BLN neutral (black dashed) and BLN tones (gray) in the 1–6-kHz sector of A1 (left) and 6–30-kHz sector of A1 (right). P27 controls: $n = 5$, recorded sites = 268; BLN-neutral maps: $n = 4$, recorded sites = 225; BLN-tones maps: $n = 4$, recorded sites = 211. Values shown are mean $\pm$ s.e.m. * $P < 0.05$, t test.