Maintenance of enriched environment-induced changes of auditory spatial sensitivity and expression of GABA_A, NMDA, and AMPA receptor subunits in rat auditory cortex

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A B S T R A C T

Enriched environment (EE) has an important role in the development and plasticity of the brain. In this study, we investigated the maintenance of early EE exposure-induced changes of spatial sensitivity, and the possible underlying mechanisms of this maintenance. We found that, compared with the age-matched control, the spatial sensitivity of A1 neurons was still enhanced after EE rats had been returned to the normal condition for 2 months. The enhancement was expressed by a sharper frequency tuning curve, smaller spatial receptive field, and a more selective directional curve of the early EE-exposed rats. Simultaneously, we detected significant increases in GABA_A receptor α1, β3 subunits; NMDA receptor NR2A, NR2B subunits; AMPA receptor GluR2 subunit protein expression; and in the ratios of GABA_Ax1/GABA_Ax3 and NR2A/NR2B. In particular, the expression ratio change of the GABA_Ax1/GABA_Ax3 was significant greater than that of NR2A/NR2B in early EE-exposed rats. These observations indicate that the persistent higher expression levels of the GABAergic and glutamatergic receptors expression induced by early EE exposure, especially enhancement of GABAergic inhibition in the auditory cortex, might be responsible for the maintenance of improved effects in auditory spatial sensitivity after the rats had been returned to the normal condition.

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1. Introduction

Enriched environment (EE) has an important role in the development and plasticity of the brain. The variety of effects on the brain, which have been demonstrated at the cellular, molecular, anatomical, physiological, and behavioral levels, suggest that EE accelerates the development and enhances the structural and functional plasticity of the brain (Berardi, Pizzorusso, Ratto, & Maffei, 2003; Cai et al., 2009; Del Arco et al., 2007; Diamond, 2001; Diamond et al., 1966; Petrosini et al., 2009; Rampon et al., 2000, 2000; van Praag, Kempermann, & Gage, 2000).

Several studies of the auditory cortex have concluded that EE increases the directional sensitivity of neurons in the rat primary auditory cortex, such as a smaller spatial receptive field, sharper frequency tuning curve and directional selective curve. The behavioral experiment also found that the EE might improve the number of correct scores, decreasing the reaction time and azimuth deviation in sound-discrimination tasks. Interestingly, the improvement of behavioral performance lasted for at least 2 months after the animals had been returned to the normal condition (Cai et al., 2009). However, it remains unknown whether and how the enhancement of auditory spatial sensitivity induced by early EE exposure maintained in the primary auditory cortex occurred.

Despite several studies, the mechanisms responsible for enhancement of spatial tuning caused by early EE remain unclear. Several mechanisms, including γ-aminobutyric acid (GABA)-mediated inhibition and N-methyl-D-aspartate (NMDA)-mediated excitation, are crucial for experience-dependent developmental plasticity. Previous studies have shown that early postnatal experience and environment significantly affect NMDA receptor expression in the visual cortex (Chen & Bear, 2007; Philpot, Sekhar, Shouval, & Bear, 2001; Quinlan, Olstein, & Bear, 1999; Quinlan, 

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Philpot, Huganir, & Bear, 1999). Previous studies have indicated that mice reared in EE from birth have higher levels of brain-derived neurotrophic factor (BDNF) protein in the visual cortex, which accelerate the development of the inhibitory GABAergic system by influencing receptive field development and synaptic plasticity (Cancedda et al., 2004; Huang et al., 1999; Sale et al., 2004, 2007). Our recent study also found that early, continuous noise rearing induces significant decreases in GAD 65 and GABA_A receptor α1 subunit expression and increases GABA_A receptor α3 subunit expression (Xu, Yu, Cai, Zhang, & Sun, 2009). These observations suggest that the developmental changes of glutamatergic and GABAergic play important roles in the structural and functional plasticity of the brain.

These findings have led us to hypothesize that the improvement of auditory spatial sensitivity induced by early EE exposure might be maintained in the auditory cortical neurons after the animal has been returned to the normal condition, and go with the developmental changes of both GABA-mediated inhibition and NMDA-mediated excitation. To test this hypothesis, we first measured the spatial sensitivity of the auditory neuron to determine whether there is any similarity to the behavioral task performance. If so, we would then examine the expression of the GABA_A receptor (regarded as an important factor in spatial sensitivity) and the NMDA receptor and AMPA receptor subunits. We hope this study will help us understand the molecular basis of the maintenance of functional plasticity in the auditory cortex after EE exposure.

2. Materials and methods

2.1. Animals

Sprague–Dawley (SD) rats were used in the present study. The EE rats with their mother were first raised in the enriched condition from postnatal day 7 (P7) to P56, and then moved to the normal reared condition until approximately P115 (Fig. 1). A reverse 12-h light/dark cycle and constant humidity and temperature conditions were maintained for both groups.

EE conditions and normal conditions were the same as in the previous study (Cai et al., 2009). The EE conditions consisted of three large cages (90L × 80W × 100H cm), each of which contained running wheels, seesaws, balls, tunnels, cubes and cone toys. There were also stairs, ramps and platforms in each cage. When rats climbed onto the platform via the stairs or ramps, they could get enough food and water. Eight speakers were fixed around the cage every 3 or 4 days to maintain novelty. Normal housing conditions comprised 2 or 3 rats per cage (45L × 35W × 35H cm). Rats had ad libitum access to food and water before the experiment, in addition to general sounds (which could also be heard by the EE rats) resulting from daily cleaning and feeding.

All studies were carried out in accordance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals and guidelines for the Use of Animals in Neuroscience Research. All efforts were made to minimize suffering and the number of animals used for the experiments.

2.2. Electrophysiology

A total of 16 SD rats were used in the electrophysiological study (eight EE and eight CON rats were used in the electrophysiological experiment). Electrophysiological recording of auditory cortical responses was conducted at approximately P115 for both EE and CON rats. The rats were anesthetized with sodium pentobarbital (40 mg/kg, IP). Anesthesia and body fluid levels were maintained throughout the recording phases with supplemental doses of sodium pentobarbital using a microinjection pump (2 mg/h). The trachea was cannulated to ensure adequate ventilation and to minimize breathing-related noises. The fourth ventricle was drained of cerebrospinal fluid to minimize cerebral edema. Under anesthesia, each rat was tied to the flat head of a 2.0-cm nail that was glued to the anterior of the rat’s exposed skull with dental cement and acrylic glue. Each rat was then placed inside a sound-proof room, and its head was fixed to the nail with a set screw. One small hole was made in the left temporal skull overlaying the auditory cortex (Bi et al., 2006; Del Arco et al., 2007; Paxinos & Watson, 1998; Xu, Yu, Cai, Zhang, & Sun, 2008; Xu et al., 2009), and cerebral dura mater was stripped. The cortex was maintained under a thin layer of viscous silicone oil to prevent desiccation. After immobilization, the rat’s eye-snout line was directed to the region of the frontal auditory space indicated by contralateral 30° and 0° elevations by adjusting the metal rod. The animal’s body temperature was monitored using a rectal probe and maintained at 38 °C by a feedback-controlled heating blanket.

The experiments were performed in a soundproof room. Acoustic stimuli were generated using the TDT III system (Tucker Davis Technologies, Alachua, FL) and transmitted by a loudspeaker, which was driven by two small electric motors using a remote control system. The loudspeaker could be located at any horizontal (azimuth) and vertical (elevation) directions in the frontal auditory space, 34 cm from the rat’s head.

Cortical auditory responses were recorded using a 3 M KCl glass micropipette electrode (diameter: 1 μm, resistance: 5–10 MΩ). Single neurons were recorded across the rat primary auditory cortex (A1), which is characterized by an anterior-to-posterior tonotopic progression from high to low frequencies (tonotopic organization) (Doron, Ledoux, & Semple, 2002; Rutkowski, Miasnikov, & Weinberger, 2003; Sally & Kelly, 1988). Once a single unit was isolated, its characteristic frequency (CF: the stimulus frequency with the lowest threshold for an evoked response) and minimum threshold (MT)—at which the probability of the neuron’s response to CF tones was 50%—at CF were determined. At the end of the experiment, recording microelectrode filled with 2% pontamine sky blue was given cathode direct current (−25 μA) for 15 min to locate the tip of microelectrode.

Frequency tuning curves (FTC) were reconstructed in detail by presenting pure tones of 79 frequencies (1–40 kHz, 50-ms duration, 5 ms rise-decay time) at each of nine sound intensities (0–80 dB SPL in 10-dB increments) contralateral to the recording site at a rate of 2 stimuli per second.
Directional selectivity of cortical neurons was examined by plotting the directional selectivity curve obtained with the CF sound set at 10 dB above each neuron’s MT value. The number of impulses discharged to CF sound at 10° increments between ±90° (ipsilateral 90° to contralateral 90°) in azimuth was recorded. The neuronal directional selectivity curves were then generated by plotting the number of impulses in response to CF sounds against azimuth angles.

The auditory spatial receptive field of each neuron was defined in terms of azimuth and elevation. A CF sound was emitted from a speaker moving across the rat’s frontal auditory space, and the stimulus intensity was adjusted to determine the response center at which the neuron had its lowest MT. The sound was then increased to 5 dB greater than the lowest MT to determine the boundary of the neuron’s spatial receptive field by systematically moving the speaker in azimuth and elevation to locations where the neuron stopped responding. The spatial receptive field determined by the stimulus amplitude at 5 dB greater than the neuron’s MT is referred to as the ±5 dB spatial receptive field.

### 2.3. Quantitative immunoblots

A total of eight SD rats were used in the immunoblot study (four EE and four CON rats were used in the immunoblot experiment). The rats used for the immunoblot experiments came from a separate group of animals—not the same as used for the electrophysiological experiment. The rats were deeply anaesthetized with an injection of sodium pentobarbital (75 mg/kg IP). Immediately after decapitation, their brains were obtained. The right and left auditory cortex were separated and immediately homogenized in ice-cold homogenization buffer (0.5 mM Dithiothreitol, 1 mM Ethylene Diamine Tetraacetic Acid, 2 mM Ethylene glycolbis (β-aminoethyether)-N,N′,N′,N′-tetra acetic acid, 10 mM N-2-hydroxyethylpiperazine-N-ethane-sulphonic acid, 10 mg/L leupeptin, 2 mg/L aprotinin, 0.1 mM Phenylmethanesulfonyl fluoride), and a portion of the homogenate was centrifuged at 14,000 g for 10 min and kept for analysis of the inhibitory GABA receptor subunits, NMDA receptor subunits and GluR2. Samples were resuspended in boiling, 1% sodium-dodecyl-sulfate (SDS) and stored at −80°C. Protein concentrations were determined using the bicinchoninic acid (BCA) assay guidelines (Pierce, Rockford, IL).

Quantitative immunoblotting assays were performed as described previously (Xu et al., 2008, 2009). Primary antibodies included anti-GABAAα1, anti-GABAAα3, anti-GABABβ3, anti-NR2A, anti-NR2B, anti-GluR2 (all from Upstate Biotechnology, Temecula, CA) and anti-β-actin (Sigma, St. Louis, MO). The relationship between optical density and protein concentration was linear over the range used in this study. The density of each band of western blotting was measured using Image Processing and Analysis in Java (Image J) software, and the relative level of each protein was calculated as the ratio of protein bands compared with the β-actin loading control band. All electrophysiological recordings and immunoblots were performed blind to experimental conditions.

### 2.4. Data analysis

#### 2.4.1. BW10 and BW20

The frequency tuning curve was assessed by constructing frequency vs intensity. The tip of each FTC was defined as neuron characteristic frequency (CF). The intensity response to the CF was the minimum threshold (MT) of each neuron. Bandwidth 10 (BW10) and bandwidth 20 (BW20) are the range of frequencies at which neurons respond to sounds of 10 dB and 20 dB intensity greater than MT, expressed in octaves.

#### 2.4.2. Azimuth angular range (AR) and preferred azimuth range 75 (PAR75)

We defined C as the spike count (number of spikes per 30 stimuli) for a given location. To facilitate the calculation of azimuth-tuning statistics, C was normalized to give the proportion of maximum response across locations, ranging from 0 (at locations yielding no spikes) to 1 (at the location yielding the maximum number of spikes) (Stecker, Mickey, Macpherson, & Middlebrooks, 2003; Xu et al., 2009). Azimuth tuning was assessed using the azimuth AR and preferred azimuth range (PAR75), which were defined as the range of locations that elicited $C > 0.5$ and $C > 0.75$.

#### 2.4.3. Azimuth-tuning depth

Azimuth tuning was assessed by the azimuth-tuning depth, which indicates the extent to which spike counts varied with azimuth. It is indicated by $\Delta C$ for modulation of spike counts, and computed as $\Delta C = 1 - C_{\text{min}}$, where $C_{\text{min}}$ is the minimum value of C across location.

#### 2.4.4. Azimuth centroid

In previous studies in cats (Stecker et al., 2003), the azimuth centroid, an estimate of a unit’s preferred location, was defined as the center of mass of the peak response. The peak response was defined as the group of contiguous locations with $C > 0.75$, and it included the overall maximum C. A further requirement for the calculation of azimuth centroid was that the response should decrease to <0.75 at some locations (i.e., some locations were not included in the peak response). The centroid was calculated by generating vectors at each of the interpolated locations within the peak response. For each vector, the angle was the interpolated location in degree, and the length was the interpolated value of C at that location. The centroid was then defined as the angle of the resultant of the vectors included in the peak response.

#### 2.4.5. Statistical analysis

The Whitney U test and independent-samples t test were used to analyze the differences between the EE group and the CON group. The Kolmogorov–Smirnov test was used to compare distributions of various spatial statistics (SPSS 17.0). The auditory receptive field was calculated according to the coordinates of each site (Matlab 8.0).

### 3. Results

#### 3.1. The enhancement of auditory spatial sensitivity of A1 neurons by early EE exposure lasted for at least 2 months after returning to normal conditions

Observations were based on 153 single neurons from the A1 of the EE rats and 157 single neurons from the age-matched control rats. Most histologically identified sites were located in layers II/III to IV of the primary auditory cortex (A1) (recording depth was among 300–1200 μm from surface). CFs were broadly distributed in each sample, ranging from 1 kHz to 30 kHz and divided into three categories stepped by 1.5 octaves.

#### 3.1.1. Frequency tuning curve (FTC)

In general, FTCs of auditory neurons within A1 were V-shaped, with a clear CF defined at the lowest threshold peak (Fig. 2A). The FTCs of the EE rats were sharper (Fig. 2Ab) than those of the CON rats (Fig. 2Aa). We measured the BW10 and BW20 values for all the recording units. We found that there were significant differences (Mann–Whitney U test, $p < 0.01$) in both BW10 and BW20 between the two groups. The BW20 values of the EE rats were...
65.8% of the CON rats, while the data from BW10 values were 51.8%. It indicates that the differences between the tips of the FTCs were more notable than the middle parts (Fig. 2B). The BW10 and BW20 were then divided into three categories according to the CFs of the recording sites. The BW10 and BW20 values of the EE rats in all categories were smaller than their counterparts, and significant differences showed up in all cases (Mann-Whitney U test, p < 0.01) (Fig. 2C).

3.1.2. AR and PAR75

Azimuth tuning was assessed using the azimuth AR and preferred azimuth range (PAR75), which were defined as the range of locations that elicited C > 0.5 and C > 0.75, respectively. The average AR of the EE rats was 47.18°, 75.9% of that of the CON rats (62.19°) (Fig. 3Aa). Distributions of AR are shown in Fig. 3Ab. We found significant differences in AR distributions between the EE and CON groups (p < 0.001). ARs in both groups showed CF dependence: the AR decreased as the CF increased. The decreasing tendency of the EE rats seemed more notable, and significant differences showed up in the middle (3.1–9.5 kHz) and high (9.5–30 kHz) frequencies (Mann-Whitney U test, p < 0.01) (Fig. 3Ac). We then calculated another value of azimuth tuning PAR75, defined as the azimuth range that elicited a response above 75% of the maximum. Average PAR75 values of the two groups were 31.93° and 42.20°, respectively, with statistical differences (Mann-Whitney U test, p < 0.01) (Fig. 3Ba). Distributions of PAR75 are shown in Fig. 3Bb. The distribution of the EE rats was centered from 10° to 50° (median, 28.14°), sharper than those of the CON rats (median, 39.58°) (Fig. 3Bc). When categorized by CF, the data showed results similar to the AR values: a decreasing tendency as CF increased, with notable differences (Whitney U test, p < .01) in the middle and high frequencies (Fig. 3Cc).

3.1.3. Azimuth-tuning depth

Azimuth-tuning depth indicated reduction in the normalized spike count across the azimuth. The average tuning depth of the EE rats was 0.86 and 0.73 of the CON rats, with statistical differences (Whitney U test, p < 0.01) (Fig. 3Ca). When we compared distributions of the tuning depths (p < 0.001) (Fig. 3Cb), positive correlation of the CF and tuning depth were observed in both groups. And CF-categorized values all showed significant differences (Whitney U test, p < 0.01) between the two groups (Fig. 3Cc).

3.1.4. Azimuth centroid

The azimuth centroid was calculated by generating vectors at each of the interpolated locations within the peak response (Xu et al., 2009). Average azimuth centroid of the two groups showed no statistical difference (EE rats, 32.54; CON rats, 34.07; p = .5789), while the CON rats (12/130, 9.23%) had twice as many noncentroid neurons (NC) as the EE rats (6/132, 4.55%) (Fig. 3Da). Distributions of azimuth centroids were significantly different (p < 0.05) between the two groups; they were distributed over a sharper range in the EE rats (Fig. 3Db).

3.1.5. Auditory spatial receptive field

The auditory spatial receptive field of each neuron can be defined by azimuth and elevation as shown in Fig. 4Ab. The spatial receptive fields of the EE rats were much smaller (Whitney U test, p < 0.01) than their counterparts (Fig. 4Aa). When classified by CF, we also found notable differences (Whitney U test, p < 0.01) in all categories. The biggest field was the low frequency of the CON rats (4217.64), and the smallest was the high frequency of the EE rats (1865.01) (Fig. 4B). The spatial receptive fields were also calculated in azimuth categories, 0–19, 20–39, and 40–60. Significant statistical differences (Whitney U test, p < 0.01) were found in all three categories (Fig. 4C).

3.2. Early EE exposure induced alterations of GABA<sub>A</sub> receptor, AMPA GluR2, and NMDA receptor subunits expression, which lasted 2 months after a return of the animals’ normal condition

To determine which factors influence maintenance effects, we examined both excitation and inhibition related receptor expressions. The receptors chosen in the present study were reported as either experience dependent or had a close relationship with spatial sensitivity (Chen & Bear, 2007; Cui, Lu, & Sun, 2001; Cui, Zhang, Cai, & Sun, 2009; Jen & Zhang, 2000; Lu et al., 2007; Xu et al., 2008, 2009).

3.2.1. GABA<sub>A</sub> receptor subunits

Expression levels of GABA<sub>A</sub>α1, GABA<sub>A</sub>α3, and GABA<sub>A</sub>β3 were quantified from whole homogenate samples. The expression level
Fig. 3. Early EE results in improved directional sensitivity in A1 neurons; this phenomenon lasted at least 2 months. Azimuth tuning was assessed by AR and PAR75, which were defined as the azimuth ranges of locations that elicited spike count above 50% and 75% of the maximum. The average values of AR (Aa) and PAR75 (Ba), the distributions of AR (Ab) and PAR75 (Bb), and the AR (Ac) and PAR75 (Bc) values of different CF categories all have statistical differences between two groups. Azimuth-tuning depth indicated reduction in the normalized spike count across the azimuth. The average tuning depth values (Ca), the distribution (Cb) and the values of tuning depth in different CF categories (Cc) all have statistical differences (n = 21, 66, 65 for the low to high frequency categories for CON rats; n = 27, 61, 63 for the EE rats). The percentage of the no-azimuth centroid neurons is shown in Da, and the distribution of the other neurons in both groups is shown in Db. Bin size = 1.5 octaves. Values shown are mean ± SEM. *p < 0.05; **p < 0.01 compared with the CON rats.

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of the GABA\textsubscript{A}a\textsubscript{1} receptor had a 63.59 \pm 10.10\% increase compared with the age-matched control (\(n = 4\) for each group, independent-samples \(t\) test, \(p < .01\)) (Fig. 5A). On the other hand, the expression level of GABA\textsubscript{A}a\textsubscript{3} was reduced 27.78 \pm 4.36\% for the EE rats compared with the CON rats (\(n = 4\) for each group, independent-samples \(t\) test, \(p < .01\)) (Fig. 5B). After enriched and normal rearing, the expression level of GABA\textsubscript{A}b\textsubscript{3}—another inhibitory receptor we investigated—received a notable increase (33.25 \pm 9.46\%) compared with the CON rats (\(n = 4\) for each group, independent-samples \(t\) test, \(p < 0.01\)) (Fig. 5C).

3.2.2. NMDA and AMPA receptor subunits

For the excitatory receptor, a 33.15 \pm 4.38\% increase in expression was found in the NR2A subunit of the EE rats, and the statistical difference was significant (\(n = 4\) for each group, independent-samples \(t\) test, \(p < .01\)) (Fig. 6A). We also found a 20.85 \pm 4.76\% increase in the expression of the NR2B subunit in the EE rats compared with the CON rats (\(n = 4\) for each group, independent-samples \(t\) test, \(p < .05\)) (Fig. 6B). Besides these two kinds of NMDA receptor, we detected another subunit of AMPA receptor GluR2. We found a slight increase (16.22 \pm 4.32\%) in the GluR2 expression (\(n = 4\) for each group, independent-samples \(t\) test, \(p < 0.05\)) (Fig. 6C).

The GABA\textsubscript{A}a\textsubscript{1}/GABA\textsubscript{A}a\textsubscript{3} ratio, which indicates the maturity of the GABA\textsubscript{A} receptor, was calculated. We found a twofold increase (independent-samples \(t\) test, \(p < 0.01\)) of this ratio compared with the age-matched control (Fig. 7A). The ratio of NR2A/NR2B was also calculated as a parameter to measure the developmental level. The ratio of NR2A/NR2B in the EE group increased 10.18\% compared with the control, and there was a significant difference between the two groups (independent-samples \(t\) test, \(p < 0.05\)) (Fig. 7B).

4. Discussion

Results of the present study indicate that the enhancement effects of auditory spatial sensitivity by early EE exposure were maintained in the primary auditory cortex after it returned to the normal condition and that there were marked increases in GABA\textsubscript{A}a\textsubscript{1}, NMDA, and AMPA receptor subunits expression in rat auditory cortex. Neurobiology of Learning and Memory (2010), doi:10.1016/j.nlm.2010.08.008
the auditory cortex, several studies have also indicated that EE might increase response strength and selectivity, decrease the threshold and latency of auditory responses, and improve the behavioral performance and auditory spatial representation of A1 neurons (Cai et al., 2009; Engineer et al., 2004; Percaccio et al., 2005, 2007; Zhang et al., 2009). Behavioral experiments have shown that EE might improve spatial memory tests and that EE rats exhibit high performance levels because of their ability to exploit procedural competencies and improved working memory abilities. In the Morris water maze, EE animals developed tuned navigational abilities faster than normal animals. In the Morris water maze, EE animals developed tuned navigational abilities faster than normal animals. For example, we found that EE animals developed tuned navigational abilities faster than normal animals (Leggio et al., 2005). We found in our study that the enhanced spatial sensitivity by EE exposure was maintained in the A1 neurons for at least 2 months. When comparing the present results with Engineer’s, we found that the basic response characteristic, such as latency of the auditory cortical response and bandwidth of the FTC, were similar (Engineer et al., 2004).

Yet, which critical factor in the EE condition induced the improvement of spatial sensitivity? We still cannot reach a positive conclusion, nor can we elicit which key factor in the EE condition induces the functional plasticity of the visual and auditory systems. An enriched environment consists of a combination of enhanced social relations, physical exercise, and interactions with nonsocial stimuli that lead to behavioral and neuronal modifications (Leggio et al., 2005). Some studies have shown that passive auditory exposure does not increase response strength or temporal processing (Condori & Weinberger, 1991). Morphological analysis of neurons also indicated that increased motor activity, separate from the other variable of an enriched environment (social interactions, cognitive stimulations), does not alter either neuronal volume or dendritic length in the cortical regions (Faherty, Kerley, & Smeyne, 2003).

In our study, although we added sounds to the EE condition (which were merely pure tones with random frequencies in random directions), we suggest that the sounds themselves, as presented in this study, might not be a critical factor in inducing improvement of spatial sensitivity. Some researchers have suggested that sensory inputs alone are not sufficient to generate plasticity, but passive stimulation paradigms with salient stimuli can enhance responsiveness and induce cortical organization under appropriate conditions (Percaccio et al., 2007). We agree with this hypothesis and suggest that, as in other studies, early EE exposure with enhanced sensorimotor, cognitive, and social stimulation results in significant changes in brain biochemistry, synaptic connectivity, and neuronal function in enriched animals (Petrosini et al., 2009). These changes provide the groundwork for the improvement of spatial sensitivity.

In our study, the data further show that enhancement of auditory spatial sensitivity of A1 neurons, induced by early EE exposure, was maintained for at least 2 months. These findings are consistent with those of Zhou and Merzenich, who reported that intensive training in adult rats refines A1 representations degraded during an earlier postnatal critical period and that the changes induced by training endured without loss for at least 2 months after training cession (Zhou & Merzenich, 2007).

Consequently, what are the molecular factors underlying the maintenance effects of EE on auditory-cortical plasticity, especially spatial sensitivity of the auditory cortical neuron? Recently, investigators of the visual cortex reported that an interaction between BDNF and the GABAergic system is thought to regulate visual cortical plasticity during development of the inhibitory GABAergic system by affecting receptive field development and synaptic plasticity (Huang et al., 1999; Sale et al., 2009). Our recent study found that early, continuous white-noise exposure impaired spatial sensitivity and significantly decreased

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GAD65 and GABA<sub>A</sub> receptor x1 subunit expression and increased GABA<sub>A</sub> receptor x3 subunit expression in the auditory cortex (Xu et al., 2009). Some studies have also reported that an adult model of age-related hearing loss experienced a weakened inhibition associated with changes in GABA receptor function, subunit composition, and levels of presynaptic GAD and GABA (Caspar, Milbrandt, & Helfert, 1995; Caspar et al., 1999). In the present study, we found that early exposure to EE increased the expression of GABA<sub>A</sub>x1 and GABA<sub>A</sub>x3 by 65.54% and 34.64%, respectively, and decreased the expression of GABA<sub>A</sub>x2 by 27.78% compared with the control animals. All these studies indicate that GABA<sub>A</sub> receptor-mediated inhibition plays a crucial role in the functional plasticity of the brain.

On the other hand, excitatory receptors, especially NMDA receptors, also regulate synaptic plasticity during the critical development period (Franks & Isaacson, 2005; Singh, Basham, Nordeen, & Nordeen, 2000; Sun et al., 2005). Several studies have shown that early experience and the postnatal environment significantly affect NMDA receptor expression in the visual cortex (Chen & Bear, 2007; Philpot et al., 2001; Quinlan, Olstein et al., 1999; Quinlan, Philpot et al., 1999). In the auditory cortex, our recent study found that NMDA receptor subunit expression levels and composition/ratio might be altered by early auditory experience. All the NR1, NR2A, and NR2B subunits were markedly increased, and chronic administration of APV significantly reduced NMDA receptor expression and the ratio of receptor subunits, indicating that changes in NMDA receptor expression might mediate the early auditory-experience plasticity (Cui et al., 2009). The present study indicates that early exposure to EE may increase the expression of the NR2A, NR2B, and GluR2 receptor subunits after enrichment cessation 2 months later.

In summation, we speculated that sensory-motor activity, induced by multisensory under-environmental-enrichment conditions, increases the expression of neurotrophins and neural growth factors such as BDNF and IGF-I. Higher levels of neurotrophin factors accelerated the development of GABAergic and glutamatergic systems, especially the GABAergic inhibitory system, which promoted a more rapid maturation in the immature cortex. Electrophysiological results showed that GABA<sub>A</sub> receptor-mediated inhibition plays a crucial role in maintaining the balance of excitation and inhibition, which likely control and regulate the expression of NMDA receptors (Wu, Ma, & Kelly, 2004). In agreement with these findings, our results show that GABA<sub>A</sub> receptors x1 and x3, NMDA receptors NR2A and NR2B, and AMPA receptor GluR2 significantly upregulated expression levels, but the expression ratio change of the GABA<sub>A</sub>x1/GABA<sub>A</sub>x3 was significantly greater than that of NR2A/NR2B in early EE-exposed rats.

The mechanisms of structural and functional plasticity induced by EE in the auditory cortex seem complex. Besides EE-regulated developmental changes of the GABAergic and glutamatergic systems, EE might also accelerate gene expression and modulate the synaptic transmission, molecular pathway, neural network formation, and morphological development of the neurons. A recent report has indicated that the environment might also affect the dentritic morphology of the auditory cortex (Bose et al., 2010). Therefore, we recommend further investigation of the mechanism of the EE affect on the development and plasticity of the brain, using cellular and molecular techniques.

The present study showed that spatial sensitivity enhancement of early exposure to EE can be maintained for at least 2 months after enrichment cessation. This maintenance was accompanied by the expression alterations of several inhibitory/excitatory receptor subunits in the primary auditory cortex of the EE rat. These findings might help reveal the molecular basis of EE-induced functional plasticity maintenance in the auditory cortex.

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**Reference**


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