Sensory experiences have important roles in the functional development of the mammalian auditory cortex. Here, we show how early continuous noise rearing influences spatial sensitivity in the rat primary auditory cortex (A1) and its underlying mechanisms. By rearing infant rat pups under conditions of continuous, moderate level white noise, we found that noise rearing markedly attenuated the spatial sensitivity of A1 neurons. Compared with rats reared under normal conditions, spike counts of A1 neurons were more poorly modulated by changes in stimulus location, and their preferred locations were distributed over a larger area. We further show that early continuous noise rearing induced significant decreases in glutamic acid decarboxylase 65 and γ-aminobutyric acid (GABA) receptor α1 subunit expression, and an increase in GABAβ receptor α3 expression, which indicates a returned to the juvenile form of GABAα receptor, with no effect on the expression of N-methyl-D-aspartate receptors. These observations indicate that noise rearing has powerful adverse effects on the maturation of cortical GABAergic inhibition, which might be responsible for the reduced spatial sensitivity.

Keywords: azimuth selectivity, developmental plasticity, GABAα receptor, GAD65, NMDA receptor, noise exposure

Introduction
The ability to localize sounds accurately and rapidly is important for humans and other species to perceive and interact with the environment. An intact auditory cortex is necessary for normal sound localization behavior in humans (Sanchez-Longo and Forster 1958; Zatorre and Penhune 2001) and animals (Jenkins and Masterton 1982; Jenkins and Merzenich 1984). The spatial tuning of neurons in the auditory cortex is an essential response property that underlies sound localization.

Sensory experiences contribute to the functional development of the mammalian auditory system during the early stages of life. Many studies have investigated the correlations between the auditory experience within the critical period and the development of cortical feature selectivity organizations (Zhang et al. 2001, 2002; Zhou and Merzenich 2007, 2008). Chang and Merzenich (2003) found that deprivation of acoustic experiences by rearing infant rats under conditions of continuous white noise from the early postnatal stage resulted in poorly developed cortical frequency receptive field structure and tonotopy in A1 of rats. However, it remains unknown whether, and if so how, the early continuous white noise rearing conditions, which also mask the spatial acoustic cues, affect the spatial tuning properties of neurons in the A1. Here, we show that rearing infant rat pups under conditions of continuous, moderate level white noise results in poor spatial tuning in A1 neurons.

Despite several studies, the mechanisms responsible for the poor spatial tuning caused by early noise rearing conditions remain unclear. A possible contribution to these mechanisms may arise from the experience-dependent developmental plasticity in neuronal circuits across the auditory cortex. Several mechanisms, including N-methyl-D-aspartate (NMDA)-mediated excitation and γ-aminobutyric acid (GABA)-mediated inhibition, are crucial for experience-dependent developmental plasticity in the sensory cortex (Michaeva and Beaulieu 1995; Knott et al. 2002; Morales et al. 2002; Heinen et al. 2004). Studies of the visual cortex reveal that visual deprivation from birth leads to reductions in the expression levels of glutamic acid decarboxylase (GAD, the enzyme that synthesizes GABA) and GABA ( Hendry and Jones 1986; Benevento et al. 1995) and indicates a return to the juvenile form of NMDA receptors (NMDARs) in the visual cortex (Quinlan, Olstein, et al. 1999; Quinlan, Philpot, et al. 1999; Chen et al. 2001).

These findings led us to hypothesize that early continuous white noise exposure may disturb the normal development of GABA-mediated inhibition and NMDA-mediated excitation and change the spatial tuning of A1 neurons. To test this hypothesis, we first examined the normal developmental expression of inhibitory GABA receptor subunits (GABAα receptor α1 and α3 subunits) and the enzyme that synthesizes GABA (GAD65) and evaluated changes in these molecules after noise rearing. We then examined the normal developmental expression of excitatory NMDARs (NMDAR NR2A and NR2B subunits) and evaluated changes in NR2A and NR2B expression after noise rearing. We hope that this study will help us understand the molecular basis of functional changes in the auditory cortex after noise rearing.

Materials and Methods
All studies were carried out in accordance with the guidelines published in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used for experiments and their suffering.

Noise Rearing A cage (70 × 45 × 35 cm3) containing rat pups (Sprague-Dawley) and their mothers was placed in a sound-attenuated room from postnatal day 7 (P7) to day 56 (P56). Rats were reared under an 8:16 h light:dark cycle and moved freely in the cage. The noise signal was produced by a white noise generator and amplified gradually to 65–70 dB sound pressure level (SPL) that was measured near the cage over the first day. Energy for the noise stimuli was essentially flat across a broad frequency spectrum (0.2–30 kHz). Continuous white noise was delivered from a speaker placed approximately 2 m above the rats (for 24 h/day) to mask most acoustic cues. The subsequent experiments were performed within 24 h after removal of the rats from the noise.
exposure at P56. The weights of all pups and mothers were continuously monitored, and there was no weight loss compared with control rats. No animal showed signs of stress or abnormal behavior. The sound level of normal control conditions was 50 dB SPL.

**Electrophysiology:** Electrophysiological recording of cortical responses was conducted at approximately P56 for noise-reared and normally reared rats. Rats were anesthetized with sodium pentobarbital (50 mg/kg). Dilute pentobarbital was injected continuously by a microinjection pump through a cannula placed in the abdomen to minimize fluctuations in the anesthetic level.

Rectal temperature was maintained at approximately 38 °C using a thermostatically controlled heating pad. 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. The flat head of a 2-cm nail was fixed to the exposed skull of each rat using acrylic glue and dental cement. After removing the temporalis muscle, the auditory cortex was exposed, and the dura was resected. The cortex was maintained under a thin layer of bicarbonate-buffered mineral oil to minimize cerebral edema. The flat head of a 2-cm nail was fixed to the exposed skull of each rat using acrylic glue and dental cement. After removing the temporalis muscle, the auditory cortex was exposed, and the dura was resected. The cortex was maintained under a thin layer of bicarbonate-buffered mineral oil to minimize cerebral edema.

Recordings were made from the A1 that is characterized by an anterior-to-posterior tonotopic progression from high-to-low frequencies (known as tonotopic organization) (Sally and Kelly 1988; Doron et al. 2002; Rutkowski et al. 2003). Cortical responses were recorded from the middle layers (III/IV) with 3 M KCl glass pipette electrodes (3–5 MΩ). A software package of TDT System III was used to calibrate the loudspeaker, generate acoustic stimuli, monitor cortical response properties online, and store data for offline analysis. Evoked spikes of a neuron or a small cluster of neurons were selected in a sequential or pseudorandom order with a spatial sampling density of 4–5 locations per second.

Experiments were performed in an electric sound-proof room. Acoustic stimuli were generated using the TDT System III (Tucker-Davis Technologies, Alachua, FL) and transmitted by a loudspeaker that could be moved in both the horizontal (azimuth) and vertical (elevation) directions to cover a sphere 20 cm in diameter. Mechanical constraints restricted measurements to the range of 90° (ipsilateral) to 0° (contralateral) along the azimuthal axis.

**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 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. Units that were modulated by less than this amount were classified as having no centroid (NC). 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.

**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 ΔC for modulation of spike counts and computed as ΔC = 1 - Cmin, where Cmin refers to the minimum values of C across location.

**Azimuth and DR**

Azimuth tuning was assessed using the PAR75, which was defined as the range of locations that elicited C > 0.75. These locations were not necessarily contiguous. Cells were classified as bounded or unbounded. Bounded cells had PAR75 that were limited to the frontal field, as was shown in Figure 1A. The PAR75 of unbounded cells extended beyond the contralateral or ipsilateral pole (90°, as shown in Fig. 1B) and could be distinguished from bounded cells because their PAR75 were underestimations of their true, unknown extent.

Azimuth selectivity was assessed by measuring the slope of the azimuth function in unbounded neurons. This was measured by the azimuth range over which a cell's response changed from 25% to 75% of the maximum and was termed DR (Fig. 1B). The DR indicates a cell's sensitivity to changes in sound source azimuth; small DR values indicate a steep slope, whereas larger values indicate a shallow slope.

**Statistical Analysis**

The Kolmogorov–Smirnov test was used to compare distributions of various spatial statistics between noise-reared and normally reared rats using the Statistical Package for Social Sciences.

**Quantitative Immunoblot**

The rats used for the immunoblot experiments come from a separate group of animals and not the same used for the electrophysiological experiment. Rats were deeply anesthetized with an injection of sodium pentobarbital (75 mg/kg). Immediately after decapitation, the rat brains were obtained. The right and left auditory cortex and visual cortex were separated and immediately homogenized in ice-cold homogenization buffer (0.5 mM Dithiothreitol, 1 mM Ethylene Diamine Tetraacetic Acid, 2 mM Ethylene glycol-bis (β-aminomethyl ether) N,N,N',N'-tetra acetic acid, 10 mM N-2-hydroxyethylpiperazine-N-ethane-sulphonacid, 10 mg/L leupeptin, 2 mg/L aprotinin, 0.1 mM Phenylmethylsulfonyl fluoride), and a portion of the whole homogenate was centrifuged at 14000 × g for 10 min and kept for analysis of the inhibitory GABAA receptor subunits and GAD65. A previously reported subcellular fraction procedure (Hollingsworth et al. 1985; Quinlan, Olstein, et al. 1999; Quinlan, Philpot, et al. 1999) was used on the remaining homogenate to obtain protein samples enriched for excitatory glutamatergic synapses (NMDA receptors). Briefly, the synaptoneurosomal was obtained by passing the homogenate through a coarse 100-μm pore nylon mesh filter followed by a fine 5-μm pore hydrophilic mesh filter (Millipore, Bedford, MA) and then centrifuged at 10000 × g for 10 min to obtain the synaptic fraction of the membrane. Both the synaptoneurosomal and whole homogenate samples were resuspended in boiling 1% sodium dodecyl sulfate and stored at −80 °C. Protein concentrations were determined using the bichinchoninic acid assay (Pierce, Rockford, IL).
Quantitative immunoblotting assays were performed as described previously (Xu et al. 2007, 2009). Primary antibodies used included anti-GAD65 (Sigma), anti-GABA\(_{\alpha1}\) (Upstate Biotechnology, Temecula, CA), anti-GABA\(_{\alpha3}\) (Upstate Biotechnology), anti-NR2A (Upstate Biotechnology), anti-NR2B (Upstate Biotechnology), and anti-\(\beta\)-actin (Sigma, St. Louis, MO). The relationship between optical density and protein concentration is linear along the range used in this study. The density of each band of western blotting was measured using Quantity One software (Bio-Rad Inc., Hercules, CA), and the relative level of each protein was calculated as the ratio of protein bands compared with the \(\beta\)-actin loading control band. For all immunoblots assays, the researcher was blinded to the experimental conditions.

**Results**

**General Observations**

Observations were based on 193 single neurons from the A1 of noise-reared rats and 166 single neurons from the A1 of control rats. Most histologically identified recording sites were located in layers III and IV. CFs were broadly distributed in each sample, ranging from 1–30 kHz, and there was no significant difference in the distribution of CF sampling between control and noise-reared rats. No significant differences were found in response latencies and respond thresholds of A1 neurons between noise-reared and control rats (data not shown).

**Early Continuous White Noise Exposure Resulted in Poor Azimuth Tuning in A1 Neurons**

We characterized the azimuth tuning of each unit by constructing azimuth-tuning curves. These curves represent a unit’s response rate as a function of stimulus location and can be used to estimate the unit’s PAR75, DR, azimuth-tuning depth, and azimuth centroid. Figure 1A,B illustrates the representative azimuth-tuning curves in normally reared (dark line) and noise-reared (grey line) rats. The azimuth-tuning...
curves observed from noise-reared rats were not as sharp as those observed from normally reared rats. Distributions of PAR75, the range of locations that elicit a robust response (specifically, a response above 75% of the maximum response), are shown in Figure 1C. The median PAR75 was 62.2° in noise-reared rats, compared with 56.5° in normally reared rats. We used the Kolmogorov–Smirnov test to compare the distributions of PAR75 between noise-reared and normally reared rats. There were significant differences in PAR75 between the 2 groups ($P < 0.001$). To examine whether the PAR75 was related to the CF of each site, we compared PAR75 from both rat groups by grouping their CF values into 3 approximately 1.5 octave-wide categories (Fig. 1E). Results showed that PAR75 was significantly different between the 2 groups across all CF ranges (Mann–Whitney $U$-test, $P < 0.05$).

For some units, the range of PAR75 extended beyond the contralateral or ipsilateral pole ($\pm 90°$, as shown in Fig. 1B), so their PAR75 was an underestimation of their true, unknown extent. These units were classified as unbounded cells distinct from bounded cells. Noise-reared rats had a greater proportion of unbounded units (45%) compared with normally reared rats (32%). The DR, the range of azimuths over which a cell's response changed from 25% to 75% of maximum, was used to evaluate the azimuth selectivity (sensitivity to changes in sound source azimuth) in unbounded neurons; Figure 1D shows distributions of DR. Units in noise-reared rats exhibited an azimuth function with shallower slope ($P < 0.001$). DR was significantly different between the 2 groups across all CF ranges (Mann–Whitney $U$-test, $P < 0.05$) (Fig. 1F). Thus, noise-reared rats seem less sensitive to changes in sound source azimuth.

The azimuth-tuning depth indicated reduction in the normalized spike count across azimuth. Distributions of azimuth-tuning depth are shown in Figure 2A. Distributions of tuning depth differed significantly between noise-reared and normally reared rats ($P < 0.001$); the median azimuth-tuning depth was 0.654 and 0.787 in noise-reared and normally reared rats, respectively. The azimuth-tuning depth was significantly different between the 2 groups across all CF ranges (Mann–Whitney $U$-test, $P < 0.05$) (Fig. 2B). This indicates that noise-reared rats have reduced azimuth selectivity.

A proportion of units were so broadly tuned that centroids were undefined when the spike count did not decrease to less than 75% of its maximum value at any location; these units are indicated as "NC." Noise-reared rats had a greater proportion of such units compared with normally reared rats; 14% of units in noise-reared rats had an undefined centroid, compared with 9% of units in normally reared rats (Fig. 2C). Distributions of centroids in the noise-reared and normally reared rats are shown in Figure 2D. The distributions of azimuth centroids were significantly different between noise-reared and normally reared rats ($P < 0.001$). The azimuth centroids were distributed over a wider range in noise-reared rats. Previous studies in the rat central auditory system showed that the preferred azimuth centroids were correlated with their CFs (Jen et al. 1987, 1989; Poon et al. 1990; Zhou and Sun 2006). In the present study, we failed to observe correlations between the azimuth centroids and CFs in normally reared and noise-reared rats.

![Figure 2](https://example.com/fig2.png)

**Figure 2.** Early continuous noise exposure results in shallower azimuth tuning and wider distribution of azimuth centroid. (A) Distribution of the azimuth-tuning depth in noise-reared and normally reared rats. Values on the y-axis indicate proportions of units per bin of 0.1 on the x-axis. Median values are plotted as gray diamonds for the noise-reared group and dark circles for the normally reared group. (B) Average tuning depth values for all recording sites in both rat groups for each 3 CF ranges. Bin size $= 1.5$ octaves. $*P < 0.05$. Error bars represent means ± standard error of the mean. (C) Percentage of units with NC. (D) Distributions of azimuth centroids for noise-reared and normally reared rats. Values on the y-axis indicate the proportion of units within a 10° range centered on the x-axis.
Early Continuous White Noise Exposure Induced Significant Decreases in GAD65 and GABAA Receptor α1 Subunit Expression and an Increase in GABAA Receptor α3 Expression, Which Indicates a Return to the Juvenile Form of GABAA Receptor

To examine whether and how early noise rearing affects the developmental expression of GABA_A receptor α1 and α3 subunits, we first determined the normal developmental expression of these molecules. Protein samples were prepared from auditory cortex of rats at P7, P12, P28, and P56 (n = 4 for each age group). During postnatal hearing development, P7 is before the critical period, P12 is the peak of the critical period according to some estimates (de Villers-Sidani et al. 2007), P28 is near the end of the critical period, and P56 is close to adulthood.

Studies in the visual cortex have revealed that in the immature cortex, the kinetics of the ionotropic GABA_A receptors are relatively slow because the receptor includes the GABA_A3 subunit and the kinetics of the ionotropic GABA_A receptor speed up 3-fold during development when the GABA_A1 subunit dominates the receptor (Laurie et al. 1992; Gingrich et al. 1995). We chose to analyze α1 and α3 subunits because the expression level of these subunits was an indicator of the maturity of GABA_A receptors.

Expression levels of 2 subunits of the ionotropic receptor (GABA_A1, GABA_A3) and GAD65 were quantified from whole homogenate samples. The whole homogenate samples were used because a large percentage of GABA receptors are extrasynaptic and the synaptoneurosome preparation enriches for excitatory synapses. The expression level of GABA_A receptor α1 that is linked with more rapid receptor decay times was lowest at P7, increased 2-fold at P28, and then increased slightly as the rat progressed to adulthood (Fig. 3B). There was a reduction in the expression level of GABA_A3 with increasing postnatal age. The GABA_A3 protein level was greatest at P7, decreased 30% at P28, and then showed further slight decrease at P56 (Fig. 3B). We calculated the GABA_A1/GABA_A3 ratio for each case because the receptor kinetics is more rapid when GABA_A receptor α1 dominates the receptor.

Figure 3. Early continuous noise exposure disturbs normal expression of GABA_A receptor subunits and GAD65. (A) Representative western blots showing developmental changes in expression for GABA_A1 and GABA_A3. (B) Changes in the expression levels of GABA_A receptors α1 and α3 during postnatal development of the rat auditory cortex. Values were normalized against the mean of the age showing the highest level of expression. (C) Developmental changes in the GABA_A1/GABA_A3 ratio. (D) Representative western blots for GABA_A1 and GABA_A3 from normal and noise-reared rats. (E) Noise rearing significantly decreases the expression of GABA_A1 and increases the expression of GABA_A3 (P < 0.05). (F) Noise rearing decreases the GABA_A1/GABA_A3 ratio. All values were normalized against the mean of the control rats. (G) Representative western blots showing developmental changes in GAD65. (H) Changes in the expression levels of GAD65 during postnatal development of the rat auditory cortex. Values were normalized against the mean of P56. (I) Noise rearing significantly decreases the expression of GAD65 (P < 0.05). All values were normalized against the mean of the control rats. *P < 0.05. Error bars represent means ± standard error of the mean.
By P28, the ratio had shifted from favoring GABA$_A$3 to favoring GABA$_A$1 (Fig. 3C).

Figure 3E shows GABA$_A$1 and GABA$_A$3 expression levels in the auditory cortex of normally and noise-reared rats. Noise rearing significantly decreased the expression of GABA$_A$1 subunit, ($n = 4$ for each group, $P < 0.05$, unpaired $t$-test), whereas noise rearing significantly increased the expression of the GABA$_A$3 subunit ($n = 4$ for each group, $P < 0.05$, unpaired $t$-test). Another well-documented consequence of noise rearing is a decrease in the GABA$_A$1/GABA$_A$3 ratio ($P < 0.05$, unpaired $t$-test) (Fig. 3F).

To further assess how early noise rearing influences the development of the GABAAergic system, we examined the normal developmental expression of GABA-synthesizing enzyme GAD65 and evaluated change in GAD65 expression after noise rearing. There was a significant increase in GAD65 expression during development of the auditory cortex (Fig. 3H). The GAD65 expression level increased about 5-fold during development. Fig. 3I shows GAD65 expression levels in the auditory cortex of normally and noise-reared rats. Noise rearing significantly decreased the expression of GAD65 ($n = 4$ for each group, $P < 0.05$, unpaired $t$-test).

**Early Continuous White Noise Exposure Had no Effect on the Expression Levels of NMDARs**

Expression of the NR2A and NR2B subunits was quantified for synaptoneurosome preparation samples. The synaptoneurosome samples showed an approximate 3-fold increase in level of the excitatory synaptic anchoring protein postsynaptic density protein 95 compared with the whole homogenate samples (Murphy et al. 2005).

There was substantial postnatal change in the NR2A and NR2B subunit expression levels in the rat auditory cortex. The expression level of NR2A was lowest at P7 and then increased progressively; it showed a 4-fold increase at P28 and then increased slightly as the rat developed toward adulthood (Fig. 4B). An opposite pattern of changes was observed for the expression level of NR2B. The NR2B expression level was greatest at P7, rapidly decreased by about 60% at P28, and then showed a further slight decrease as the rat developed toward adulthood (Fig. 4B). Previous studies in the visual cortex have shown that the ratio of NR2A/NR2B is an important measure because it is related to the decay time of the NMDA-mediated excitatory postsynaptic currents (EPSCs). As the NR2A/NR2B ratio increases, the NMDAR decay time decreases (Flint et al. 1997). We calculated the NR2A/NR2B ratio for each case and the average values for each group. There was a progressive increase in the ratio of synaptic NR2A/NR2B during development of the auditory cortex (Fig. 4C). During the early developmental stages, synaptic NMDARs were dominated by the NR2B subunit. The NR2A/NR2B ratio continued to increase with development, and by P28 the ratio had clearly shifted to be dominated by NR2A expression.

In our experiment, we did not find any significant difference in the expression of NR2A and NR2B subunits of NMDARs or the NR2A/NR2B ratio ($n = 4$ for each group, in all cases $P > 0.05$, unpaired $t$-test) between noise-reared and normally reared rats (Fig. 4E,F).

In addition, there were no differences in NR2A, NR2B, GAD65, GABA$_A$1, and GABA$_A$3 expression levels in the visual cortex of noise-reared and normally reared rats ($n = 4$ for each group, in all cases $P > 0.05$, unpaired $t$-test) (Fig. 5A,B,C).

**Discussion**

The results in the present study show that rearing infant rat pups under conditions of continuous, moderate level, white noise markedly attenuated the spatial sensitivity in the A1. Compared with normally reared rats, spike counts of A1 neurons in noise-reared rats were more poorly modulated by noise-reared rats.
changes in stimulus location, and their preferred locations were distributed over a wider range. We further show that early continuous noise rearing induced significant decreases in GAD65 and GABA\textsubscript{A} receptor α1 subunit expression and an increase in GABA\textsubscript{A} receptor α3 subunit expression, which may be the reason for the decrease in spatial sensitivity.

It has been reported that the spatial receptive fields of neurons recorded in the A1 of awake cats are typically more sharply tuned than those recorded under anesthesia (Mickey and Middlebrooks 2003). It should be noted that anesthesia might affect the spatial tuning; however, anesthesia would not be expected to bias estimations made in noise-reared and normally reared rats because of identical anesthetic conditions during recording for both rat groups.

Previous findings have indicated that noise exposure can cause changes in the subcortical central auditory system. Exposure of gerbils to omnidirectional white noise during the critical period of hearing development resulted in abnormal development of the interaural time difference tuning of auditory neurons in the brain stem (Seidl and Grothe 2005). We cannot exclude the possibility that the observed post-exposure changes in spatial tuning of the A1 are partially due to feed-forward responses reflecting experience-dependent changes in subcortical sources.

One pitfall of our study is that although we found noise exposure attenuated the spatial sensitivity in the horizontal plane, we have no idea about how the spatial sensitivity in the vertical plane changed after noise exposure. In our experiment, rats experienced noise coming from above them during the critical period. It is possible that neurons were tuned preferentially to the sound direction that was experienced during development. Such an effect could also contribute to the poor spatial resolution in the horizontal plane noted here, which was not experienced (or experienced to a lesser extent) during development. In future studies, location selectivity in the vertical plane should be investigated.

This report presents analysis of the normal postnatal development of NMDA and GABA\textsubscript{A} receptors plus GAD65 expression and the effects of early noise rearing on the expression of these molecules in the rat auditory cortex. These are key components of the neural mechanisms that underlie experience-dependent developmental plasticity in the sensory cortex, and the relative expression levels of these components will affect critical period plasticity. There were significant changes in the expression levels of synaptic NMDAR subunits during development; the NR2B expression level decreased rapidly, whereas there was a progressive increase in the NR2A expression level, and there was a change in the NR2A/NR2B ratio from being dominated by NR2B during the early stages of development to dominance by NR2A during the later stages. There was also a significant downregulation in the GABA\textsubscript{A} receptor α3 subunit expression in favor of an upregulation of the α1 GABA\textsubscript{A} receptor subunit. In agreement with the postsynaptic changes, there was an increase in GAD65 expression presynaptically.

Studies in the visual cortex revealed that the NR2 subunits of NMDAR affect functioning of the receptor largely by changing the opening time of the ion channel and affecting the decay time of the EPSC. A receptor composed of NR2B subunits (which was prevalent in infants) is very sluggish, about 380-millisecond decay, but becomes faster (~190 ms) when NR2A (which was prevalent in adults) is inserted into the receptor (Monyer et al. 1992, 1994; Flint et al. 1997; Roberts and Ramoa 1999). It is believed that shortening of the NMDAR-mediated current underlies the developmental reduction of ocular dominance plasticity (Carmignoto and Vicini 1992; Flint et al. 1997). In the immature cortex, the kinetics of the ionotropic GABA\textsubscript{A} receptors is relatively slow (Gingrich et al. 1995) because the receptor includes the GABA\textsubscript{A} receptor complex. Furthermore, the inclusion of GABA\textsubscript{A} receptor speeds up during development when the GABA\textsubscript{A} receptor subunit dominates the receptor complex. The kinetics of the GABA\textsubscript{A} receptor speeds up during development when the GABA\textsubscript{A} receptor subunit dominates the receptor complex. Furthermore, the inclusion of GABA\textsubscript{A} receptor subunit contributes to the transition to shorter, more phasic inhibition that results in faster inhibitory postsynaptic currents decay in the cortex (Iwai et al. 2003).

Previous studies have reported that rearing rat pups under conditions of continuous white noise causes the spectral and temporal response selectivity and cortical inhibition to be sustained in a degraded and immature state and the critical period window to be extended until normal acoustic inputs are restored (Chang and Merzenich 2003; Chang et al. 2005).
agreement with these findings, our results showed that continuous noise rearing significantly decreased the GAD65 expression level and was associated with a return to the juvenile form of GABA<sub>A</sub> receptor. These results indicate that white noise rearing that acts as a form of normal auditory experience deprivation maintains kinetic development of inhibition in an immature state and might cause the poor A1 spatial sensitivity seen in the present study and distortions of A1 tonotopicity reported by Chang and Merzenich (2003).

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. 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 (Hensch et al. 1998). Rearing animals in the presence of continuous moderate level noise led to a delayed maturation of the inhibitory receptive fields of auditory cortical neurons (Chang et al. 2005). These results plus our present result indicate that exposure to patterned sound inputs is crucial to inhibitory dimension of A1 function maturation.

Like continuous white noise rearing, visual deprivation by dark rearing during the early stages of development markedly affects the development of direction selectivity in the striate cortex (Leventhal and Hirsch 1980; Sherman and Spear 1982). Also in the visual system, dark rearing from birth reduces the GAD and GABA expression levels (Hendry and Jones 1986; Benevento et al. 1995), increases the GABA<sub>A</sub> receptor α1 and α3 subunit expression levels (Chen et al. 2001), and returns to the juvenile form of NMDARs in the visual cortex (Quinlan, Olstein, et al. 1999; Quinlan, Philpot, et al. 1999).

Speechley et al. (2007) reported that rats housed in continuous white noise for the first 50 days of postnatal life exhibited greater long-term potentiation (LTP) than controls reared in unaltered acoustic environments. Thus, the absence of patterned auditory stimulation during early postnatal life appears to retard sensory-dependent thalamocortical synaptic strengthening, as indicated by the preferential readiness for synaptic potentiation over depression (Speechley et al. 2007). In our studies, we further found that early continuous noise rearing reduced levels of inhibition, with no effect on the NMDAR subunit composition. This result is not consistent with the work by Quinlan et al showing that dark rearing facilitates plasticity/LTP, which is due to changes in NR2b/NR2B subunit of the NMDAR (Quinlan, Olstein, et al. 1999; Quinlan, Philpot, et al. 1999). We believe that dark rearing and noise rearing are somewhat different. Dark rearing typically deprived both the patterns and overall intensity of stimulation, whereas noise rearing only masked the patterned auditory information with no effect on the overall intensity of stimulation. Under noise conditions, the auditory cortex also can receive auditory input, but the auditory functional circuit cannot develop properly that is more related with the inhibitory system. Our result also suggests that the development of NMDA-mediated excitation mainly relies on the overall intensity of stimulation, unlike the GABA-mediated inhibition, which mainly relies on the stimulation pattern, rather than the presence of stimulation.

Recently, it has been reported that auditory deprivation caused by wearing ear plugs causes age-dependent changes in the expression levels of the NR2B and NR1 NMDAR subunits (Bi et al. 2006; Lu et al. 2008). However, the 2 deprivation techniques, ear plugs and white noise, may differ in their effects on auditory information transmission and processing. Ear plugs only attenuate ambient sound, whereas rearing of infant rats in continuous noise effectively masks normal environmental patterns of sound inputs, interferes with the sensing auditory information in the auditory system, but has no effect on the overall intensity of stimulation.

In summary, we have shown that rearing infant rat pups under conditions of continuous, moderate level white noise significantly decreases the GAD65 and GABA<sub>A</sub> receptor α1 subunit expression levels and increases the GABA<sub>A</sub> receptor α3 subunit expression level, which indicates a return to the juvenile form of GABA<sub>A</sub> receptor subunits and may cause the decrease in spatial sensitivity.

Funding
New Century Excellent Talents in University of State Education Ministry of China (NCET-07-0298); National Natural Science Foundation of China (30570595; Shanghai Pujiang Program (06PJ14036); Shanghai Shuguang Program (No.05SG28); PhD Program Scholarship Fund of East China Normal University 2008 (20080035).

Notes
Conflict of Interest: None declared.
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References


