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Research Report

Sensory experience determines enrichment-induced plasticity in rat auditory cortex

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Our previous studies demonstrated that only a few days of housing in an enriched environment increases response strength and paired-pulse depression in the auditory cortex of awake and anesthetized rats [Engineer, N.D., Percaccio, C.R., Pandya, P.K., Moucha, R., Rathbun, D.L., Kilgard, M.P., 2004. Environmental enrichment improves response strength, threshold, selectivity, and latency of auditory cortex neurons. J Neurophysiol. 92, 73–82 and Percaccio, C.R., Engineer, N.D., Pruette, A.L., Pandya, P.K., Moucha, R., Rathbun, D.L., Kilgard, M.P., 2005. Environmental enrichment increases paired-pulse depression in rat auditory cortex. J Neurophysiol. 94, 3590–3600]. Multiple environmental and neurochemical factors likely contribute to the expression of this plasticity. In the current study, we examined the contribution of social stimulation, exercise, auditory exposure, and cholinergic modulation to enrichment-induced plasticity. We recorded epidural evoked potentials from awake rats in response to tone pairs and noise bursts. Auditory evoked responses were not altered by social stimulation or exercise. Rats that could hear the enriched environment, but not interact with it, exhibited enhanced responses to tones and increased paired-pulse depression. The degree to which enrichment increased response strength and forward masking was not reduced after a ventricular injection of 192 IgG-saporin. These results indicate that rich auditory experience stimulates physiological plasticity in the auditory cortex, despite persistent deficits in cholinergic activity. This conclusion may be beneficial to clinical populations with sensory gating and cholinergic abnormalities, including individuals with autism, schizophrenia, and Alzheimer's disease.

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1. Introduction

Environmental enrichment increases social interactions, physical exercise, and sensory stimulation. Over the past 50 years, enriched environments have been used to demonstrate that the structure, chemical composition, and function of the entire brain can change across the lifespan (van Praag et al., 2000; Diamond, 2001). Animals housed in enriched environments exhibit increases in brain weight, cortical thickness, glial cell to neuron ratio, dendritic branching, number of synapses per neuron, and levels of neurotrophins compared to animals housed in standard laboratory conditions (Diamond et al., 1966; Bennett et al., 1969; Greenough et al., 1973; Katz and Davies, 1984; Turner and Greenough, 1985; Ickes et al., 2000). Enrichment also increases

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the levels of acetylcholine (ACh) receptors, acetylcholinesterase (AChE), choline acetyltransferase (ChAT), and monoamines in multiple brain regions (Bennett et al., 1964; Por et al., 1982; O'Shea et al., 1983; Park et al., 1992; Naka et al., 2002). The anatomical and neurochemical plasticity associated with environmental complexity enhances recovery from several forms of brain damage (Johansson, 2003). Enrichment increases the responsiveness of cortical neurons to tactile, visual, and auditory stimuli (Beaulieu and Cynader, 1990a; Coq and Xerri, 1998; Engineer et al., 2004). For example, environmental enrichment dramatically increases the strength of cortical responses to tones and noise bursts in both young and adult rats (Engineer et al., 2004). Enrichment also alters temporal processing in sensory cortex (Beaulieu and Cynader, 1990b; Percaccio et al., 2005). In rat auditory cortex, paired-pulse depression (PPD) increases during environmental enrichment and returns to control levels when housed in standard conditions (Percaccio et al., 2005). Recent evidence suggests these changes result from strengthened glutamatergic synapses in supragranular auditory cortex (Nichols et al., 2007). Collectively, these results indicate that enriched environments have a profound effect on the form and function of cortical circuits.

Given the complexity of enriched environments, it is possible that many environmental factors contribute to the observed changes in cortical responses. For example, the increased level of physical activity typical of rats housed in a complex environment could contribute to our previous observations of enrichment-induced plasticity. Wheel running is associated with increases in thickness of motor cortex, angiogenesis in both cerebellar and motor cortex, neurogenesis in the dentate gyrus of the hippocampus, neurotrophin levels, dopamine, long-term potentiation, and resistance to injury (de Castro and Duncan, 1985; Black et al., 1990; Stummer et al., 1994; Neeper et al., 1996; van Praag et al., 1999; Anderson et al., 2002; Swain et al., 2003; Farmer et al., 2004). Similarly, group housing significantly increases cortical weight, neurotrophic factor levels, neuronal density, and behavioral recovery after brain injuries, while isolation rearing results in neuroanatomical, neurochemical, physiological, and behavioral abnormalities (Rosenzweig et al., 1978; Einon et al., 1981; Turner and Greenough, 1985; Geyer et al., 1993; Risedal et al., 2002; Gordon et al., 2003; Preece et al., 2004; Stranahan et al., 2006). Although exercise or social stimulation can generate many of the benefits that accompany general enrichment, it is not known whether they are sufficient to increase response strength and PPD in auditory cortex.

Several studies indicate that cortical plasticity is regulated by the behavioral relevance of environmental stimuli. Rats that observe, but do not interact with, an enriched environment do not exhibit increased brain weight and exploratory behavior typical of rats housed in the environment (Ferchmin and Bennett, 1975). Monkeys exposed to sensory inputs without behavioral meaning do not exhibit the cortical map plasticity observed in monkeys who use stimuli to make behavioral judgments (Recanzone et al., 1993). Nucleus basalis (NB) neurons, which provide the major source of cholinergic innervation to the cortex, are activated as a function of the behavioral importance of environmental stimuli (Richardson and DeLong, 1991). Cholinergic modulation is necessary for the acquisition of behaviorally relevant information (Berger-Sweeney et al., 2000; Ferriera et al., 2001; Kudoh et al., 2004). ACh application and electrical activation of NB facilitate cortical plasticity when repeatedly paired with sensory stimuli (Metherate et al., 1987; Metherate and Weinberger, 1989; Kilgard and Merzenich, 1998a, b; Verdier and Dykes, 2001). Cholinergic receptor antagonists and NB lesions prevent experience-dependent and injury-induced plasticity in auditory, visual, somatosensory, and motor cortices (Sato et al., 1987; Metherate and Weinberger, 1989; Delacour et al., 1990; Juliano et al., 1990, 1991; Webster et al., 1991; Gu and Singer, 1993; Ivliev, 1999). These results suggest that cholinergic neurons may be required for the induction of enrichment-induced plasticity.

Earlier studies have suggested that sensory experience, physical activity, social interaction, and/or cholinergic modulation could be responsible for the increased response strength and PPD in the auditory cortex of enriched rats documented in our previous studies. The experiments in this report were designed to replicate these results and establish how passive exposure to sensory stimuli, exercise, social stimulation, and acetylcholine contribute to enrichment-induced plasticity in the auditory cortex.

2. Results

Adult female Sprague–Dawley rats were chronically implanted with a ball electrode over left primary auditory cortex and a ground screw over the cerebellum to record auditory evoked responses. After a brief recovery period, rats in studies 1 and 2 were differentially housed for a period of several weeks (Figs. 1 and 2). In study 1, we determine the relative contributions of sensory exposure, social stimulation, and physical activity to enrichment-induced plasticity by comparing the responses from a group of rats housed in each of these environments to the responses of a group of rats housed in the standard environment. In study 2, another group of rats was randomly assigned to either receive a cholinergic or a sham lesion and to be housed in either the enriched or standard environment (4 groups) to test whether a significant degree of cholinergic depletion would prevent enrichment-induced plasticity. First, we present the data from rats with sham-lesions in study 2 to replicate our previous results.

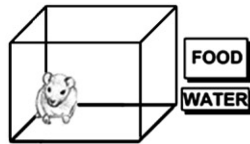
2.1. *Enrichment-induced plasticity*

2.1.1. *Tone-evoked response strength*

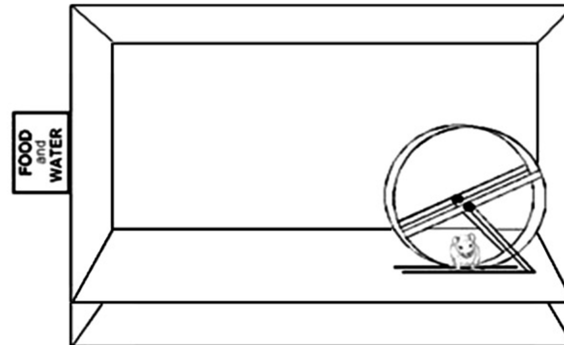
The morphology of the population average evoked potential of the enriched group was very similar to that documented in our previous studies (Engineer et al., 2004; Percaccio et al., 2005). The evoked response to a 70-dB tone consisted of negative peaks 25 (N1a), 40 (N1b), and 160 ms (N2) after sound onset and a positive peak 85 ms (P1) after onset (Fig. 3A). We compared the average of each individual's mean evoked potential recorded before enrichment, during enrichment, and after return to the standard housing condition. The amplitudes of the N1b, P1, and N2 peaks in the population average response to the average tone before enrichment were −14, 24, and −18 mV, respectively. During enrichment, the amplitudes of the N1b, P1, and N2 peaks increased by 250%, 136%, and 37%, respectively. Since the shape of an individual's evoked responses often differed from the population average, we used the root mean square (RMS) of the N1–P1 complex (30–100 ms after tone onset) for each rat to quantify the power of the

average evoked potential in each individual. The RMS voltage increased for 10 out of 11 rats when they were moved from the standard to the enriched condition and decreased for 10 out of 11 rats after they were moved from the enriched back to the standard condition (Fig. 3B). During enrichment, the average RMS evoked response

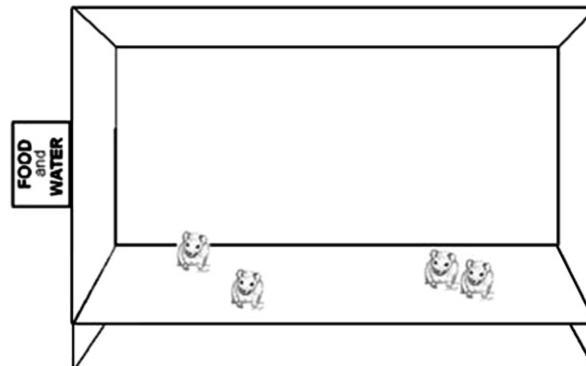
A) STANDARD ENVIRONMENT



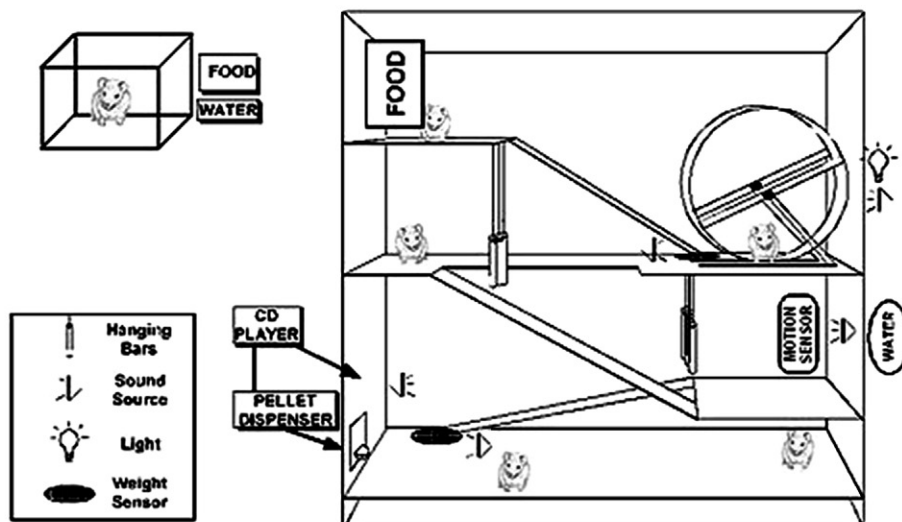
B) EXERCISE ENVIRONMENT



C) SOCIAL ENVIRONMENT



D) PASSIVE EXPOSURE E) COMPLEX ENVIRONMENT



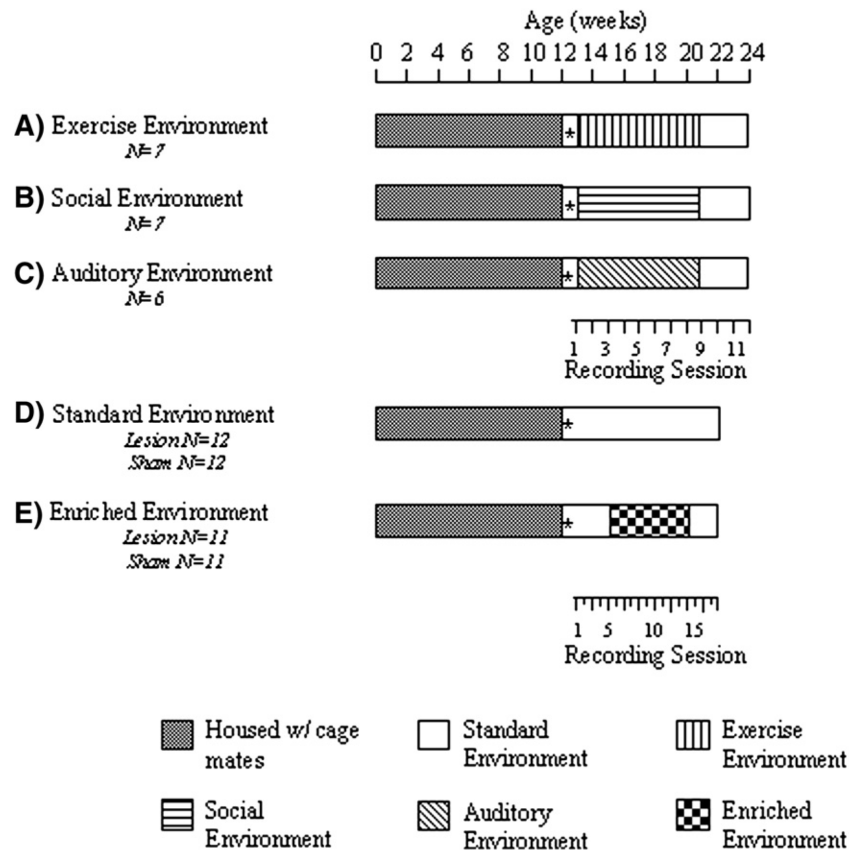


Fig. 2 – Experimental time lines. Asterisks indicate chronic electrode implantation. (A–C) Middle latency evoked potential data were collected over 12 recording sessions when rats were housed in either the (A) exercise, (B) social, or (C) auditory environment. Data were first collected in the standard environment 2 days after chronic implantation of recording electrodes and weekly thereafter. Rats were differentially housed for 8 weeks and then returned to the standard condition for 3 weeks. (D, E) Evoked potential data were collected the day after implantation and every 3–4 days thereafter for 62 days from each rat in the standard and enriched environments. Rats in the standard groups were injected with 192 IgG-saporin or the inactive control and housed singly in the standard environment for 62 days. Rats in the enriched groups were injected with 192 IgG-saporin or the inactive control substance and housed in the standard environment for 13 days, moved to the enriched environment for 35 days, and then returned to the standard environment for 14 days.

increased by 73%. Each individual's average evoked response during enrichment was divided by the average response while in the standard environment to quantify proportional changes in response strength and reduce variability due to electrode placement. A plasticity index value of 1 indicates a 2-fold increase in the magnitude of the evoked potential compared to week 1, while a value of −1 would indicate that the magnitude of the evoked potential was halved. The average tone-evoked plasticity index was 0.79±0.19 (mean±SE) during enrichment (Fig. 3C), and was significantly larger than during standard housing (Fig. 4, $p<0.01$).

2.1.2. *Paired-pulse depression*

During enrichment, the response to the second of 2 tones separated by 200 ms was 36±2% of the response to the first (Fig. 5). The amount of PPD was significantly greater during enrichment than during standard housing at every interval tested

Fig. 1 – Schematics of the 5 environmental housing conditions. (A) The standard environment consisted of 1 rat housed in a small hanging cage. (B) The exercise environment consisted of 1 rat housed in a medium sized cage with an exercise wheel. (C) The social housing condition consisted of 4 female rats housed in a medium sized cage. The acoustic environment of conditions A–C included cage noises and vocalizations from 20 to 30 other rats housed in the main rat colony room. (D) In the auditory exposure condition, 1 rat was housed in a hanging cage in the same room as the enriched environment. (E) The enriched environment consisted of 4–8 rats housed in a large cage with devices that generated different sounds when rats ran on the wheel, crossed a motion detector path, stepped on weight sensors, or passed through hanging bars. A CD player played 74 sounds, including tones, noise bursts, musical sequences, and other complex sounds in random order. Some of these sounds were associated with delivery of a sugar reward.

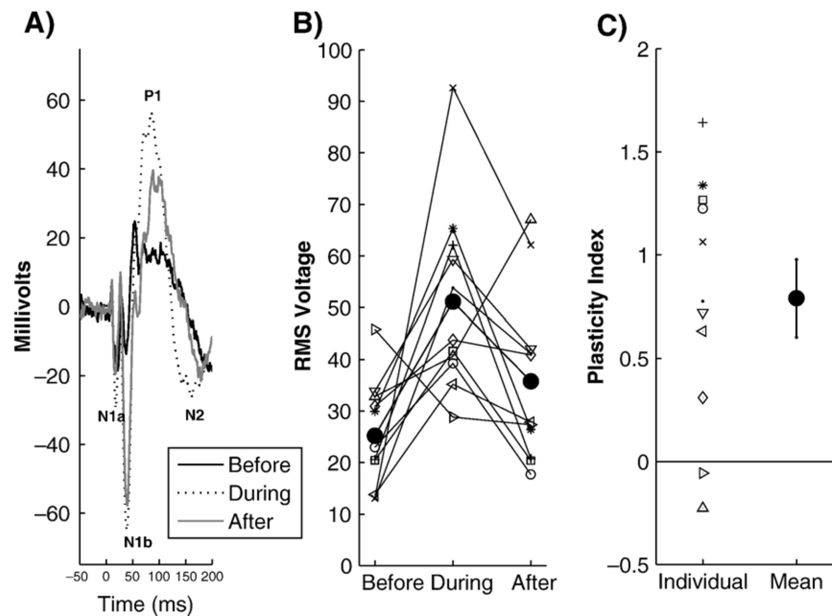


Fig. 3 – Enrichment-induced plasticity. (A) Mean auditory evoked potential in response to a 70-dB 9-kHz tone recorded from an individual rat in the sham-enriched group before, during, and after enrichment. During enrichment, the amplitudes of the N1b, P1, and N2 peaks increased by 250%, 136%, and 37%, respectively. (B) Root mean square was used to quantify the power of the average evoked potential in each individual. RMS voltage increased for 10 out of 11 rats when they were moved from the standard to the enriched condition and decreased for 10 out of 11 rats after they were moved from the enriched back to the standard condition. The mean RMS evoked response of individual rats in the sham-enriched group increased by 73%. (C) The plasticity index represents the logarithm base 2 of the average response of each rat during enrichment normalized to their response during periods of housing in the standard environment. A plasticity index value of 1 indicates a 2-fold increase in the magnitude of the evoked potential compared to week 1, while a value of −1 would indicate that the magnitude of the evoked potential was halved. The relative changes in tone-evoked plasticity for each individual rat is compared to the mean tone-evoked plasticity (0.79±0.19) for the enriched group.

(Table 2, $p<0.01$). Collectively, these results confirm our earlier observations that housing in an enriched environment increases response strength and PPD compared to standard-housed rats (Engineer et al., 2004; Percaccio et al., 2005).

2.2. *Study 1: Environmental factors contributing to cortical plasticity*

Neurophysiologic responses were recorded from rats housed in environments designed to determine the contribution of exercise ($n=7$), social stimulation ($n=7$), and sensory exposure ($n=6$) to the enrichment-induced plasticity documented in our previous studies (Engineer et al., 2004; Percaccio et al., 2005).

2.2.1. *Tone response strength*

There was a significant effect of housing condition on the strength of the cortical evoked response to tones ($F_{3,\ 29}=2.93$, $p<0.05$). The average tone-evoked potential amplitude of the auditory exposure group increased 2-fold, while the response of rats in the exercise and social groups were not altered compared to responses from rats in the standard environment (Fig. 6; Table 1, $p<0.05$).

2.2.2. *Paired-pulse depression*

There was a significant effect of housing condition on PPD for the 200-ms interstimulus interval ($F_{3,\ 29}=4.65$, $p<0.01$). Compared to when the rats were housed in the standard environment, PPD increased during periods of auditory exposure, but not during periods of increased physical activity or social interactions (Fig. 7; Table 2, $p<0.05$). Although similar trends were observed for the 500-, 100-, and 50-ms ISI stimuli, the degree of PPD was not significantly different, possibly due to floor and ceiling effects.

2.2.3. *Noise burst response strength*

Neither physical activity, social stimulation nor auditory exposure significantly affected the response amplitude to either the quiet noise burst ($F_{3,\ 29}=1.42$, $p>0.05$), the ramped noise burst ($F_{3,\ 29}=1.13$, $p>0.05$), or the loud noise burst ($F_{3,\ 29}=1.36$, $p>0.05$).

2.3. *Study 2: Cholinergic contribution to environmental plasticity*

Evoked potentials were recorded from rats with cholinergic or sham lesions housed in standard laboratory conditions or an enriched environment to determine the contribution of ACh to environmental plasticity.

2.3.1. *Lesion confirmation*

Since enrichment increases cholinergic markers, we injected 2.6 μg of the immunotoxin 192 IgG-saporin into the left lateral ventricle to determine whether cholinergic inputs from the basal forebrain contribute to this form of cortical plasticity (Bennett

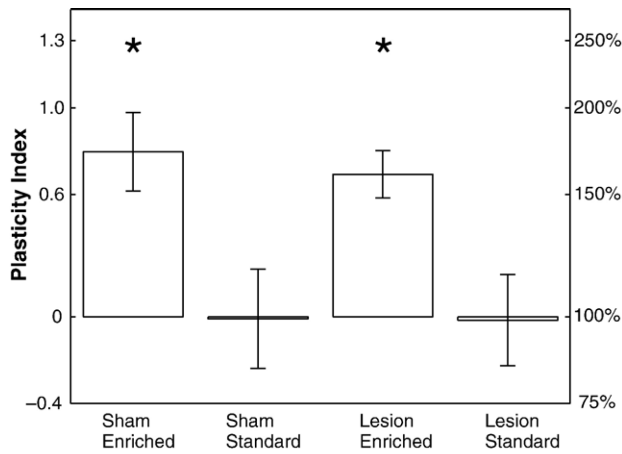


Fig. 4 – Environmental plasticity index for sham-enriched, sham-standard, lesion-enriched, and lesion-standard groups. Despite a significant reduction in cholinergic activity in the cortex, the strengthening of tone-evoked responses occurred to the same extent in both sham and lesion-enriched rats. During enrichment, the population average of the tone-evoked responses was significantly larger than when housed in the standard environment. Asterisks indicate significant increases in the cortical evoked response ($p<0.01$). Error bars represent standard error of the mean.

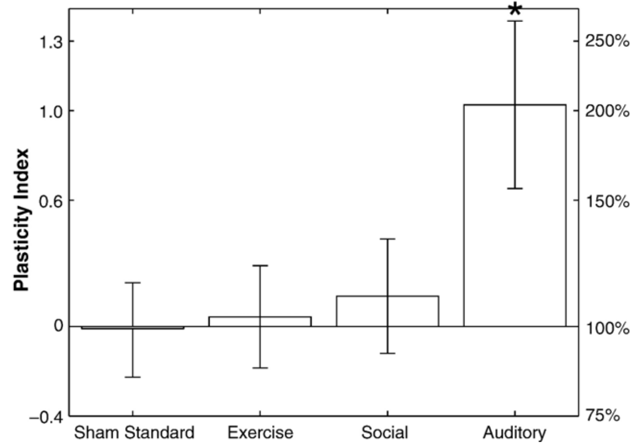


Fig. 6 – Environmental plasticity index for the exercise, social, and auditory exposure groups. During the period of differential housing, the average evoked potential amplitude of the auditory exposure group increased 2-fold, while the response of rats in the exercise and social groups were not altered compared to standard housing. The asterisk indicates a significant increase in the tone-evoked response ($p<0.05$). Error bars represent standard error of the mean.

et al., 1964; Por et al., 1982; Park et al., 1992). We confirmed that cholinergic activity was reduced in each animal by staining for AChE and determined the percent of AChE reduction by dividing the intensity of ipsilateral staining by the intensity of contralateral staining, and subtracting from 1 (for representative examples, see Figs. 8A, B). Every rat that received an injection of the cholinergic immunotoxin exhibited a reduction of cortical AChE staining of at least 25%, while only 16% of the rats that received a sham injection and 0% of controls exhibited a reduction of 25% or more. On average, ipsilateral AChE staining was reduced relative to contralateral AChE staining by 54±7% ($p<0.0001$) in the group of rats with cholinergic lesions, by 6±6% in the sham group, and by 0±6% in the control group. While it is not straightforward to estimate the percent of destruction of cholinergic NB neurons that project to cortex from diffuse AChE staining, previous studies have shown that 192 IgG-saporin doses between 2 and 2.7 μg eliminate 60–85% of ChAT and AChE immunoreactive fibers in cortical areas (Waite et al., 1995; Walsh et al., 1995; Galani et al., 2002). Previous work has also indicated that decreases in AChE levels are well correlated with decreases in ChAT levels and in basal ACh release (Gil-Bea et al., 2005).

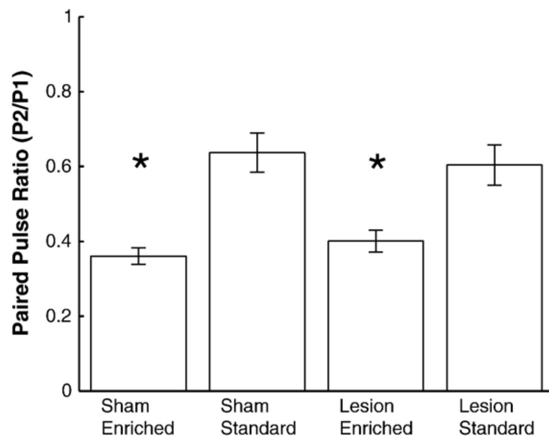


Fig. 5 – Paired-pulse ratios (response to the second pulse divided by the response to the first pulse) for sham-enriched, sham-standard, lesion-enriched, and lesion-standard groups. The response to the second of 2 tones separated by 200 ms was reduced by 36±2% in the sham-enriched group, 64±5% in the sham-standard group, 40±3% in the lesion-enriched group, and 60±5% in the lesion-standard group. Paired-pulse depression significantly increased when rats with either sham or cholinergic lesions were housed in the enriched environment compared to standard housing. Asterisks indicate significant increases in PPD ($p<0.01$). Error bars represent standard error of the mean.

2.3.2. *Tone response strength*

There was a significant effect of housing condition on the strength of the cortical response to tones ($F_{3,\ 42}=5.21$, $p<0.01$). Despite a significant reduction in cholinergic activity in the cortex, the strengthening of evoked responses occurred to the same extent in both sham- and lesion-enriched rats (Fig. 4, Table 1, $p<0.01$). Neither rats with sham nor cholinergic lesions exhibited response plasticity when housed in standard conditions.

2.3.3. *Paired-pulse depression*

There was a significant effect of housing condition on PPD for every interstimulus interval (500 ms: $F_{3,\ 42}=3.82$, $p<0.05$; 200 ms: $F_{3,\ 42}=11.59$, $p<0.00001$; 100 ms: $F_{3,\ 42}=7.52$, $p<0.0001$; 50 ms: $F_{3,\ 42}=5.44$, $p<0.005$). Specifically, for the rats with cholinergic lesions, the amount of PPD was significantly greater in enriched compared to standard-housed rats at 200

Table 1 – Plasticity index values

	Tone	Quiet noise burst	Ramped noise burst	Loud noise burst
Study 1: Environmental factors				
Sham standard	−0.01±0.24	−0.01±0.22	0.05±0.21	0.03±0.19
Exercise	0.04±0.24	−0.20±0.24	−0.32±0.26	−0.29±0.25
Social	0.14±0.26	0.23±0.17	0.14±0.17	0.17±0.17
Auditory exposure	*1.03±0.39	0.49±0.33	0.24±0.26	0.32±0.27
Study 2: Cholinergic damage				
Sham enriched	*0.79±0.19	*0.69±0.16	0.55±0.16	0.38±0.15
Sham standard	−0.01±0.24	−0.01±0.22	0.05±0.21	0.03±0.19
Lesion enriched	*0.68±0.11	*0.72±0.16	0.57±0.12	0.43±0.14
Lesion standard	−0.02±0.22	−0.10±0.16	−0.03±0.15	−0.04±0.14

Plasticity index values for each experimental group computed from auditory cortex potentials evoked by 4 different sounds. Values represent mean and standard error. Student's *t*-tests (alpha level adjusted with Bonferroni correction) were used to determine statistical significance (*$p<0.01$).

Table 2 – Paired-pulse depression values

	Interstimulus interval			
	500 ms	200 ms	100 ms	50 ms
Study 1: Environmental factors				
Sham standard	27±4%	36±5%	36±8%	60±5%
Exercise	19±2%	29±5%	34±4%	46±4%
Social	22±5%	47±3%	43±5%	55±6%
Auditory exposure	32±2%	*56±2%	58±6%	68±2%
Study 2: Cholinergic damage				
Sham enriched	*42±3%	*64±2%	*64±3%	*78±3%
Sham standard	37±4%	36±5%	36±8%	60±5%
Lesion enriched	34±4%	*60±3%	*68±3%	74±4%
Lesion standard	20±7%	40±5%	34±9%	50±9%

Paired-pulse depression (1−[response of 2nd tone/response of 1st tone]) values of tone-evoked potentials for each experimental group at each interval tested. Values represent mean and standard error. Student's *t*-tests (alpha level adjusted with Bonferroni correction) were used to determine statistical significance (*$p<0.01$).

and 100 ms interstimulus intervals (Table 2; $p<0.001$). The response to the second of two tones separated by 200 ms was 40±3% of the response to the first (Fig. 5, lesion enriched). Collectively, these results indicate that a significant reduction in cholinergic activity does not prevent enrichment-induced changes in response strength or PPD in auditory cortex.

2.3.4. *Noise burst response strength*

There was a significant effect of housing condition on the response to the quiet noise burst ($F_{3,\,42}=6.53$, $p<0.001$), but not for the ramped ($F_{3,\,42}=3.95$, $p>0.05$), or the loud ($F_{3,\,42}=2.55$, $p>0.05$) noise bursts. The response of rats with reduced cholinergic activity housed in the enriched environment to the quiet noise burst was significantly larger than the response of rats housed in the standard environment (Table 1; $p<0.01$).

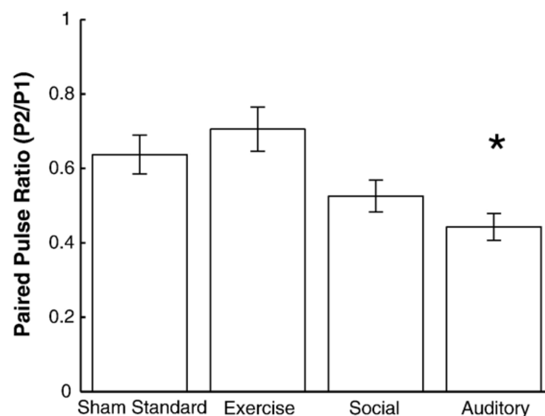


Fig. 7 – Paired-pulse ratios (response to the second pulse divided by the response to the first pulse) for the exercise, social stimulation, and auditory exposure groups. Paired-pulse depression was increased during periods of auditory exposure, but was not significantly different from standard housing during periods of exercise or social housing. The asterisk indicates a significant increase in PPD ($p<0.05$). Error bars represent standard error of the mean.

2.4. *Correlation analysis*

The strength of response to the first tone and the degree of PPD is positively correlated at every interstimulus interval tested (500 ms: $r=0.81$; 200 ms: $r=0.86$; 100 ms: $r=0.90$; 50 ms: $r=0.83$). Increased cholinergic depletion did not decrease either response strength or PPD plasticity (*p*-value >0.05). Both housing in an enriched environment and experiencing a variety of auditory input from afar increased cortical response strength and PPD, while the groups housed in the standard animal colony room had smaller responses to the first tone and less PPD (Fig. 9).

3. Discussion

Our previous studies demonstrated that housing rats in an enriched environment increases the cortical response to tone and noise burst stimuli and increases PPD to repeated tones (Engineer et al., 2004; Percaccio et al., 2005). This study was designed to evaluate the contribution of exercise, social stimulation, auditory experience, and cholinergic modulation to enrichment-induced plasticity. Collectively, the current results confirm that enrichment substantially increases response strength and PPD, identify sensory experience as the most important factor, and demonstrate that a reduction in unilateral cholinergic activity does not prevent enrichment-induced plasticity in auditory cortex.

Both social stimulation and exercise result in anatomical, neurochemical, physiological, and behavioral changes that are similar to those caused by complete environmental enrichment (Turner and Greenough, 1985; Renner and Rosenzweig, 1986; van Praag et al., 1999; Risedal et al., 2002). If the anatomical and neurochemical plasticity that results from enrichment contributes to the physiological changes we have documented in auditory cortex, then exercise and social stimulation would have increased

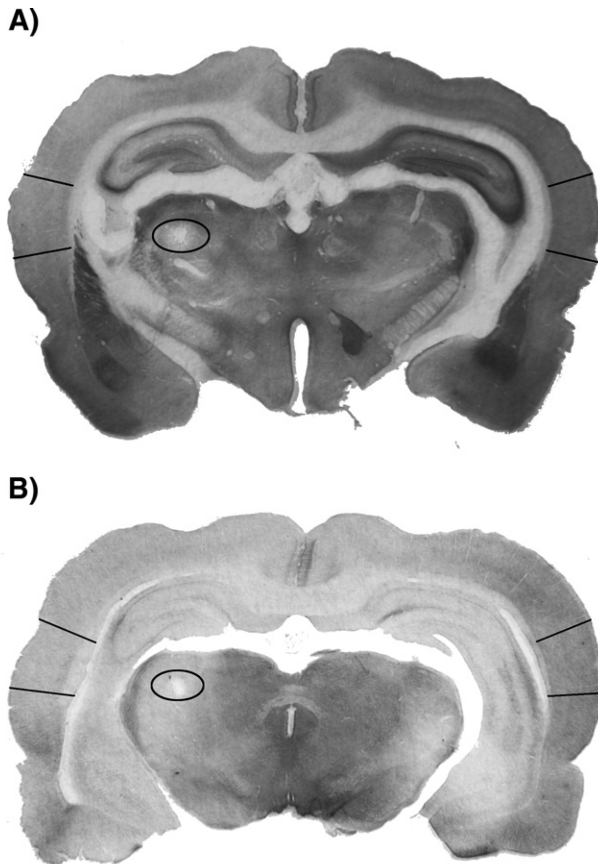


Fig. 8 – Examples of AChE-stained sections to verify the reduction in cholinergic activity in primary auditory cortex. The circled pin hole indicates the left/ipsilateral hemisphere and were made prior to sectioning the tissue since free floating sections were used for the staining procedure. Percent of AChE reduction is the intensity of ipsilateral staining divided by the intensity of contralateral staining, subtracted from 1. The outline indicates the region of interest. (A) Anterior section (~3.60 mm from bregma) and (B) posterior section (~4.52 mm from bregma) in a different rat. In these particular sections, the staining of the ipsilateral hemisphere relative to the contralateral hemisphere was reduced by 59% in section A and by 43% in section B.

response strength and PPD in auditory cortex. Our observation that neither exercise nor social interactions are sufficient to increase auditory cortical plasticity suggests that the plasticity induced by exercise and social housing occur primarily in brain regions activated by these conditions. For example, exercise stimulates anatomical plasticity in motor cortex (Anderson et al., 2002). Given the strong connection between somatosensory and motor modalities, perhaps exercise in isolation increases sensorimotor cortical processing. Since social interactions increase the expression of neurotrophic factors in the frontal cortex and amygdala (Gordon et al., 2003), they may be associated with enhanced evoked responses in frontal cortex. The fact that passive exposure to sounds can increase physiologic responses in auditory cortex suggests the possibility that associated anatomical changes are restricted to auditory cortex. Additional studies are needed to evaluate these hypotheses.

Although our results indicate that sensory exposure can stimulate plasticity in auditory cortex, several earlier studies have shown that passive auditory exposure does not increase response strength or temporal processing. Habituation, for example, results in a long-lasting frequency specific decrease in cortical responses to repeated tone presentations (Condon and Weinberger, 1991). Exposing monkeys to meaningless tones while they are engaged in a tactile discrimination task does not alter the organization of the A1 frequency map (Recanzone et al., 1993). Likewise, exposure to noise burst trains does not alter response strength or latency of auditory cortex neurons (Bao et al., 2003). These results indicate that sensory inputs alone are not sufficient to generate plasticity. However, passive stimulation paradigms with salient stimuli can enhance responsiveness and induce cortical organization under the appropriate conditions. A protocol which synchronously stimulates a patch of skin improves tactile discrimination abilities in parallel to inducing reorganization in the somatosensory cortex of young and elderly human subjects (Dinse et al., 2006). In visual cortex, repeated exposure to a specifically oriented stimulus selectively increases the evoked response to this stimulus (Frenkel et al., 2006). The neural bases for these interactions are likely to be quite complicated and potentially include modulation of neurotransmitter release, stress hormone levels, and arousal—the same factors that contribute to behavioral learning (Kuriki et al., 2006; Seitz and Dinse, 2007).

The most likely explanation for the passive exposure-induced plasticity in our study is that the enriched environment was sufficiently interesting to cause the rats in the adjacent cages to actively attend to the events associated with it. Whereas the sounds in the earlier studies were typically simple and highly repetitive, our enriched environment was designed to be sufficiently complex so that the sounds generated by it were neither

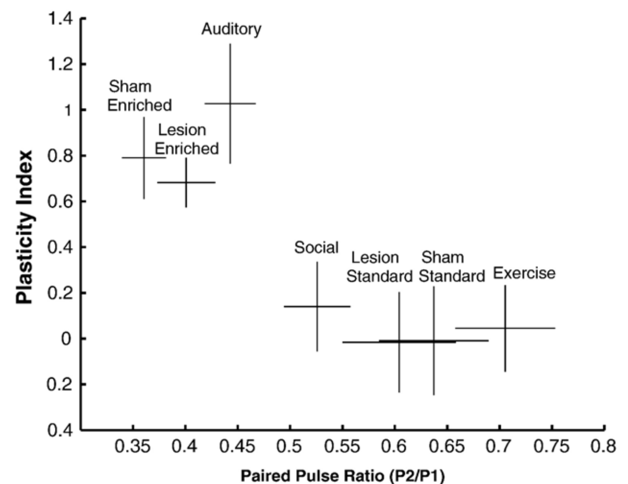


Fig. 9 – Mean plasticity index of each group as a function of the average paired-pulse ratio for the 200-ms interstimulus interval. Rats in the sham-enriched, lesion-enriched, and auditory exposure conditions exhibit large response magnitude plasticity and greater paired-pulse depression than rats in the exercise, social and standard-housed groups.

predictable (i.e., boring) nor random (i.e., meaningless). Exposure to the enriched environment may have commanded attention and generated plasticity because the interactions between the rats and the sound sources were optimally unpredictable and interesting to the rats observing the environment. In addition, the social interactions in the enrichment cage, including vocalizations, are situationally dependent, and likely an interesting component of acoustic enrichment. However, the singly and group-housed rats in the standard environment heard many vocalizations, but did not have increased response plasticity. Rats group housed in a large cage with added objects have greater brain weights than rats individually housed in the enriched cage or rats socially housed in a standard cage (Rosenzweig et al., 1978). Collectively, these results indicate that vocalizations, in and of themselves, are not sufficient to induce these forms of anatomical or physiological plasticity.

The importance of social interaction for learning complex stimuli is well documented in the animal and human literature. Auditory learning in songbirds is enhanced by the presence of a live tutor compared to an audiotaped recording of the song (Baptista and Gaunt, 1997). Language development in infants is also enhanced by the presence of a more experienced partner, compared to televised or audiotaped material (Kuhl et al., 2003). In both cases, tutors may be necessary to provide important acoustic and social cues necessary to learn complex stimuli. In our study, the influence of social interaction may have been diminished due to the nature of the test stimulus. Future studies are needed to determine the importance of social interactions to increase perception of complex stimuli in rats.

Regardless of which component of the environment is the most critical, enrichment increases attention and arousal that stimulate the release of important neuromodulators. Acetylcholine, dopamine, and norepinephrine promote many forms of plasticity both independently and in concert (Hasselmo, 1995; Gu, 2002). While several forms of cortical plasticity are blocked by removing cholinergic inputs, other forms require removal of more than one neuromodulator (Bear and Singer, 1986; Baskerville et al., 1997; Conner et al., 2003; Kamke et al., 2005). For example, the reorganization that follows digit amputation or nerve transection is blocked by excitotoxic NB lesions that destroy both cholinergic and GABAergic inputs to the cortex (Juliano et al., 1991; Webster et al., 1991). Ocular dominance plasticity can also only be prevented if cholinergic reduction is combined with concurrent norepinephrine depletion (Bear and Singer, 1986). Targeted destruction of cholinergic NB neurons that project to cortex does not impair cortical reorganization following cochlear damage, indicating that another neurotransmitter may co-mediate plasticity in auditory cortex (Kamke et al., 2005). These observations suggest that the additional destruction of GABAergic innervation from NB or noradrenergic inputs from locus coeruleus may be required to block the cortical effects of environmental enrichment. Future studies that combine lesion protocols are needed to distinguish the relative contribution of each neurotransmitter to environmental plasticity.

3.1. *Technical considerations*

Although considerable evidence suggests that multiple neurotransmitters regulate plasticity, it is possible that the cholinergic lesion technique used in this study failed to block enrichment-induced plasticity because spared cholinergic fibers were sufficient to stimulate plasticity. Over time, there may be substantial cholinergic marker recovery after a unilateral injection (Waite et al., 1995; Casamenti et al., 1988). Although it is possible that bilateral cholinergic lesions would interfere with enrichment-induced plasticity, this seems unlikely since the NB projection to the cortex is homolateral, and unilateral ICV injection of 192 IgG-saporin (6 μg) is sufficient to block whisker pairing plasticity in somatosensory cortex (Wenk et al., 1980; Baskerville et al., 1997). We only injected 2.6 μg because higher doses damage Purkinje cells and do not result in a significantly greater reduction of cholinergic innervation (Waite et al., 1995; Walsh et al., 1995; Waite and Chen, 2001). In addition, 2.5 μg was sufficient to prevent auditory cortex map plasticity after NB stimulation–tone pairing (Kilgard and Merzenich, 1998a). Since blocking map plasticity in motor cortex impairs experience-dependent plasticity (performance on the skilled reaching task) (Conner et al., 2003), it is reasonable to expect this dose of immunotoxin to impair enrichment-induced plasticity in the auditory cortex. However, the normal development of enrichment-induced plasticity in rats with cholinergic lesions suggests either that ACh is not involved in forms of learning that do not require sustained attention, or that there were effective compensatory mechanisms, including upregulation of intrinsic or surviving cholinergic neurons and/or the involvement of other neuromodulatory systems. The most likely explanation for why our results differ from earlier plasticity studies is that anatomical and neurochemical forms of enrichment-induced plasticity counteracted the effects of a substantial reduction in cholinergic activity (see also Paban et al., 2005). Although we did not measure physiological, anatomical, histological, or chemical differences between enriched and standard rats with lesions beyond what is described in studies 1 and 2 and are unable to determine the degree of compensation, cholinergic activity was still significantly reduced more than 20 weeks after the injection. It remains a possibility, however, that a more complete block of cholinergic projections, achieved with either higher doses of toxin or with bilateral lesions, might prevent enrichment-induced plasticity.

Previous experiments have shown that behavioral performance is disrupted when 75–85% of cholinergic input to the cortex is depleted (Wrenn and Wiley, 1998); however, we chose to use a partial cortical cholinergic deafferentation model after an extensive review of the clinical literature. Post-mortem studies of patients with Alzheimer's disease report that cholinergic markers are reduced 29–72% compared to tissue from individuals without Alzheimer's disease (Gil-Bea et al., 2005). Our cholinergic lesion achieved a similar level of depletion in AChE (54%). Specifically, this study was designed to test the hypothesis that cholinergic dysfunction on a scale comparable to that seen in clinical populations

would prevent the physiological benefit of an enriched environment.

3.2. *Clinical implications*

Sensory gating deficits are associated with several clinical populations, including schizophrenia, autism, and dyslexia (Buchwald et al., 1992; Erwin et al., 1991; Nagarajan et al., 1999). Sensory training (i.e., a focused form of enrichment) has been suggested as a treatment for temporal processing deficits found in some central auditory processing disorders (Merzenich et al., 1996, 1999; Tallal et al., 1996; Nagarajan et al., 1998; Tremblay et al., 2001; Hayes et al., 2003; Warrier et al., 2004). For example, individuals with dyslexia often have too much PPD, which may explain the difficulty they have processing rapid acoustic transitions necessary for the development of phonetic awareness and literacy (Nagarajan et al., 1999; Tallal et al., 1998). Our observation that sensory stimulation can alter both response strength and paired-pulse depression supports the use of sensory training to renormalize cortical processing.

Several of the disorders associated with sensory gating deficits also have cholinergic abnormalities, including patients with autism, schizophrenia, and Alzheimer's disease (Cullum et al., 1993; Freedman et al., 1994; Jessen et al., 2001; Perry et al., 2001, 2006; Gil-Bea et al., 2005). It has been suggested that cholinergic dysfunction alters PPD (Buchwald et al., 1992; Adler et al., 1998). There is a strong correlation between reductions in cholinergic activity and the degree of cognitive impairment in Alzheimer's disease (Gil-Bea et al., 2005; Perry et al., 1978). The loss of cholinergic cells may also underlie the cognitive decline associated with Rett syndrome, an autistic spectrum disorder (Wenk, 1997). Our data suggest that sensory gating deficits are not caused by reduced cholinergic activity and cast doubt on the hypothesis that both the sensory gating deficits and the cognitive dysfunction associated with schizophrenia, autism, and Alzheimer's disease arise from a single cholinergic deficit. For instance, abnormal activity of dopamine also results in sensory gating deficits (Braff and Geyer, 1990). Although the perceptual consequences of environmental enrichment are not well documented, our observations that environment can significantly influence PPD and that enrichment-induced plasticity may be independent of cholinergic function suggest that focused, intensive sensory enrichment may alter sensory gating despite persistent cholinergic abnormalities. For example, exposure to tactile stimuli ameliorates the age-related decline of tactile acuity observed in elderly subjects (Dinse et al., 2006). These results indicate that passive exposure to sensory stimuli may offer new perspectives for therapy.

3.3. *Ceiling effect*

While physiological plasticity studies in humans often probe neural responses with intense stimuli (i.e., Ponton et al., 2001), more reliable cortical plasticity may be observed in many situations using stimuli that do not elicit saturated responses (i.e., Tremblay et al., 2001). To confirm this hypothesis and determine how environmental plasticity differs when measured with broadband sounds, we recorded evoked potentials in response to three different noise bursts. Averaged across all rats in this report, the 70-dB loud noise burst evoked a larger response and resulted in less plasticity than the 70-dB ramped noise burst, which evoked a larger response and resulted in less plasticity than the 50-dB noise (Table 1). Despite the better signal to noise ratio in recordings evoked by noise bursts, the greatest proportional change in evoked response was recorded with a narrowband 70-dB tone, presumably because it stimulates less than the maximal neural response. Similarly, the use of submaximal stimuli may more effectively distinguish reliable differences in cortical processing among different clinical populations (Wible et al., 2002; Hayes et al., 2003; Sharma et al., 2004; Warrier et al., 2004; Gilley et al., 2006).

3.4. *Conclusions*

These experiments confirm that enrichment enhances response strength and PPD in auditory cortex, highlight the importance of sensory experience, and demonstrate that a significant reduction in cholinergic activity does not prevent enrichment-induced plasticity. Neither social interactions nor running on a wheel stimulated plasticity compared to standard housing; however, experiencing the enriched environment from a distance increased the response to the first tone and the degree of PPD, suggesting that sensory stimulation has the potential to generate plasticity that may be beneficial for several clinical disorders.

4. Experimental procedures

4.1. *Chronic implantation*

Eighty-one adult female Sprague–Dawley rats were chronically implanted with a ball electrode over left primary auditory cortex (approximately 4.8 mm posterior, 4 mm ventral, and 7.5 mm lateral to bregma) and a ground screw over the cerebellum (Paxinos and Watson, 1998) as described in our previous publications (Engineer et al., 2004; Percaccio et al., 2005). Briefly, surgical anesthesia was induced with sodium pentobarbital (50 mg/kg, i.p.), and supplemental doses of dilute pentobarbital were administered subcutaneously if needed (0.2 ml; 8 mg/ml). Rats received injections of atropine (1 mg/kg), dexamethasone (4 mg/kg), and antibiotics (ceftriaxone 20 mg/kg) before and after surgery. Body temperature was maintained at 37°C. Four to five structural screws were used to anchor the implant on the skull. The 4-pin connector was held in place with dental acrylic. Fifteen rats were excluded from the experiment due to implant malfunction. Experimental attrition was roughly equal across the 7 groups.

4.2. *Study 1: Environmental factors contributing to cortical plasticity*

4.2.1. Environmental conditions

4.2.1.1. Standard environment. Rats in the standard environment were housed 1 per cage (25 L × 15 W × 18 H cm; Fig. 1A) in a colony rack of 30 cages. The acoustic environment of this condition consisted of cage noises and vocalizations from 20 to 30 other singly and group-housed rats in the same room.

4.2.1.2. Exercise environment. Rats in the exercise environment were housed 1 per cage (61 L×41 W×36 H cm; Fig. 1B) with a running wheel (34.5 in circumference) in the same room as the standard-housed rats. On average, rats in this condition ran one-half mile per day (1023 revolutions).

4.2.1.3. Social environment. Rats in the social environment were housed 4 per cage (61 L×41 W×36 H cm; Fig. 1C) in the same room as the standard-housed rats.

4.2.1.4. Auditory exposure. Rats were housed 1 per cage (25 L×15 W×18 H cm; Fig. 1D) in a colony rack in the same room as the enriched environment (see Study 2). The acoustic environment of rats in this condition consisted of sounds described as part of the enriched environment, as well as vocalizations from rats housed in the enrichment cage and the other rats in the auditory exposure group. Although the intensity of the sounds generated by the enriched environment were 5–20 dB quieter for rats in the auditory exposure group, most sounds were audible from any location in the room.

4.2.2. Time course

Auditory evoked responses were collected from rats in the exercise ($n=7$), social ($n=7$), or exposure ($n=6$) groups during 12 recording sessions (Figs. 2A–C). Data were first collected in the standard environment 2 days after chronic implantation of recording electrodes and weekly thereafter. Rats were differentially housed for 8 weeks and then returned to the standard condition for 3 weeks.

4.2.3. Stimulus presentation and data collection

Recordings were made during the dark cycle in all housing conditions to encourage rats to be as alert as possible. However, the rats did spend some time sleeping. EEG recordings indicate that rats were in slow wave sleep no more than 25% of the time during each recording session. Overall, no differences in activity level, exploration, time spent sleeping, or arousal levels were noted across experimental groups during recording sessions.

Auditory evoked potential data were collected from each rat in a sound-attenuated booth. Acoustic stimuli included pairs of 25 ms 9 kHz tones with interstimulus intervals of either 500, 200, 100, or 50 ms (70 dB SPL with 3 ms rise and fall times), and 3 different 100 ms long noise bursts (loud: 70 dB SPL with 3 ms rise and fall times; quiet: 50 dB SPL with 3 ms rise and fall times; ramped: 70 dB SPL with 50 ms rise and fall times). The noise bursts with different rise and fall times were presented to test the hypothesis that there is a graded response to the stimulus depending on the level of activation of neurons in auditory cortex. Tones and noises were presented from a speaker centered above the cage and randomly interleaved every 10 s. Signals were low-pass filtered (800 Hz), amplified (10,000×), and displayed on an oscilloscope. Data acquisition computers collected and averaged cortical responses to 125 presentations of each stimulus. The evoked response to a 70-dB tone consisted of negative peaks 25 (N1a), 40 (N1b), and 160 ms (N2) after onset and a positive peak 85 ms (P1) after sound onset, while the evoked response to a 70-dB noise burst consisted of negativities 40 and 140 ms after noise onset (N1 and N2) and positivity 75 ms after onset (P1). Laminar recording studies have shown that the asynchronous participation of supragranular and infragranular pyramidal cells is responsible for the generation of the evoked-response complex (Barth and Di, 1990). The surface-recorded Pl is primarily produced by supragranular cells and the Nl by infragranular cells. The N2 is produced by temporally overlapping contributions from both cell groups. The spatial distribution of these peaks suggests they are generated by neurons in primary auditory cortex, consistent with the location of the ball electrode (Barth and Di, 1990). Trials (<1%) with excessive motion artifacts (>0.1 mV) were discarded prior to analysis of the mean evoked potential.

4.3. Study 2: Cholinergic contribution to environmental plasticity

4.3.1. Injection technique

Cholinergic neurons were selectively killed with 192 IgG-saporin (Advanced Targeting Systems) immediately before chronic implantation. Injections were made into the left lateral ventricle to target p75 NGFr-positive cells in the basal forebrain including the medial septum, vertical and horizontal limb of the diagonal band of Broca (which project to the hippocampus), and the substantia innominata and NB (which project to the cortex) (Kiss et al., 1988; Wiley et al., 1991, 1995; Heckers et al., 1994). When the antibody binds to the receptor, the immunotoxin is internalized and saporin inhibits protein synthesis (Wiley et al., 1995). Twenty-eight rats received an injection of the active immunotoxin and 27 rats received an injection of unconjugated saporin. An injection of 2.6 μg of toxin or control (in a volume of 1.7 μl) was made at 0.8 mm posterior from bregma, 1.4 mm lateral on the left hemisphere, and 3.8 mm below the pial surface according to Paxinos and Watson (1998). The brain was allowed to settle for 5 min before and after injection of the fluid (at a rate of 1 μl/min.). A similar dose of immunotoxin (2.5 μg) was sufficient to prevent auditory cortex map plasticity induced by NB stimulation (Kilgard and Merzenich, 1998a).

4.3.2. Environmental conditions

4.3.2.1. Enriched environment. Four to eight rats were housed together in a large cage (76 L×45 W×90 H cm) with 4 levels connected by ramps in a separate room from the standard rat colony (Fig. 1E). Touch plates at the bottom of 2 ramps triggered different tones (2100 or 4000 Hz) when rats stepped on the plates. Chains, wind chimes, and bells were hung across the entrance of each ramp so that a unique sound was elicited when rats passed from one level to the next. A motion detector emitted an electronic chime each time a rat crossed the infrared beam in front of the water source. An exercise wheel emitted a tone (3000 Hz Piezo Speaker) and activated a small green light emitting diode with each rotation. Each movement-triggered sound had unique spectral and temporal characteristics that provided behaviorally meaningful information about the location and activity of other rats in the cage. A CD player presented randomly selected sounds

every 2 to 60 s, including simple tones, amplitude-modulated and frequency-modulated tones, noise bursts, and other complex sounds (rat vocalizations, classical music, rustling leaves, etc.). Seven of the 74 sounds activated a dispenser (Med Associates) that delivered a sugar pellet to encourage attention to the sounds. The rewarded tracks included interleaved tones of different carrier frequencies (25 ms tones of 4, 5, 9, 12, 14, and 19 kHz with interstimulus intervals ranging from 50 ms to 2 s) and frequency modulated sweeps (1 octave up or down in a 140- or 300-ms sweep with interstimulus intervals ranging from 80 to 800 ms). The power spectrums of sounds in the enriched environment spanned the entire hearing range of the rat (1–45 kHz) and were less than 75 dB SPL. The sound sources added to the enriched environment were provided 24 h a day and were designed to be more diverse and more behaviorally relevant than sounds in the standard condition.

Our definition of a complex sound was any sound louder than 50 dB SPL with more than one onset separated by no more than 300 ms. The average interval between the components of complex sounds in the enriched environment was significantly shorter compared to the standard environment (108 ± 73 vs. 133 ± 80 ms, mean ± SD, $p < 0.001$). The total number of sounds (>50 dB SPL) was not significantly different between the enriched and standard environments (23 ± 23 vs. 19 ± 15 sound onsets per minute, mean ± SD).

The standard and enriched environmental conditions are identical to those described in our previous studies (Engineer et al., 2004; Percaccio et al., 2005). Rats in all housing conditions (i.e., Study 1 and Study 2) were on a reversed 12-h light/dark cycle and heard the sounds of room traffic, feeding, and cleaning while they were most active. Constant temperature and humidity were maintained and food and water were provided ad libitum for all rats. As in our earlier studies, all the rats used in this study were female; however, since we documented that enrichment-induced plasticity is equivalent in young and adult rats, all of these rats were housed with littermates at Charles River Laboratories until approximately 12 weeks of age (Engineer et al., 2004).

The enriched environment used in our experiments was designed to increase sensory exposure, social stimulation, and physical activity compared to standard-housed rats (Engineer et al., 2004; Percaccio et al., 2005). In addition to being housed in a large group, a vasectomized male rat was introduced into the cage to encourage natural social interactions, since rats reach sexual maturation by 12 weeks of age (Lewis et al., 2002). We estimate that each rat housed in the enrichment cage ran one-fifth of a mile per day (360 rotations). Although the additional exercise due to movements up and down the levels of the large cage were not quantified, we estimate that rats in the enriched and exercise groups received approximately the same amount of physical activity. In this study, we determine the relative contributions of sensory exposure, social stimulation, and physical activity to enrichment-induced plasticity.

We observed no evidence that the enriched environment, or any of the housing conditions, caused excessive stress (i.e., excessive licking, hair loss, fighting, etc.). Rats actively explored the enrichment cage, voluntarily activated sound sources, and engaged in playful behavior. Our behavioral observations and EEG recordings indicate that the different housing conditions did not significantly interfere with normal sleep–wake behavior. Although some forms of enriched housing increase release of corticosterone (Moncek et al., 2004), the observation that removal of the adrenal or pituitary glands do not reduce the beneficial effects of enrichment suggests that stress hormones are not critical factors in environmental plasticity (Rosenzweig et al., 1972; Devenport et al., 1992). However, since we did not measure glucocorticoid levels this study, it is uncertain if the sounds added to our enriched environment caused stress.

4.3.3. *Time course*

Rats were randomly assigned to 1 of 4 groups: lesion enriched, sham enriched, lesion standard, and sham standard. Groups of rats housed in the enriched environment (lesion: $n = 11$; sham: $n = 11$) were injected with 192 IgG-saporin or the inactive control and housed in the standard environment for 13 days, moved to the enriched environment for 35 days, and then returned to the standard environment for 14 days (Fig. 2E). Rats in the standard groups (lesion: $n = 12$; sham: $n = 12$) were injected with 192 IgG-saporin or the inactive control and housed singly in the standard environment for 62 days (Fig. 2D). Evoked potential data were collected the day after implantation and every 3–4 days thereafter. Data from the first 13 days after implantation were not included in our analysis, since the effects of the lesion develop over this time (Book et al., 1992; Waite et al., 1994; Motooka et al., 2001).

4.3.4. *Stimulus presentation and data collection*

Stimulus presentation and data collection were identical to Study 1.

4.3.5. *Histology*

Rats were sacrificed in groups of 3 (1 rat with a cholinergic lesion, 1 rat with a sham injection rat, and 1 uninjected rat from the exercise, social, or exposure group), and their brain sections processed simultaneously to minimize variability. All solutions were warmed to room temperature prior to use. Rats were overdosed with urethane and transcardially perfused with 100 ml of 0.9% NaCl and 0.5% sodium nitrate in 0.1 M phosphate buffer (pH 7.4) followed by 200–400 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) and 100 ml of 10% sucrose in 0.1 M phosphate buffer. Brains were removed, post-fixed for 24 h in 4% paraformaldehyde, and stored in 30% sucrose for 48 h. Brains were sectioned 50 μm thick on a freezing microtome into 0.1 M phosphate buffer. Free-floating sections were stored overnight in a refrigerator.

4.3.6. *Histochemistry*

AChE-stained sections were examined to determine the extent of cortical cholinergic denervation. The staining protocol was modified from Karnovsky and Roots by Dr. Larry Cauller at the University of Texas at Dallas (Karnovsky and Roots, 1964). All solutions were made fresh each time and filtered prior to use. Free-floating sections were agitated throughout the procedure to ensure adequate exposure to each bath of solution. Phosphate buffer (pH 7.4) was replaced with 0.1 M sodium acetate buffer (pH 6.0) in several washes. The pre-incubation solution (two 10-min baths) consisted of

1.47 g/ml of sodium citrate, 0.48 g/ml of cupric sulfate, and 0.17 g/ml of potassium ferricyanide dissolved in sodium acetate buffer. Acetylthiocholine iodide (0.5 g/ml) and ethopropazine (0.05 g/ml) were added to a clean bath of preincubation solution of the same batch to create the incubation solution. Sections incubated for 1 h, were detoxified in 0.05 M Tris buffer (pH 7.4), mounted out of 10% phosphate buffer onto gelatin-coated slides, and photographed for subsequent quantitative analysis.

Throughout this study, adequate measures were undertaken to minimize any pain or discomfort of rats. All protocols and recording procedures conformed to the guidelines published in the NIH *Guide for the Care and Use of Laboratory Animals* (NIH publication no. 86-23, revised 1987) and the European Communities Council Directive, and were approved by the University Committee on Animal Research at the University of Texas at Dallas.

4.4. *Analysis*

Response strength: The response to the first tone of the 500-ms, 200-ms, and 100-ms stimuli tone pairs were averaged (resulting in 375 presentations) to increase the reliability of the evoked response. The RMS of the evoked potential (30–100 ms after sound onset) was used to quantify the magnitude of the auditory evoked response. Peak to peak analysis and analysis of individual peak components yielded the same general pattern of results; however, the power of the evoked response yielded more consistent results given the inherent variability in and difficulty labeling individual evoked potential components. To eliminate variability in evoked potential amplitude due to individual differences in electrode position and recording characteristics, the size of the average evoked response during the period of differential housing was divided by the average evoked response during periods of standard housing. As in our previous study, changes were quantified using the plasticity index, which is simply the logarithm base 2 of this ratio (such that −1 and +1 indicate 2-fold decreases and increases, respectively) (Engineer et al., 2004). Differences in the plasticity index were compared using one-way analysis of variance (ANOVA). Post hoc *t*-tests were used to determine whether differences in response strength were statistically significant (alpha = 0.0166). Alpha level was determined using the Bonferroni correction for the three multiple comparisons in each study (Study 1: sham standard vs. auditory, exercise, and social; Study 2: sham standard vs. sham enriched, lesion enriched, and lesion standard). Comparisons in both studies are to the responses of rats housed in the standard environment, and error measures represent standard error of the mean.

Paired-pulse depression: For each rat, the response to the second tone was calculated by subtracting the response to a single tone (derived from the first pulse of the 500-ms tone pair) from the overlapping response to 2 tones separated by shorter (200, 100, or 50 ms) interstimulus intervals. We estimated the waveform of the response 500 ms after a single tone onset by using the waveform 500 ms after onset of the 50-ms pair of tones since we did not present the tone in isolation. The waveform was indistinguishable from baseline 500 ms after onset of the 50-ms pair. Response strength was quantified as the RMS of the evoked potential 10–175 ms after each tone onset (N1–P1 complex). Paired-pulse ratios were calculated by dividing the response to the second tone by the response to the first. The degree of PPD was determined by subtracting this ratio from 1. Differences in the PPD of each group were compared using one-way ANOVA and *t*-tests as for plasticity indices (above).

Acetylcholinesterase: Sections were imaged in 36-bit color (12-bit RGB) using a high-resolution digital camera (DVC Co., Austin, TX, USA, Mod. 1310/1312) and Neurolucida ™ software (Microbrightfield, Inc., Williston, VT). Using Adobe Photoshop (6.0), we quantified AChE optical density (inverse of mean luminosity) across all layers of primary auditory cortex, according to Paxinos and Watson Rat Brain Atlas (Paxinos and Watson, 1998). The brightness and contrast settings of all images were held constant during image acquisition and analysis. AChE staining was estimated by subtracting the background staining observed in nearby white matter. The reduction of AChE staining was determined by averaging approximately 10 sections from each rat through the extent of primary auditory cortex to control for local variations in staining. The intensity of cortical AChE staining on the ipsilateral side relative to the contralateral side was used to confirm immunolesions in rats with 192IgG-saporin injections compared to rats with sham lesions and uninjected control rats.

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