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# Environmental Enrichment Increases Paired-Pulse Depression in Rat Auditory Cortex

Cherie R. Percaccio,\* Navzer D. Engineer,\* Autumn L. Pruette, Pritesh K. Pandya, Raluca Moucha, Daniel L. Rathbun, and Michael P. Kilgard

Neuroscience Program, School of Behavioral and Brain Sciences, University of Texas at Dallas, Richardson, Texas

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**Percaccio, Cherie R., Navzer D. Engineer, Autumn L. Pruette, Pritesh K. Pandya, Raluca Moucha, Daniel L. Rathbun, and Michael P. Kilgard.** Environmental enrichment increases paired-pulse depression in rat auditory cortex. *J Neurophysiol* 94: 3590–3600, 2005. First published August 10, 2005; doi:10.1152/jn.00433.2005. Temporal features are important for the identification of natural sounds. Earlier studies have shown that cortical processing of temporal information can be altered by long-term experience with modulated sounds. In a previous study, we observed that environmental enrichment dramatically increased the response of cortical neurons to single tone and noise burst stimuli in both awake and anesthetized rats. Here, we evaluate how enrichment influences temporal information processing in the auditory cortex. We recorded responses to repeated tones and noise bursts in awake rats using epidural evoked potentials and in anesthetized rats using microelectrodes. Enrichment increased the response of cortical neurons to stimuli presented at slow rates and decreased the response to stimuli presented at fast rates relative to controls. Our observation that enrichment substantially increased response strength and forward masking is consistent with earlier reports that long-term potentiation of cortical synapses is associated with increased paired-pulse depression. Enrichment also increased response synchronization at slow rates and decreased synchronization at fast rates. Paired-pulse depression increased within days of environmental enrichment and was restored to normal levels after return to standard housing conditions. These results are relevant to several clinical disorders characterized by abnormal gating of sensory information, including autism, schizophrenia, and dyslexia.

## INTRODUCTION

Temporal information is essential for identifying many natural sounds, including animal vocalizations and speech. Auditory cortex is involved in the perception of temporal distinctions (Merzenich et al. 1993). Humans, non-human primates, and rodents exhibit temporal processing deficits after auditory cortex lesions (Clark et al. 2000a,b; Fitch et al. 1994; Harrington et al. 2001; Herman et al. 1997; Mummery et al. 1999).

Sensory enrichment has been suggested as a treatment for temporal processing deficits found in some central auditory processing disorders. Training improves cortical processing of temporal information in humans and animals (Beitel et al. 2003; Hayes et al. 2003; Merzenich et al. 1996; Nagarajan et al. 1998; Recanzone et al. 1992; Tallal et al. 1996; Tremblay et al. 2001; Warrier et al. 2004). Collectively, these results indicate that cortical temporal information processing can be

altered by an intense schedule of exposure to modulated stimuli.

In our previous study, we demonstrated that rats exposed to an enriched environment exhibited improved selectivity, sensitivity, latency, and magnitude of primary auditory cortex responses (Engineer et al. 2004). The experiments reported here document the effects of sensory enrichment on the processing of temporally modulated acoustic stimuli. An earlier study reported that environmental enrichment increased the preferred temporal frequency of cat primary visual cortex neurons (Beaulieu and Cynader 1990). To test whether enrichment would increase or decrease the preferred modulation frequency of auditory cortex neurons, we collected extracellular recordings from anesthetized rats and surface evoked potentials from unanesthetized rats housed in standard and enriched conditions.

## METHODS

All protocols and recording procedures conformed to the Ethical Treatment of Animals (National Institutes of Health) and were approved by the committee on Animal Research at the University of Texas at Dallas.

### *Environmental conditions*

Thirty-five female Sprague-Dawley rats were used in this study. Rats in both housing conditions were on a reverse 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. All the animals used in this study were housed with their mothers and littermates until weaning at 4 wk of age. The environmental conditions are identical to those described in our previous study (Engineer et al. 2004).

The standard housing condition consisted of 1–2 rats per cage (26 × 18 × 18 cm, Fig. 1A). The acoustic environment of this condition included vocalizations from 20 to 30 other rats housed in the same room. In the enriched environment, four to eight rats were housed together in a single large cage in a separate room from the main rat colony (Fig. 1B). Because rats reach sexual maturation between 8 and 12 wk of age, after 1 mo in the enriched environment, a vasectomized male rat was introduced into the cage to encourage natural social interactions.

Animals in the enriched environment were housed in a large cage (76 × 45 × 90 cm) with four levels connected by ramps. Touch plates at the bottom of two ramps triggered different tones (2,100 or 4,000

\* These authors contributed equally to this work.

Address for reprint requests and other correspondence: M. P. Kilgard, Neuroscience Program, School of Behavioral and Brain Sciences, GR 41, University of Texas at Dallas, Richardson, Texas 75083-0688 (E-mail: kilgard@utdallas.edu).

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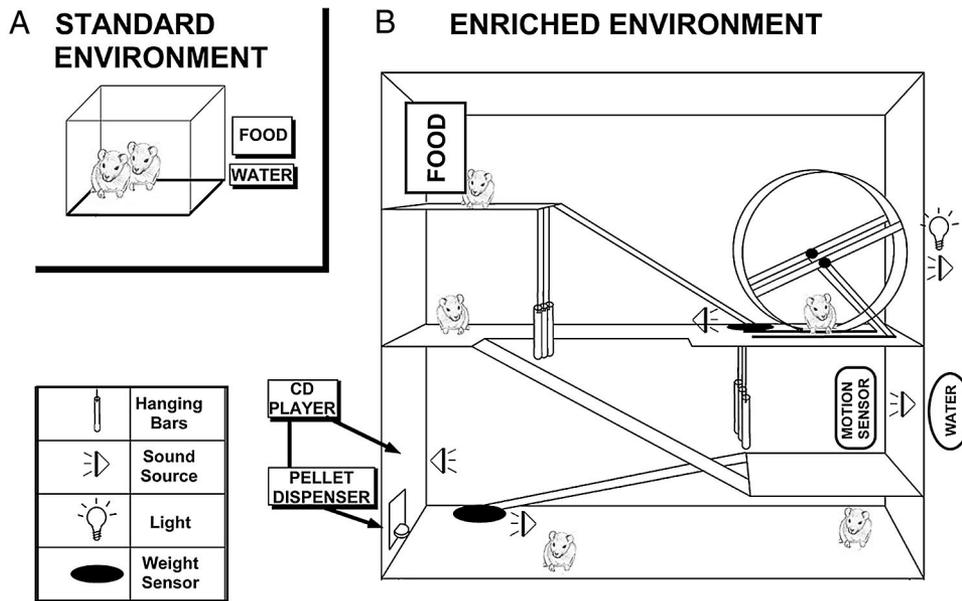


FIG. 1. Schematic of standard and enriched housing conditions. *A*: standard environment consisted of 1 or 2 rats housed in hanging cages within an animal colony room. *B*: enriched environment consisted of 4–8 rats housed in a large cage with devices that generated different sounds when rats crossed a motion detector path, stepped on weight sensors, or passed through hanging bars. In addition, each rotation of the running wheel triggered a brief tone and light flash. 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. While the sounds in the enriched environment were more diverse and behaviorally relevant, rats in both conditions heard approximately the same number of sounds each day.

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 (3,000-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 2–60 s, including simple tones, amplitude- and frequency-modulated tones, noise bursts, and other complex sounds (rat vocalizations, classical music, rustling leaves, etc.). Seven of the 74 sounds activated a pellet dispenser (Med Associates) that delivered a sugary food reward to encourage attention to the sounds. The rewarded tracks included interleaved tones of different carrier frequencies (25-ms-long 4-, 5-, 9-, 12-, 14-, and 19-kHz tones 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 <75 dB SPL. The sound sources added to the enriched environment were provided 24 h/day and were designed to be more diverse and to provide more behaviorally relevant information than sounds in the standard condition. The average interval between the components of complex sounds in the enriched environment was significantly shorter compared with the standard environment [ $108 \pm 73$  vs.  $133 \pm 80$  (SD) ms,  $P < 0.001$ ]. 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 total number of sounds (>50 dB SPL) was not significantly different between the enriched and standard environments  $23 \pm 23$  vs.  $19 \pm 15$  (SD) sound onsets/min.

Although it is possible that the enriched environment was mildly stressful, we observed no evidence of distress in any of the rats at anytime (i.e., excessive licking, hair loss, fighting, etc.). Rats actively explored the environment, voluntarily activated sound sources, and engaged in playful behaviors. Additionally, our behavioral observations and electroencephalographic (EEG) data indicate that enrichment did not interfere with normal sleep-wake behavior.

Experiment 1

EXTRACELLULAR RECORDINGS. Thirty-day-old female Sprague-Dawley rats were randomly assigned to either the enriched environment ( $n = 8$ ) or the standard condition ( $n = 6$ ). The enriched rats were raised four to five per cage (in 2 sessions). Rats in the standard condition were raised two per cage. Acute microelectrode mapping was performed after 8 wk in each environment (Fig. 2, *A* and *B*). Although acute experiments were interspersed, it should be noted that

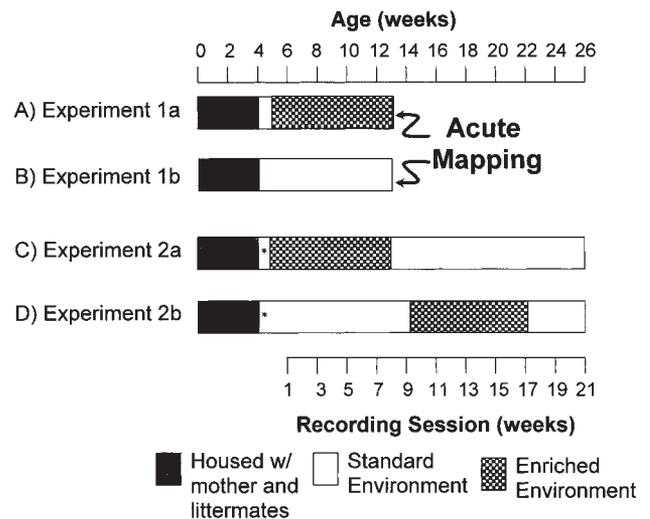


FIG. 2. Experimental time-lines. *A* and *B*: *experiment 1*. Extracellular recordings were compared from rats housed in the enriched environment or the standard condition for 8 wk. *C* and *D*: *experiment 2*. Evoked potentials from auditory cortex were recorded weekly for 5 mo. \*, indicates chronic electrode implantation. In *experiment 2a* (early enriched), rats were housed in the enriched condition for 8 wk (from 4 to 12 wk of age) and then switched to the standard condition and housed singly until 26 wk of age. In *experiment 2b* (late enriched), rats were housed singly in the standard condition for 9 wk (from 4 to 13 wk of age), then switched to the enriched condition for 8 wk, and finally, back to the standard condition until 26 wk of age. Although enriched rats experienced 2 novel housing conditions, we observed no evidence of distress in any of the rats at any time. Rats actively explored the environment, voluntarily activated sound sources, and engaged in playful behavior. A male rat was introduced to the enriched environment after 4 wk of housing to encourage natural, social interactions.

some experimenters were not blind to the identity of the animal because of the unkempt state of the rats' fur typical of enriched animals. However, the sampling density and depth of recordings made in enriched and standard housed rats were indistinguishable, and all analysis was performed blind to housing condition.

**ACUTE SURGERY.** Surgical anesthesia was induced with pentobarbital sodium (50 mg/kg ip). A state of areflexia was maintained throughout the surgery and recording phases with supplemental doses of dilute pentobarbital (0.2–0.5 ml; 8 mg/ml ip). The interval between supplements varied depending on the anesthetic state of the animal but was typically every 1–1.5 h. Anesthesia depth was evaluated by heart rate, breathing rate, corneal reflexes, and response to toe pinch. These indicators were indistinguishable between the two groups. Circulatory function was monitored with electrocardiograph (EKG) and pulse oximetry. Fluid balance was maintained with a 1:1 mixture of 5% dextrose and Ringer lactate (~0.5 ml/h). Body temperature was maintained at 37°C. The trachea was cannulated to ensure adequate ventilation and minimize breathing sounds. Humidified air was delivered to the open end of the cannula. After the cisterna magna was drained to minimize cerebral edema, the right auditory cortex was exposed and the dura removed. Because previous studies implicated the importance of the right hemisphere in processing temporally modulated sounds, this hemisphere was selected for recording responses to complex tone trains (Wetzel et al. 1998).

The cortex was maintained under a layer of viscous silicon oil to prevent desiccation during the 24- to 30-h experiment. Penetration locations were referenced using cortical vasculature as landmarks. A detailed map of auditory cortex was generated from 50 to 100 microelectrode penetrations. A pair of parylene-coated tungsten microelectrodes (FHC, 250- $\mu$ m separation, 2 M $\Omega$ ) was lowered 550  $\mu$ m below the pial surface (layer 4/5) of the auditory cortex. Action potentials from a small cluster of neurons were collected at each penetration site.

Action potentials were recorded simultaneously from two tungsten microelectrodes. The neural signals were filtered (0.3–15 kHz) and amplified (10,000 times). As in our earlier experiment, potentials above ~0.18 mV were considered to be action potentials (Pandya et al. 2005). The borders of A1 were defined based on continuous topography of characteristic frequency (CF) and short response latency. Sites with high thresholds, long latencies, broad tuning, and discontinuities in CF topography were considered non-A1 and excluded from further analysis (Kilgard et al. 2001). Criteria for identifying nonA1 sites were subjectively applied by well-trained blind observers.

**STIMULUS PRESENTATION AND DATA ANALYSIS.** Sounds were delivered in a shielded double-walled sound-attenuated chamber via a speaker (Motorola model No. 40–1221) positioned directly opposite the contralateral (left) ear at a distance of 10 cm. Frequency and intensity calibrations were performed with an ACO Pacific microphone (PS9200-7016) and Tucker-Davis SigCal software. After collecting frequency-intensity tuning curves at each site, Brainware (Tucker-Davis Technologies) presented tones at 12 repetitions of 14 modulation rates (3–20 Hz) and noise bursts at four repetition rates (5, 10, 15, and 20 Hz). The tone frequency was selected to generate the strongest response at each recording site. A 2-s silent period separated each randomly interleaved train. All stimuli were presented at 70 dB SPL and were 25 ms long with 3-ms rise and fall times.

Temporal processing was quantified using three different measures. Tone and noise burst repetition-rate transfer functions (RRTF) were derived at each site by quantifying action potentials per stimulus. Action potentials per stimulus was simply the average response occurring between 8 and 38 ms after the second, third, fourth, fifth, and sixth sounds in each train minus the spontaneous rate. The responses to tone and noise burst trains were also quantified using vector strength and Rayleigh statistic measures (Liang et al. 2002).

Vector strength (VS) quantifies the degree of synchronization between action potentials and repeated tones pips, and is calculated with the formula

$$VS = \frac{1}{n} \sqrt{x^2 + y^2}; x = \sum_{i=1}^n \cos \theta_i; y = \sum_{i=1}^n \sin \theta_i; \theta_i = 2\pi \frac{t_i}{T}$$

where  $n$  = total number of action potentials,  $t_i$  is the time of occurrence of the  $i$ th action potential, and  $T$  is the interstimulus interval. A value of 1 indicates perfect synchronization and 0 indicates no synchronization. Rayleigh statistic ( $2nVS^2$ , where  $n$  is the total number of action potentials) is a circular statistic that essentially combines the previous two measures (action potentials and vector strength) to assess the statistical significance of the vector strength (Mardia and Jupp 2000). Values  $>13.8$  ( $P < 0.001$ ) are considered significant (Liang and Wang 2002). The number of evoked action potentials, vector strength, and Rayleigh statistic at each repetition rate and the best rate for each measure were compared across groups using unpaired two-tailed  $t$ -test.

### Experiment 2—evoked potential recordings

**EXPERIMENTAL TIME COURSE.** Evoked potentials were recorded each week from an electrode implanted over left auditory cortex. Twenty-one female Sprague-Dawley rats were randomly assigned to one of two groups. Rats in *experiment 2a* ( $n = 12$ ) were housed in the enriched environment for 8 wk (4–12 wk of age) and then moved to the standard condition and housed singly until 26 wk of age (Fig. 2C). Rats in *experiment 2b* ( $n = 9$ ) were housed singly in the standard condition for 9 wk (4–13 wk of age), then moved to the enriched environment for 8 wk, and then back to the standard condition until 26 wk of age (Fig. 2D). In this series of experiments, four to eight rats were housed in the enriched environment at any given time.

**CHRONIC IMPLANTATION.** Rats were chronically implanted at 28 days of age with a ball electrode over A1 and a ground screw over the cerebellum. Surgical anesthesia was induced with pentobarbital sodium (50 mg/kg ip). A state of areflexia was maintained throughout the surgery, and supplemental doses of dilute pentobarbital were administered subcutaneously if needed (0.2 ml; 8 mg/ml). Anesthesia level was monitored by response to toe pinch. Atropine (1 mg/kg) and dexamethasone (4 mg/kg) were administered subcutaneously to minimize lung secretions and brain edema during the procedure and recovery. Animals received antibiotic injections (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.

Although some implants remained firmly in place for 5 mo, many implants were lost due to skull growth after implantation. Implanted rats were housed singly when not in the enriched environment to minimize the tendency of rats in small cages to damage implants by grooming excessively.

**STIMULUS PRESENTATION.** Middle-latency-evoked potential data were collected once each week for 21 wk from each rat in a sound-attenuated booth. Recordings were made during the dark cycle in both 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 level were noted between enriched and standard rats during recording sessions.

Acoustic stimuli included pairs of 25-ms, 9-kHz tones with interstimulus intervals of 500, 200, 100, or 50 ms. Tones pairs were presented from a speaker centered above the cage and randomly interleaved every 10 s. The stimuli used for the awake recordings corresponds to the 2-, 5-, 10-, and 20-Hz intervals used in *experiment*

1. All stimuli were 70 dB SPL with 3-ms rise and fall times. Signals were low-passed filtered (800 Hz), amplified (10,000 times), and displayed on an oscilloscope for monitoring. Data-acquisition computers collected cortical responses to 125 tone pair presentations. Trials with excessive motion artifacts ( $>0.1$  mV) were discarded prior to analysis of the mean evoked potential.

### Data analysis

The first negative peak in the evoked response is referred to as N1 ( $\sim 30$  ms). The second negative peak is N2 ( $\sim 140$  ms). The first positive peak is referred to as P1 ( $\sim 75$  ms; Fig. 6A). Only the eight rats (of the 21 implanted) that maintained their implants for the duration of the 5-mo study were included in this analysis. 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 two 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 (Fig. 7D). Response strength was quantified as the root-mean-square of the evoked potential 10–175 ms after each tone onset (N1–P1 complex). Paired-pulse depression was quantified as the response to the second tone divided by the response to the first. Paired *t*-test were used to determine whether differences in evoked potential response strength and paired-pulse depression were statistically significant ( $\alpha = 0.01$ ).

## RESULTS

Neurophysiologic responses were recorded from rats housed either in standard laboratory conditions or in an enriched environment. Action potentials were recorded from small groups of A1 neurons at  $>700$  sites in 14 rats. Twenty-one rats were implanted with EEG electrodes, and evoked responses were recorded from auditory cortex each week for  $\leq 5$  mo. Although similar environmental plasticity was observed in all the rats, only the eight rats that maintained their implants for the duration of the 21-wk study were included in the analysis reported in the following text.

### Experiment 1

**EXTRACELLULAR RECORDINGS: REPETITION RATE TRANSFER FUNCTIONS.** Responses from animals housed in enriched ( $n = 8$  rats; 462 sites) and standard ( $n = 6$  rats; 263 sites) conditions indicate that environment substantially altered A1 responses to modulated noise bursts and tones that had no special significance in the enriched environment.

A representative example of a dot raster plot from a single site is shown in Fig. 3, *A* and *B*. Each point represents an action potential. *A* shows responses to tone trains, *B* shows responses to noise burst trains. The number of action potentials evoked per sound declines rapidly at repetition rates  $>12$  Hz (Fig. 3C).

The mean RRTF from enriched and standard rats is shown in Fig. 4A. At slower rates ( $<8$  Hz), A1 neurons from enriched rats responded with significantly more action potentials per tone compared with naïve rats. At faster rates however, A1 neurons from enriched rats responded with significantly fewer action potentials per tone. The rate that evoked the maximum

number of action potentials per tone at each site (best modulation rate) was significantly decreased in enriched rats compared with standard rats ( $6.3 \pm 0.2$  vs.  $7.5 \pm 0.3$  Hz,  $P < 0.001$ ). The limiting rate (defined as the highest rate at which the response was 50% of the response to the best modulation rate) was also significantly lower in enriched rats ( $10.4 \pm 0.2$  vs.  $12.3 \pm 0.2$  Hz,  $P < 0.000001$ ).

The degree of synchronization between tone trains and action potentials was quantified with vector strength and the Rayleigh statistic (see METHODS). Enrichment significantly decreases the degree of phase locking at high repetition rates and increases it at low rates (Fig. 4B). The rate at which maximum phase locking occurred was lower in enriched rats compared with standard rats ( $8.92 \pm 0.14$  vs.  $10.66 \pm 0.23$ ,  $P < 0.000001$ ). The average rate that resulted in the highest Rayleigh statistic for each site was also lower ( $7.6 \pm 0.1$  and  $8.9 \pm 0.2$ ,  $P < 0.000001$ ). Both groups showed significant phase locking at all rates (Fig. 4C). The average vector strength at the best modulation rate for each site was not significantly different between the two groups ( $0.88 \pm 0.01$  vs.  $0.88 \pm 0.01$ ,  $P > 0.05$ ). The observation that enriched rats exhibited increased response strength and synchronization at slow rates but decreased response strength and synchronization at fast rates indicates that enrichment decreased the preferred modulation rates of A1 neurons.

A1 neurons from enriched rats also responded with more action potentials to noise bursts when presented at rates  $<10$  Hz (Fig. 5). Once again, the average vector strength at the best rate for each site was not significantly different between the two groups ( $0.86 \pm 0.01$  vs.  $0.87 \pm 0.01$ ,  $P > 0.05$ ).

### Experiment 2

**EVOKED POTENTIAL RECORDINGS: TONE-EVOKED RESPONSES.** To determine if enrichment also increases response strength and paired-pulse depression in unanesthetized rats and whether or not this increase is reversible, we recorded auditory evoked potentials weekly during periods of standard and enriched housing. We previously reported that the grand mean average cortical evoked potential increased significantly during enrichment and reversed within a week when rats were returned to standard housing conditions. Chronic recordings from unanesthetized rats indicate that paired-pulse depression is also increased during periods of enrichment and returns to normal levels during standard housing (Fig. 6, *A* and *B*).

To improve the signal-to-noise ratio, we compared the average of each individual's mean evoked potential for all presentations during the standard housing condition, including before and after enrichment, with all presentations during the enriched housing condition. The amplitudes of the N1, P1, and N2 peaks in the grand mean average response to the first tone during enrichment were 211, 243, and 167% of their amplitude during standard housing (Fig. 7A). The amplitudes of each peak in response to a second tone presented 500 ms later were 163, 182, and 167% of their amplitude during standard housing (Fig. 7A). These results suggest that enrichment causes greater paired-pulse depression by increasing the N1 and P1 responses of the first tone compared with the second tone.

The surface potential in response to the second of two tones separated by 500 ms was smaller than the first when rats were housed in the standard and the enriched conditions. The peak-

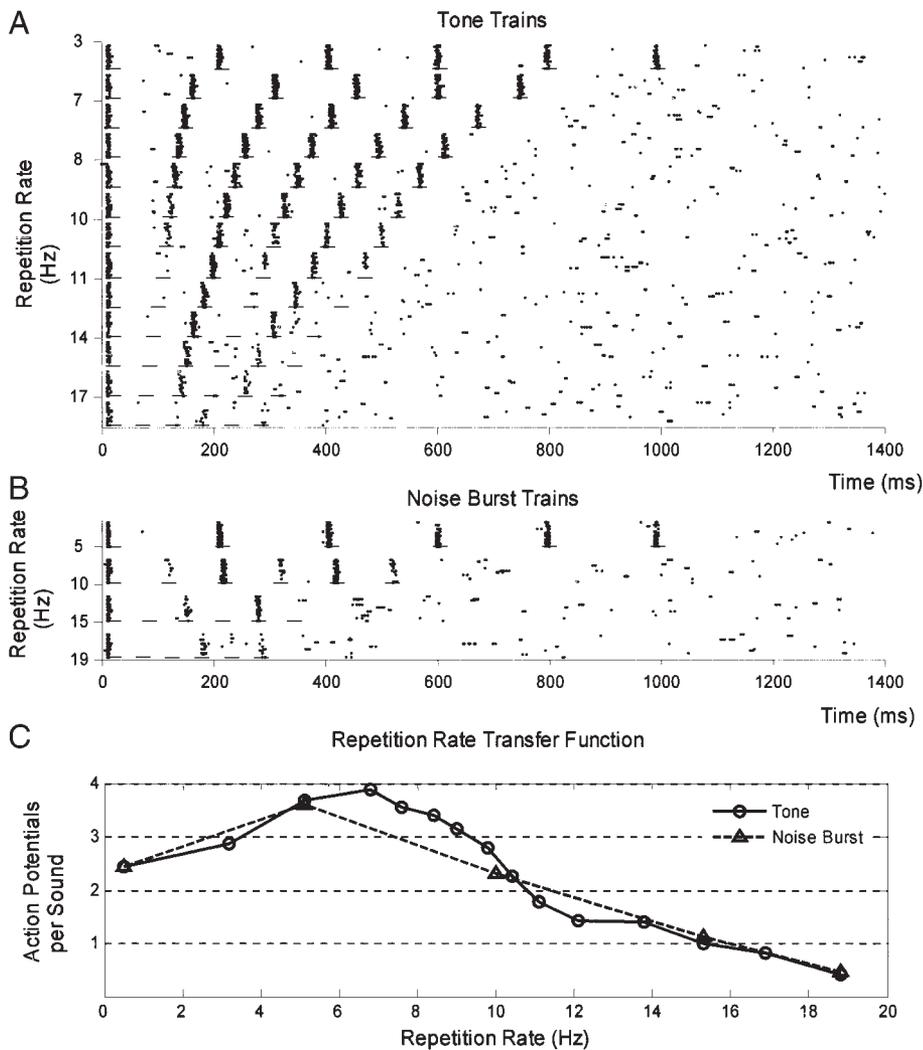


FIG. 3. Responses of a single site in an enriched rat to repeated tone and noise burst trains. *A* and *B*: in the spike dot-raster plots, action potential times are indicated for each of the 12 repetitions of each train. Tones were presented at 14 different repetition rates (3–19 Hz), and noise burst trains were presented at 4 rates (5–19 Hz). Horizontal lines indicate sound onset in panels *A* and *B*. The tone frequency of 19 kHz was selected because it was close to the site's best frequency of 23 kHz. *C*: repetition rate transfer functions (RRTF) for tones (—) and noise burst trains (- - -) for the same sites shown in *A* and *B*.

to-peak amplitude of the response to the second tone was reduced by 11% during standard housing and by 37% during enrichment (Fig. 7*A*). Because the evoked responses of some rats were different from the grand mean average, we used the root mean square of the N1–P1 complex to quantify the power of the average evoked potential in each individual. Paired-pulse depression was quantified as the ratio of the response to the second tone divided by the response to the first. For the 500-ms inter-stimulus interval, this ratio was  $64 \pm 4\%$  for rats during periods of housing in the enriched environment compared with  $87 \pm 7\%$  for rats during periods of housing in the standard condition ( $P < 0.05$ ; Fig. 9). Collectively, these results indicate that enrichment significantly increases the response to the first tone and significantly enhances the degree of paired-pulse depression of the second tone 500 ms later compared with standard housing.

During enrichment, paired-pulse depression was even greater at interstimulus intervals  $< 500$  ms. Because the response to a single tone lasts 250 ms, the response to the second tone overlapped at shorter interstimulus intervals (Fig. 7, *B–D*). To quantify the response to the second tone, we subtracted the waveform from the response to the first tone alone. This analysis assumes that the two responses summate linearly. The response to the second tone decreased more rapidly as the

interstimulus interval was shortened in enriched compared with standard housed rats. During enrichment, the grand mean average peak-to-peak amplitude evoked by the second of two tones separated by 200, 100, or 50 ms was reduced by 60, 70, or 80%, respectively, compared with the response to the first tone (Fig. 8*A*). In contrast, during standard housing, the response to the second tone was reduced by 35, 24, or 41% (Fig. 8*B*). The root mean square power of the evoked response of individual rats also showed the same pattern. Significant paired-pulse depression was observed at each of these intervals regardless of housing condition ( $P < 0.05$ ); however, there was significantly more paired-pulse depression when rats were housed in an enriched environment compared with standard housing ( $P < 0.01$ ; Fig. 9). During enriched housing, the power of the response to the second tone was reduced by 50% when the interval was decreased from 500 to 50 ms. During standard housing, the response only decreased by 20%. While paired-pulse depression increased as the interstimulus interval decreased when rats were housed in the enriched environment ( $P < 0.01$ ), there was no significant difference in the degree of paired-pulse depression occurring during standard housing. These results indicate that enrichment substantially increases the degree of response suppression caused by preceding sounds.

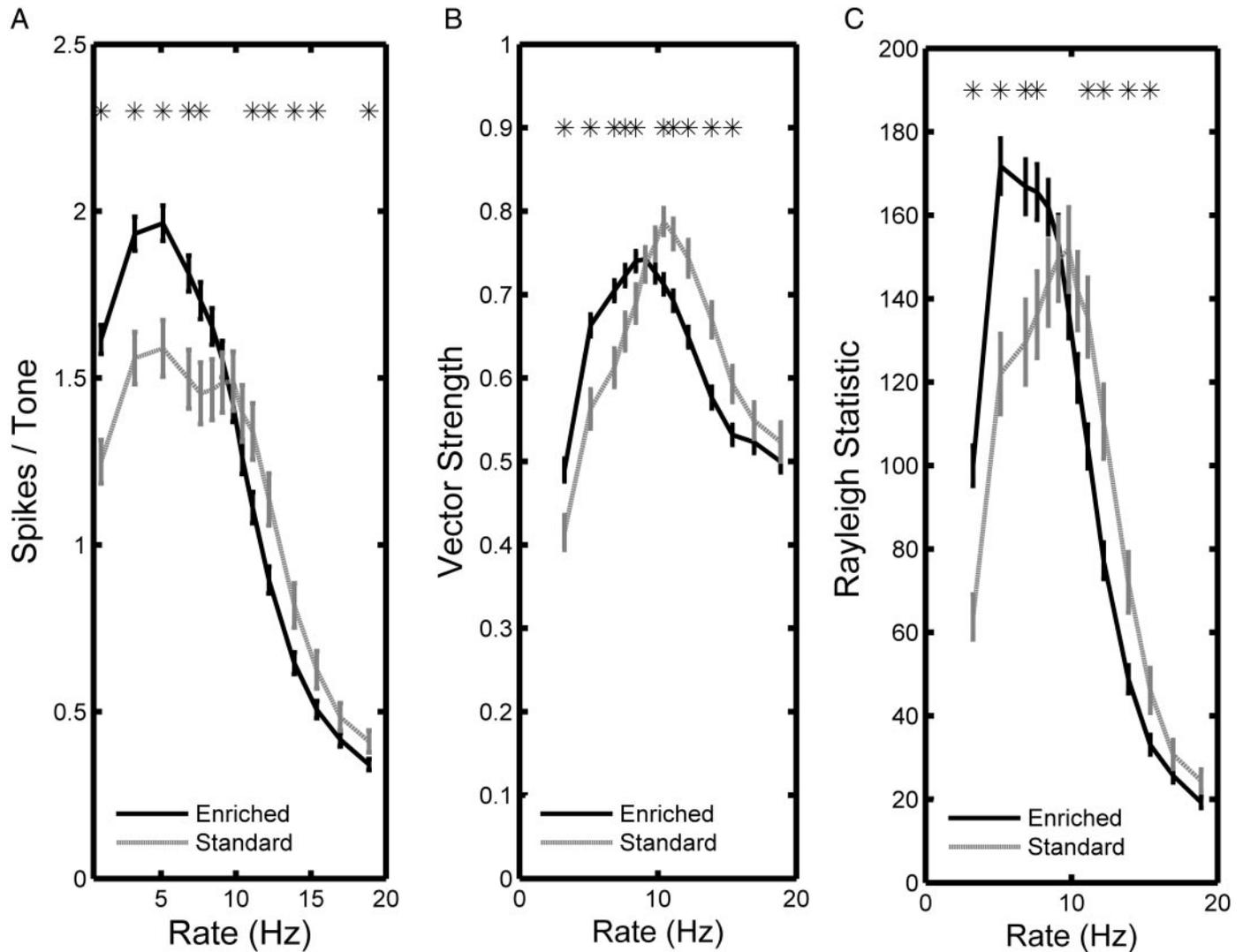


FIG. 4. *A*: average repetition rate transfer functions for tones in enriched (solid black) and standard (dashed gray) rats. The tone frequency was set to the best frequency for each site. *B*: vector strength quantifies the degree of synchronization between action potentials and repeated tone onset. *C*: Rayleigh statistic combines the degree of synchronization with the number of action potentials. These results indicate that compared with standard housed controls, enriched rats exhibited increased response strength and synchronization at slow rates but exhibited decreased response strength and synchronization at fast rates. Significant differences between the groups are indicated with an asterisk ( $P < 0.05$  by 2-tailed, unpaired  $t$ -test). Error bars indicate SE.

DISCUSSION

Our previous study demonstrated that environmental enrichment substantially increases the response of rat auditory cortex neurons to tone and noise bursts stimuli (Engineer et al. 2004). The current study was designed to evaluate the neurophysiologic consequences of environmental enrichment on temporal information processing in the auditory cortex. Action potentials from anesthetized rats were collected in response to tone and noise burst trains of varying repetition rates. Compared with standard housed rats, A1 responses from enriched rats were stronger and more synchronized at rates <10 Hz and weaker and less synchronized at higher rates. Evoked potentials were also recorded from auditory cortex in awake rats to compare the degree of paired-pulse depression during housing in standard and enriched conditions. Paired-pulse depression increased while rats were housed in the enriched environment and returned to normal levels within days of transfer back to standard housing. Collectively, these studies demonstrate that

environment can substantially alter temporal processing in the auditory cortex of anesthetized and awake rats.

Temporal processing

Neurons in the visual, auditory, and somatosensory cortex fail to respond at high modulation rates. Repetition rate transfer functions in the primary auditory cortex of naïve monkeys, cats, and rats are predominantly low- or band-pass (Bao et al. 2004; Beitel et al. 2003; Eggermont 1999; Gaese and Ostwald 1995; Kilgard and Merzenich 1998). Neurons with shorter latencies typically exhibit faster recovery from forward masking and higher best repetition rates than neurons with longer latencies (Brosch and Schreiner 1997; Kilgard and Merzenich 1999; Schreiner et al. 1997). Our observation that enrichment increases time to peak and decreases best repetition rate of A1 neurons is consistent with this correlation. The enrichment-induced decrease in the maximum following rate and increased paired-pulse depression is not due to a greater number of

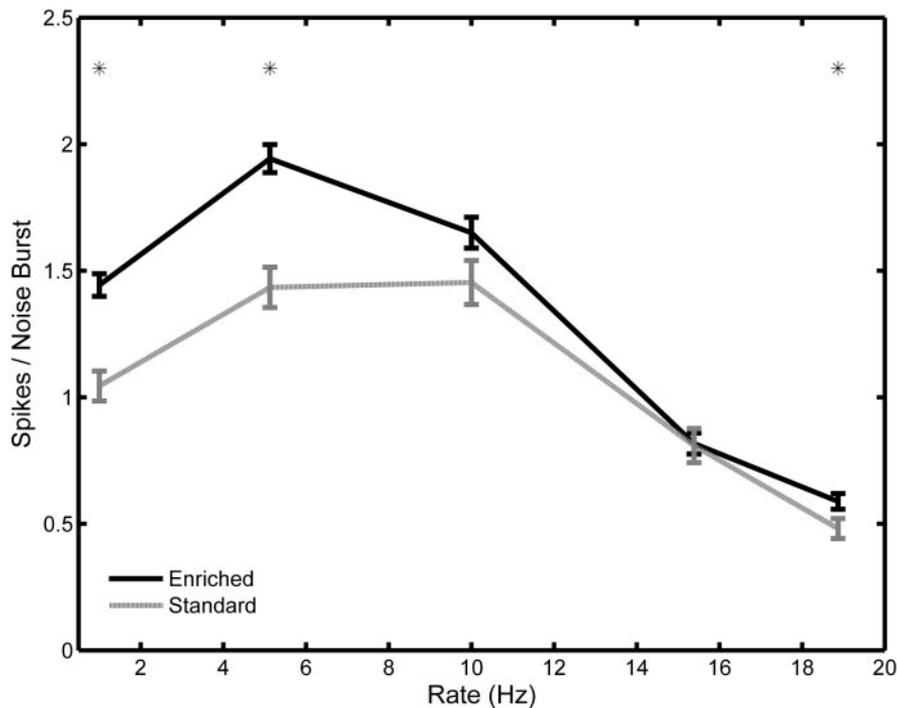


FIG. 5. Average repetition rate transfer functions for noise burst trains in enriched (solid black) and standard (dashed gray) rats. At slow rates (<10 Hz), the number of action potentials evoked per noise burst was greater for enriched rats compared with standard housed rats. Significant differences between the groups are indicated with an asterisk ( $P < 0.05$  by 2-tailed, unpaired  $t$ -test). Error bars indicate SE.

sounds with slow modulation rates in the enriched environment compared with the standard environment. In fact, the average modulation rate in the enriched environment was higher due to the greater complexity of the sound sources (music, chimes, etc).

One potential consequence of enrichment may be more accurate perceptual judgments to sounds with modulation rates <10 Hz, given the greater response strength and synchroniza-

tion to these sounds. However, the greater paired-pulse depression observed in enriched rats may impair their ability to detect stimuli in a forward masking protocol. Detailed psychophysical experiments will be needed to fully evaluate the perceptual consequences of environmental enrichment. In any case, enriched housing provides sensory stimulation and social interactions that are common in natural environments, and it is likely that the greater paired-pulse depression observed in

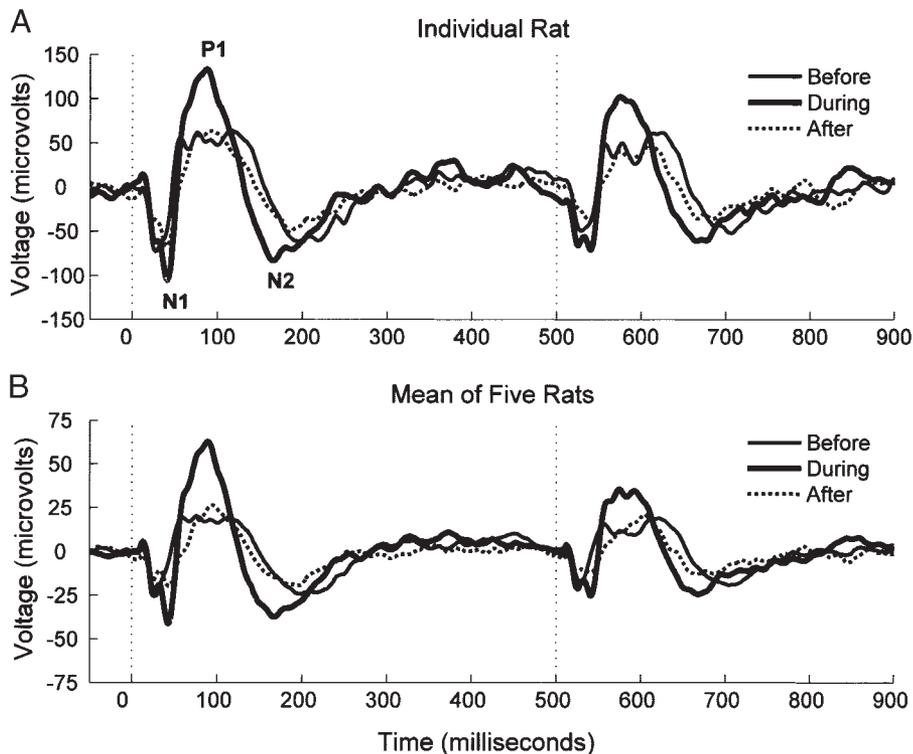


FIG. 6. *A*: mean auditory evoked potentials from an example rat before, during, and after enrichment in response to 2 70-dB 9-kHz tones separated by 500 ms. *B*: grand mean average auditory evoked potentials from 5 rats before, during, and after enrichment (*experiment 2b*). These results indicate that response amplitude and paired-pulse depression are increased during periods of enrichment and return to normal levels during standard housing.

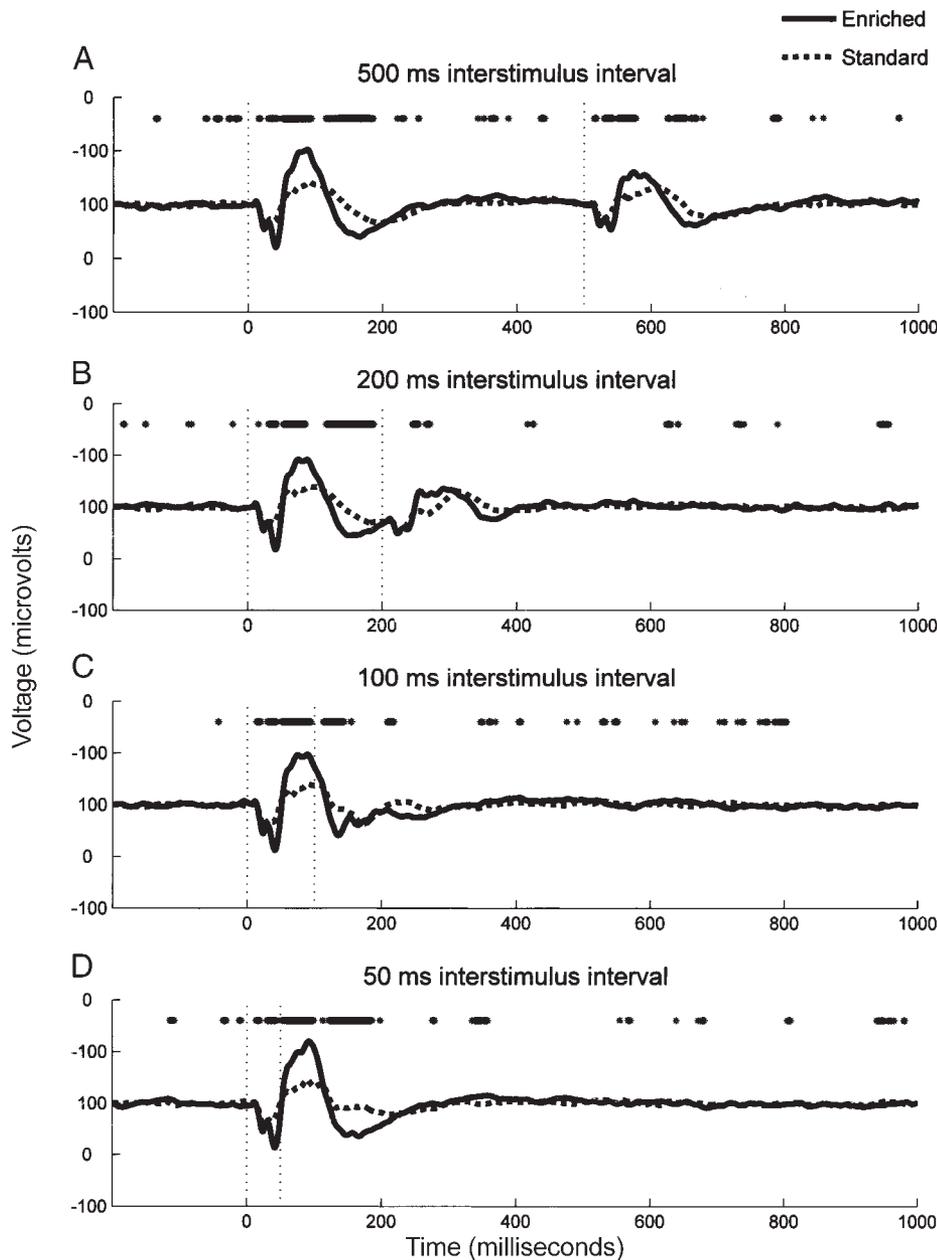


FIG. 7. Grand mean average responses of enriched (solid) and standard (dashed) rats to 2 9-kHz tones separated by 500-, 200-, 100-, and 50-ms intervals. The times during which the enriched and standard potentials are significantly different from each other are indicated with dots (paired 2-tailed  $t$ -test  $P < 0.01$ ). These results indicate that paired-pulse depression increases more rapidly as the interval is decreased during enriched compared with standard housing.

enriched rats more accurately reflects typical cortical temporal information processing compared with results from animals housed in standard laboratory conditions.

*Potential mechanisms*

Plasticity of synaptic, cellular, or network properties may contribute to the changes in temporal processing induced by environmental conditions (Gilbert 1998; Katz and Shatz 1996; Syka 2002). Two distinct types of excitatory synapses have been described in layer II/III auditory cortex neurons of standard housed rats (Atzori et al. 2001). Two of three have weak connection strengths with a low probability of release, while the remaining synapses have strong connection strengths and a high probability of release. The strong synapses exhibited substantially more paired-pulse depression than the weak syn-

apses, presumably due to faster depletion of the synaptic vesicle pool. Earlier studies have shown that long-term potentiation of cortical synapses is often accompanied by higher probability of release and increased paired-pulse depression (Markram and Tsodyks 1996). If enrichment increases the proportion of synapses with a high release probability, both our earlier observation of increased response strength and our current observation of increased response depression could be explained by a single mechanism.

Enrichment could also strengthen paired-pulse depression by increasing long-lasting inhibition. However, in cat visual cortex enrichment decreases, rather than increases, the number of inhibitory synapses and increases, rather than decreases, the ability of neurons to follow rapid stimuli (Beaulieu and Colonnier 1987; Beaulieu and Cynader 1990). Although enrichment resulted in increased responsiveness and selectivity sim-

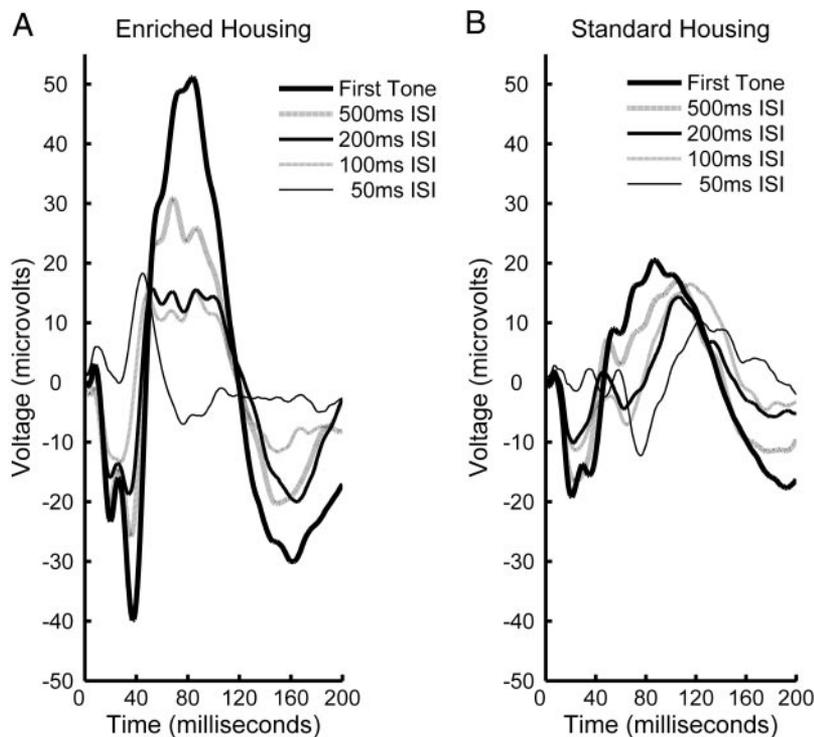


FIG. 8. Overlays of the mean response evoked by each tone when presented in isolation or 500 to 50 ms after another tone. For the 50-, 100-, and 200-ms intervals, the responses of each rat were subtracted from their response to a single tone (derived from the 1st pulse of the 500-ms tone pair). These difference functions make it easier to see that paired-pulse depression increases more rapidly as the interval is decreased during enriched compared with standard housing.

ilar to that seen in rat auditory cortex, the opposite effects on temporal processing suggest different synaptic mechanisms may be involved.

Recent *in vivo* findings indicate that at interstimulus intervals  $>150$ -ms synaptic depression, rather than inhibition, is the primary mechanism responsible for paired-pulse depression in rat auditory cortex (Tan et al. 2004; Wehr and Zador 2003, 2005). Although it is possible that enrichment could strengthen paired-pulse depression by increasing long-lasting inhibition (or even the amplitude of postresponse hyperpolarization or oscillatory conductance periodicity), each of these explanations would also require an increase in excitatory drive to explain the stronger response to isolated sounds (D'Angelo et al. 2001; Gaese and Ostwald 1995; He 2003).

The increased paired-pulse depression observed in enriched rats is likely to be a consequence of the increased response magnitude to the first tone in a pair. Several experiments have shown that greater stimulus intensity causes greater paired-pulse depression. Increasing tone amplitude typically increased the duration of forward masking (Brosch and Schreiner 1997). In addition, we observed more paired-pulse depression of evoked potentials with louder (90 dB) tones compared with quieter (50 dB) tones in standard housed rats (unpublished data). These results suggest enrichment has the same effect on response strength and paired-pulse depression as increasing stimulus intensity.

Although numerous cellular mechanisms are possible, increased probability of synaptic release is the most likely explanation because a single mechanism (well documented with *in vitro* studies of auditory cortex) would explain both the increased response strength and decreased temporal after rate observed in enriched rat auditory cortex. Clearly, further studies are needed to characterize the cellular and molecular basis of enrichment-induced plasticity.

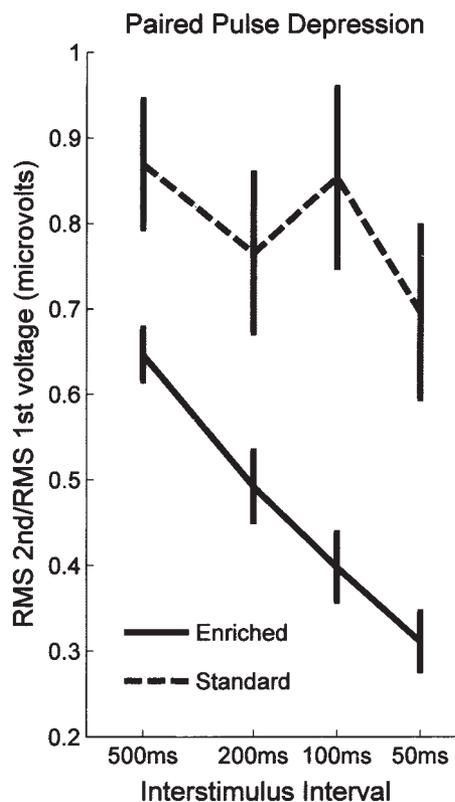


FIG. 9. Average paired-pulse depression ratios for each group as a function of interstimulus interval. The paired-pulse depression ratio was quantified as the response to the 2nd tone divided by the response to the 1st, where response strength was quantified as the root-mean-square of the evoked potential 10–175 ms after each tone onset (N1–P1 complex). Error bars indicate SE. Evoked potentials exhibited significant paired-pulse depression in both groups at all rates tested. Paired-pulse depression was significantly greater during enrichment, compared to standard housing, at all rates tested. Paired-pulse depression was significantly greater at 50 ms compared with 500 ms during enrichment but not during standard housing.

Finally, because both response strength and paired-pulse depression increase during the transition from an alert state to a quiet awake state, it is possible that enriched rats spend more time in the quiet awake state than standard housed rats (Castro-Alamancos 2004). However, because the enrichment-induced increases in response strength and paired-pulse depression are maintained under general anesthesia, we believe that differences in global state are unlikely to explain our observations.

*Technical considerations*

Both standard and enriched rats have significant paired-pulse depression to subsequent auditory stimuli. However, the degree of paired-pulse depression was different depending on the method of data recording. For surface potentials recorded in awake rats, the time required for the amplitude of the second response to recover to 2/3 of the first response was 10 times longer in enriched compared with standard housed rats (500 vs. 50 ms). In contrast, for action potentials recorded under anesthesia, the time required for the response to the second tone to recover to 2/3 of the first was only 17% longer (83 vs. 71 ms). Three factors could be responsible for the longer time course of paired-pulse depression for evoked potentials compared with action potentials. First, anesthesia may eliminate possible neuromodulatory differences that could be engaged in awake rats during periods of differential housing. Second, nonprimary auditory regions, which likely contribute to evoked potentials, are not included in our microelectrode data. Nonprimary auditory cortex neurons are known to exhibit greater paired-pulse depression (Eggermont and Ponton 2002) and may be more sensitive to environmental conditions. Third, evoked potentials are generated by summed synaptic potentials, which appear to be potentiated during enrichment. Recordings of action potentials would show smaller differences due to the threshold-based mechanism of action potential generation. Further studies will be needed to determine which of these differences is responsible for the greater scale of environmental plasticity observed with evoked potentials.

Multiple factors influence the degree of environmental-induced plasticity including physical activity, social experience, and behavioral relevance of sensory events (van Praag et al. 2000). Social interactions significantly increase brain weight (Ferchmin and Bennett 1975), and wheel running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus (van Praag et al. 1999). Additional experiments are ongoing to evaluate which specific factors contribute to the physiologic changes observed in this study.

*Clinical relevance*

Several clinical populations exhibit abnormal cortical responses to rapidly presented acoustic signals. Unmedicated patients with schizophrenia exhibit weak responses and less paired-pulse depression compared with normal controls, possibly reflecting their inability to filter incoming sensory stimuli and reported sensations of being “overwhelmed” or “flooded” by sensory stimulation (Braff and Geyer 1990; Erwin et al. 1991; Siegel et al. 1984). Paired-pulse depression is restored to normal when patients are treated with the antipsychotic clozapine (Adler et al. 2004). Individuals with autism also exhibit

reduced responses to sensory stimulation that fail to adapt at increased repetition rates (Buchwald et al. 1992). These differences may contribute to abnormal gating of sensory information, abnormal arousal levels, and inattentiveness characteristic of the autistic population.

In contrast, individuals with dyslexia exhibit too much paired-pulse depression. The response to the first acoustic stimulus is stronger, but the response to second and subsequent stimuli is significantly weaker compared with controls (Nagarajan et al. 1999). These differences were especially pronounced at short interstimulus intervals, which is consistent with observations that dyslexic individuals have substantial difficulties processing the rapid spectrotemporal transitions present in language. Although the perceptual consequences of environmental enrichment are not well documented, our observation that environment can significantly influence paired-pulse depression supports earlier hypotheses that focused, intensive sensory enrichment may alter sensory gating in patients with schizophrenia, autism, or dyslexia (Baranek 2002; Gunatilake and Silva 2004; Tallal et al. 1998).

*Conclusion*

In summary, enrichment strengthens the response of primary auditory cortex neurons to isolated sounds but increases the degree of paired-pulse depression. Both forms of plasticity could be explained if enrichment increases the probability of synaptic release, although several other mechanisms are possible, and may act in concert. A better understanding of the cellular basis for enrichment-induced paired-pulse depression would be helpful in designing effective treatments for neurological disorders characterized by temporal processing abnormalities.

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