Pulsed Noise Experience Disrupts Complex Sound Representations

Michele N. Insanally, Badr F. Albanna, and Shaowen Bao
Helen Wills Neuroscience Institute, University of California, Berkeley, California

Submitted 24 September 2009; accepted in final form 25 February 2010

Insanally MN, Albanna BF, Bao S. Pulsed noise experience disrupts complex sound representations. J Neurophysiol 103: 2611–2617, 2010. First published March 3, 2010; doi:10.1152/jn.00872.2009. Cortical sound representations are adapted to the acoustic environment. Early exposure to exponential frequency-modulated (FM) sweeps results in more neurons selective to the experienced sounds. Here we examined the influence of pulsed noise experience on the development of sound representations in the primary auditory cortex (AI) of the rat. In naive animals, FM sweep direction selectivity depends on the characteristic frequency (CF) of the neuron—low CF neurons tend to select for upward sweeps and high CF neurons for downward sweeps. Such a CF dependence was not observed in animals that had received weeklong exposure to pulsed noise in periods from postnatal day 8 (P8) to P15 or from P24 to P39. In addition, AI tonotopicity, tuning bandwidth, intensity threshold, tone-responsiveness, and sweep response magnitude were differentially affected by the noise experience depending on the exposure time windows. These results are consistent with previous findings of feature-dependent multiple sensitive periods. The different effects induced here by pulsed noise and previously by FM sweeps further indicate that plasticity in cortical complex sound representations is specific to the sensory input.

INTRODUCTION

Early experience has a significant influence on complex sound processing at both the behavioral and neuronal representational levels. Juvenile songbirds must hear tutor song early in life to accurately reproduce the learned song later in development (Marler and Peters 1982). Experience-dependent plasticity has been shown for the learning of the zebra finches’ own song (Doupe and Solis 1997; Volman 1993). Electrophysiological studies show that neurons in the auditory forebrain of songbirds are selective for natural sounds (Grace et al. 2003; Margoliash 1986; Müller and Leppelsack 1985; Woolley et al. 2005), and such selectivity develops gradually (Amin et al. 2007). In addition to shaping perceptual behaviors, early experience also alters sensory neuronal representations (Brainard and Knudsen 1993; de Villers-Sidani et al. 2007, 2008; Fugiolini et al. 1994; Han et al. 2007; Kim and Bao 2009; Merzenich et al. 1984; Polley et al. 2004; Simons and Land 1987; Takahashi et al. 2006; Van der Loos and Woolsey 1973; Wiesel and Hubel 1963; Zhang et al. 2001). In mammals, it has recently been shown that the maintenance and refinement of FM sweep selectivity are experience dependent (Razak et al. 2008). The development of complex sound features emerges in a series of sensitive periods within a month-long critical period window. Early exposure to frequency-modulated (FM) sweeps alters frequency and bandwidth representations, and later exposure changes FM sweep selectivity (Insanally et al. 2009). This study did not, however, rule out the possibility that the observed effects were mainly caused by the broadband nature of the selected FM stimulus and not specific to the features of the stimulus. Studying the effect of exposure to other broadband sounds on complex sound development is necessary to address this problem.

Broadband white noise is a stimulus that has been widely used to study critical period plasticity. For example, early exposure to continuous environmental noise has been shown to prolong critical period duration (Chang and Merzenich 2003). Additionally, rearing rats during the first month of life in pulsed white noise disrupts tonotopicity, degrades frequency tuning, and reduces temporal correlation (Grecova et al. 2009; Zhang et al. 2002). It has also been shown that critical period closure in the auditory cortex (AI) is modulated by sound input dynamics; exposing animals to spectral band-notched noise results in critical period closure for the portion of AI representing those absent frequencies while exposing animals to band-pass noise kept the critical period open for those frequencies (de Villers-Sidani et al. 2007). These studies indicate that broadband noise experience alters acoustic representations and influences critical period dynamics. Based on early findings of multiple sensitive periods for different acoustic features, one would hypothesize that restricting broadband noise exposure to early versus late developmental time windows would differentially impact rudimentary versus complex feature representations, respectively.

Here, we studied the effects of pulsed white noise exposure in different developmental time windows on the feature selectivity that occurs during each sensitive period described in an earlier study (Insanally et al. 2009). Our results indicate that cortical map tonotopicity and multiple response parameters are differentially altered by pulsed noise experience depending on the exposure windows. These results are consistent with pre-
vious findings of multiple sensitive periods in the AI. The differences between the effects of noise exposure and those induced by FM sweeps indicate that experience-dependent complex sound representations are adaptive to the specific experienced stimulus.

**Methods**

**Acoustic rearing**

The UC Berkeley Animal Care and Use Committee approved all procedures used in this study. Litters of rat pups (female, Sprague-Dawley) and their mothers were placed in a sound-attenuation chamber for a brief period, comprising one of four time windows (P8–P15, P16–P23, P24–P31, and P32–P39). Pulsed trains of uniform white noise were generated with a LabView program and I/O card and played through a Vifa speaker (XT25TG30-04) placed in the sound attenuation chamber 24 h/d. The peak sound pressure level was measured with a BK microphone and conditioning amplifier over a 5-ms window and in the 1–32 kHz range and was set at 65 dB SPL. Figure 1 shows the spectral density of the noise. Each noise pulse was 50-ms long with 5-ms on- and off-ramps. Each noise pulse train consisted of six noise pulses played at 6 Hz. Trains were separated by 500 ms quiet periods. In addition, the acoustic environment may have also contained vocalization sounds produced by the animals, mainly in a frequency range >32 kHz. After the exposure period, the animals were returned to normal animal rooms.

**Electrophysiological recording**

The AI of pulsed noise-exposed and naïve animals (4 animals for each group) were mapped under pentobarbital anesthesia (50 mg/kg for induction, 10–20 mg/kg supplemental, as needed; intraperitoneal injections) at ages P40–P60. Animals in different groups were roughly age matched. The AI was defined by its tonotopic organization and reliable neuronal responses to tone pips of selective frequencies. Neurons were evenly sampled from the AI using parylene-coated tungsten microelectrodes (2 Mohm) advanced 500–600 μm below the pial surface (layer 4/5), and responses to 25-ms tone pips of 50 frequencies (1–32 kHz, 0.1 octave spacing) and eight sound pressure levels (0–70 dB SPL, 10 dB steps) were recorded three times to reconstruct the response-frequency tuning curve. Responses to a series of exponential FM sweeps (frequency range, 0.5–50 kHz; sweep rates, 30, 45, 60, 75, and 90 octaves/s, at 60 dB SPL, in both up and down...
directions) were also recorded three times to determine the neuron selectivity for FM sweep rates. Tone pips and FM sweeps were intermixed and presented in a pseudorandom order through a custom made STAX speaker. A Tucker-Davis Technologies system (TDT System 3) was used for speaker calibration, sound delivery, and electrophysiological recording.

**Analysis**

The receptive field was analyzed automatically with a custom-made program. Briefly, all responses in the receptive field were thresholded at 28% of the maximum response magnitude and smoothed with a 3 × 3 median filter. A frequency-intensity response area was defined as three continuous pixels of responses of at least one spike per tone over the threshold. The characteristic frequency (CF) and tuning bandwidth of each neuron were derived from all response areas of the receptive field. The CF was calculated as the center of mass, and the tuning bandwidth was defined as the bandwidth of all response areas. Responsiveness of a neuron to tones was quantified with the sum of all spikes in the response areas over the threshold. A neuron was considered nonresponsive to tones if there was not a response area in the receptive field. To generate CF maps, Voronoi tessellation was performed to create tessellated polygons, with each polygon corresponding to an electrode penetration site, and assigned the CF of the site. The magnitudes of responses to the exponential FM sweeps were determined in 30-ms response windows that were automatically set to best fit the response latencies at all the different sweep rates. Under the assumption that the neuronal response was triggered by a specific latency of the response to the sweep at the FM Rate, and $T_{min}$ is the minimal response delay. A regression analysis was performed on FM Rates and Ts (peak response latencies for each FM rate derived from 20-ms smoothed PSTH) to derive $T_{min}$ and f. The expected latency was calculated as $T_{exp} = \log_2(f_{min}) \times FM_{Rate}^{-1} + T_{min}$. A 30-ms window centered at $T_{exp}$ was used as the response window. The number of spikes in the response window was counted as the response magnitude. Spontaneous firing was determined from the 30-ms prestimulus period and was subtracted from the response to the FM sweeps. $T_{min}$ and f are derived separately for up- and down-sweeps. For down-sweeps, $FM_{Rate} \times (T - T_{min}) = \log_2(f_{max} \times f^{-1})$, and $T_{exp} = \log_2(f_{max} \times f^{-1}) \times FM_{Rate}^{-1} + T_{min}$. Our visual inspection indicated that the response windows consistently captured the phasic cortical responses to the sweeps.

A sweep direction selectivity index (SDSI) was calculated as $(R_{up} - R_{down})/(R_{up} + R_{down})$, in which $R_{up}$ is response magnitude to the up-sweep, and $R_{down}$ is response magnitude to the down-sweep. To avoid bias by small responses, we calculated a $\chi^2$-based statistic of up-sweep selectivity: $\chi^2_{SDSI} = (R_{up} - R_{down})^2/(R_{up} + R_{down})^2$. 

**Determining the locations of recorded sites on the tonotopic axis**

A line connecting the site with the highest CF and that with the lowest CF was defined as the tonotopic axis. The maps were rotated to orient the tonotopic axis horizontally. For all recorded sites in each map, a linear regression was performed in the form of $\log(CF) = k(X - c)$, in which CF is the characteristic frequency in kilohertz, and X is the x-coordinate of the recorded site. $X - c$ is used as the location of the recorded site on the tonotopic axis.

---

**FIG. 3.** Exposure to pulsed noise alters AI receptive field properties. A: number of nonresponsive sites along the tonotopic axis. The bin size is 5% of the length of AI along the tonotopic axis. B and C: the size of the response areas as a function of the CFs. D and E: number of spikes per tone in the response areas. F: response threshold. Error bars show SE. *P < 0.05; **P < 0.01.
Results

Exposure to pulsed noise disrupts cortical frequency tuning and tonotopy

We exposed rat pups to pulsed white noise (50-ms noise pulses, 5-ms ramps, 65 dB SPL, 6 pps with 500 ms of silence between trains) in one of four time windows (P8 –P15, P16–P23, P24–P31, and P32–P39) and examined cortical sound representations from P40 to P60. Comparison of frequency-intensity receptive fields indicates that exposure to pulsed noise during P8–P15 led to a greater number of presumptive AI sites that did not respond to tones (as marked in gray in Fig. 2 during P8 –P15 led to a greater number of presumptive AI sites that did not respond to tones (as marked in gray in Fig. 2A; also see receptive field 5 in Fig. 2B). Such poorly responsive sites were less frequent in P16–P23, P24–P31, and P32–P39 groups and were not seen in naïve animals (P8–P15, 19 ± 5; P16–P23, 3 ± 1; P24–P31, 4 ± 1; P32–P39, 3 ± 1; P < 0.0005 between P8 and P15 and all other groups; P > 0.2 between any 2 groups of naïve, P16–P23, P24–P31, or P32–P39). Because AI sites are generally defined by their response to tones, defining nonresponsive AI sites was somewhat arbitrary, especially for the bordering sites. However, the large number of nonresponsive sites in P8–P15 animals was not likely caused by oversampling of non-AI sites because the sizes of AI were not significantly different among the five groups (P8–P15, 1.18 ± 0.05 mm²; P16–P23, 1.15 ± 0.06 mm²; P24–P31, 1.09 ± 0.11 mm²; P32–P39, 1.18 ± 0.03 mm²; naïve, 1.21 ± 0.04 mm²; P > 0.5). In addition, the nonresponsive sites were located mostly in the AI area defined by tone selective sites and the appropriate tonotopic gradient. These nonresponsive sites were approximately evenly distributed along the tonotopic axis—a Kolmogorov-Smirnov test showed that the distribution of the nonresponsive sites was not significantly different from a uniform distribution (P > 0.5; Fig. 3A).

To determine whether the occurrence of the nonresponsive sites was caused by a general reduction of cortical responses in all neurons or a reduction of responses in a specific subpopulation of neurons, we quantified the size of the frequency-intensity response area of the tone-responsive neurons and found that the P8–P15 group had significantly smaller response areas compared with the P16–P23, P32–P39, and naïve groups (P < 0.05; Fig. 3, B and C). In addition, the response magnitude to tones in the response area was reduced in the P8–P15, P24–P31, and P32–P39 groups compared with the naïve group (P < 0.01; Fig. 3, D and E). Among the noise-exposed groups, the response magnitude was lower for the P24–P31 group than the P8–P15 and P16–P23 groups (P < 0.01) and was lower for the P32–P39 group than the P16–P23 group (P < 0.05). Thus the occurrence of nonresponsive sites might be caused by a general reduction of the response area but not the response magnitude.

Analysis of the receptive field indicates that the noise exposure also altered tone response threshold (Fig. 3F). Neurons from the P8–P15 group had significantly higher thresholds than did the P16–P23, P24–P31, and P32–P39 groups (P < 0.05; Fig. 3F). The threshold of the P32–P39 group was significantly lower than that of the naïve control group.

The basic tonotopic gradient from caudal low frequencies to rostral high frequencies was present in all groups (Fig. 2C). However, fine tonotopic organization was disrupted in P8–P15 animals, as indicated by the wider scattering of points in its CF-tonotopic axis plot (Fig. 2C). The residual of a linear regression analysis was significantly greater for P8–P15 group than for other groups (P8–P15, 0.29 ± 0.02 mm; P16–P23, 0.17 ± 0.01 mm; P24–P31, 0.19 ± 0.01 mm; P32–P39, 0.21 ± 0.01 mm; naïve, 0.20 ± 0.01 mm; P < 0.0001).

Unlike exposure to downward FM sweeps during P8–P15, which results in reduced representations of frequencies <4 kHz (Insanally et al. 2009), exposure to pulsed noise did not cause a distortion in the proportional allocation of cortical area to any given range of CFs (Fig. 2, A and C, and 5C).

Exposure to pulsed noise broadens frequency tuning bandwidth

We calculated the frequency tuning bandwidth from neurons that had frequency-intensity receptive fields. The bandwidths...
of neurons in the P16–P23 animals were significantly broader than those of all other groups at sound pressure levels 50–70 dB (Figs. 2B and 4A; *P < 0.05). To determine whether the bandwidth differences were an epiphenomenon of the noise exposure-induced threshold shifts (Fig. 3F), we measured the bandwidth relative to the response threshold. The bandwidth was significantly broader for the P16–P23 group than for all the other groups at 40 dB above threshold (Fig. 4B; *P < 0.05). These findings indicate that exposure to a broadband stimulus in P16–P23 results in broader receptive field tuning, as shown in a previous report (Insanally et al. 2009). In addition, the bandwidth was significantly narrower for the P32–P39 group than for all the other groups at 30 and 40 dB above threshold (Fig. 4B; *P < 0.05).

Exposure to pulsed noise alters FM sweep direction selectivity

We recorded cortical responses to up and downward sweeps at five rates (30, 45, 60, 75, and 90 octaves/s) from tone responsive neurons (see METHODS for definition). The nonresponsive AI neurons were either weakly or not responsive to the exponential FM sweeps and were not analyzed further (mean response magnitude to all 10 FM sweeps sounds was 0.86 ± 0.07 spikes/sound for nonresponsive AI sites and 3.26 ± 0.21 spikes/sound for responsive AI sites, *P < 0.0001). We calculated a χ²-based sweep direction selectivity index (χ²-SDSI) that measures the level of sweep direction selectivity (see METHODS). Neurons that are selective for up-sweeps will have a positive χ²-SDSI, and those selective for down-sweeps will have a negative χ²-SDSI. Previous studies reported that high-CF neurons tend to select for downward sweeps and low-CF neurons for upward sweeps (Heil et al. 1992; Zhang et al. 2003). We observed this CF dependence of sweep direction selectivity in AI neurons of naïve animals for high sweep rates at 75 and 90 octaves/s (Fig. 5) but not for low sweep rates at 30, 45, and 60 octaves/s. Exposure to pulsed noise during P8–P15, P24–P31, and P32–P39 resulted in a loss of CF dependence of sweep direction selectivity. Figure 5A depicts the χ²-SDSI at the sweep rate of 90 octaves/s as a function of CF. A linear regression analysis showed that the slopes of the regression line are significantly negative for the P16–P23 and naïve control groups (*P < 0.05) and are not different from 0 for P8–P15, P24–P31, and P32–P39 groups (**P > 0.05). When grouped by CFs into 1-octave bins, χ²-SDSI is a decreasing function of CF only for the P16–P23 and naïve control groups (*P < 0.05, comparing CF < 8 vs. > 16 kHz) but not for the other groups (**P > 0.05). The same results were seen for the sweep rate of 75 octaves/s (*P < 0.05 for the P16–P23 and naïve control groups but not the other groups). Compared between groups, the χ²-SDSI is significantly lower for P16–P23 and naïve groups than for the other groups at 16–32 kHz and is significantly lower for the P8–P15 group than for the other groups at 8–16 kHz (**P < 0.05; Fig. 5B). This loss of CF dependence is not caused by a general loss of direction selec-

![Fig. 5. Exposure to pulsed noise alters frequency-modulated (FM) sweep representation. A: χ²-sweep direction selectivity index (SDSI) at 90 octaves/s as a function of CF for P8–P15, P16–P23, P24–P31, P32–P39, and control animals. Each point represents a neuron for which sweep responses have been recorded. The linear regression line is plotted, and the 95% CI of the slope is indicated. Only the P16–P23 and the control groups had significantly negative slopes. B: average χ²-SDSI at 90 octaves/s as a function of CF for all groups. Note the lack of CF dependence in P24–P31 and P32–P39 groups. The P8–P15 group also lacked downward FM direction selectivity at 16–32 kHz. The χ²-SDSI index value is different (*P < 0.05) between the low (1–8 kHz) and high (16–32 kHz) frequency bins for P16–P23 and control groups but not for the other groups. C: number of recorded sites in each frequency bin. The 16–32 kHz bin may have included neurons responsive to 16–32 kHz tones but tuned to higher than 32 kHz. D: cumulative distributions of the χ²-SDSI at 90 octaves/s. The distributions are not significantly different among the 5 groups. E: the magnitude of responses to the best FM sweeps. *P < 0.05.](http://jn.physiology.org/)

*J Neurophysiol* • VOL 103 • MAY 2010 • www.jn.org
tivity because the strength of direction selectivity, as measured by the mean absolute value of the $\chi^2$-SDSI, is not different among the five groups for any of the sweep rates ($P > 0.1$). Furthermore, the general distributions of the $\chi^2$-SDSI are not different among the five groups (Fig. 5D; pairwise Kolmogorov-Smirnov tests, $P > 0.05$). We also calculated the magnitude of the cortical responses to the best FM sweep, the one that most strongly activated the neuron. All noise-exposed animals showed weaker sweep responses compared with naïve animals (Fig. 5E; $P < 0.05$). In addition, the sweep response was weaker for the P8–P15 group than for the P16–P23 group ($P < 0.05$).

Discussion

Our results are in general agreement with a previous report of multiple sensitive periods for the developing auditory cortex (Insanally et al. 2009). In addition, we showed that the same response features may be altered in different sensitive periods. For example, bandwidth tuning was broadened by pulsed noise exposure during P16–P23 and narrowed during P32–P39. Similarly, the frequency dependence of sweep direction selectivity was disrupted by pulsed noise exposure during P8–P15 and P24–P39. Because these response features are determined by multiple (e.g., excitatory and inhibitory) cortical circuits (Galindo-Leon et al. 2009; Razak and Fuzessery 2009; Zhang et al. 2003), our results are consistent with multiple sensitive periods for the development of these different circuits.

The differences between the noise-induced effects reported here and FM sweep-induced effects reported in our earlier paper (Insanally et al. 2009) indicate that plasticity effects of simple and complex sound representations depend on the spectrototemporal structures of the sensory input. For example, rearing rat pups in downward FM sweeps during P8–P15 resulted in reduced representations of frequencies below 4 kHz (Insanally et al. 2009). In contrast, animals reared in pulsed noise during the same period showed apparently normal low-frequency representations but disrupted tonotopicity. In addition, exposure to FM sweeps and pulsed noise during P24–P31 and P32–P39 also produced different outcomes to sweep selectivity of the AI neurons. Exposure to downward FM sweeps resulted in a significant increase in the number of neurons selective for downsweeps (Insanally et al. 2009), whereas exposure to pulsed noise eliminated the CF dependence of $\chi^2$-SDSI. Animals that had been exposed to pulsed noise during P8–P15 also lacked CF dependence of $\chi^2$-SDSI. Because sweep selectivity is presumably mediated by temporally offset excitatory and inhibitory receptive fields, altered excitatory receptive fields in P8–P15 animals (Figs. 2 and 3) may contribute to the disruption of sweep direction selectivity.

Exposing rat pups to pulsed noise from P9 to P28 resulted in reduced threshold and narrower tuning bandwidth are unexpected. Broadband noise activates both the excitatory and inhibitory response areas of the receptive field, and the interactions between the excitation and inhibition are likely important in determining the specific form of cellular plasticity that pulsed noise induces. It is possible that the period from P32 to P39 represents a unique window, in which broadband noise-activated excitation and inhibition interact to shape stronger connections from a narrower range of thalamic input. It is also possible that broadband noise enlarges sideband inhibition to shape narrower frequency tuning, and subsequent homeostatic excitatory plasticity leads to lower response thresholds.

To standardize data analysis, we used a program to automatically determine neuronal response properties such as CF, bandwidth, and sweep response magnitude. The CF was calculated as the center of mass of the frequency-intensity receptive field (see METHODS). These CFs are biased by responses at high-intensity levels and tend to be lower in frequency than the CFs determined at the threshold sound level. Nevertheless, the center-of-mass CFs are highly correlated with the threshold CFs. In addition, the center-of-mass CFs may be more relevant in analyzing CF dependence of the FM sweep selectivity, because the FM sweeps were played at 60 dB SPL.

Sweep direction selectivity is presumably mediated in part by sideband inhibition (Galindo-Leon et al. 2009; Razak and Fuzessery 2009; Zhang et al. 2003). Sideband inhibition for high-CF neurons is located mostly on the low frequency side, giving rise to its selectivity for downward sweeps. Likewise, low-CF neurons, having sideband inhibition mostly on the high-frequency side, prefer upward sweeps. A recent study indicates that the development of GABA-mediated sideband inhibition is experience dependent, and both the development and maintenance of FM direction selectivity requires appropriate experience (Razak and Fuzessery 2009; Razak et al. 2008). It is likely that the pulsed noise used in this study dominated the acoustic environment and masked/diluted the FM sound features in the natural acoustic environment, thereby disrupting normal development of inhibitory circuits and the CF dependence of sweep direction selectivity.

GRANTS

This work was supported by National Institute of Deafness and Other Communication Disorders Grants DC-007883 and DC-009259. M. N. Insanally is supported by the National Science Foundation Graduate Research Fellowship Program.

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


No conflicts of interest are declared by the authors.

REFERENCES


