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Research paper

Neonatal nicotine exposure impairs development of auditory temporal processing

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ABSTRACT

Accurate temporal processing of sound is essential for detecting word structures in speech. Maternal smoking affects speech processing in newborns and may influence child language development; however, it is unclear how neonatal exposure to nicotine, present in cigarettes, affects the normal development of temporal processing. The present study used the gap-induced prepulse inhibition (gap-PPI) of the acoustic startle response to investigate the effects of neonatal nicotine exposure on the normal development of gap detection, a behavioral testing procedure of auditory temporal resolution. Neonatal rats were injected twice per day with saline (control), 1 mg/kg nicotine (N-1 mg) or 5 mg/kg nicotine (N-5 mg) from postnatal day 8–12 (P8–P12). During the first month after birth, rats showed poor gap-PPI in all three groups. At P45 and P60, gap-PPI in control rats improved significantly, whereas rats exposed to nicotine exhibited less improvement. At P60, the gap-detection threshold in the N-5 mg group was significantly higher than in the control group, suggesting that neonatal nicotine exposure affects the normal development of gap-detection acuity. Additionally, 1 h after receiving an acute nicotine injection (1 mg/kg), gap-PPI recorded in adult rats from the N-5 mg group showed a temporary significant improvement. These results suggest that neonatal nicotine exposure reduces gap-PPI implying an impairment of the normal development of auditory temporal processing by inducing changes in cholinergic systems.

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1. Introduction

The processing of complex acoustic signals, such as speech and music, requires a central auditory system able to detect rapid changes of sound intensity and spectral fluctuations (Metherate et al., 2005; Purcell et al., 2004). A reduced spectral and temporal resolution, as seen in elderly listeners, can lead to difficulties in understanding speech (Strouse et al., 1998). High spectral and temporal resolutions are critical for normal development of child language and speech comprehension (Nicholas and Geers, 2006). Children with language impairment score poorly on auditory processing tests, such as auditory word discrimination, temporal integration and auditory synthesis (Stollman et al., 2003). In addition, Trehub et al. found that children with poor temporal resolution are more likely to have language learning disabilities than children with better temporal resolution (Trehub and Henderson, 1996). Recent studies have found that prelingually deaf children who received cochlear implants also require precise temporal processing capability in order to acquire language. Cochlear implant users who exhibited poor language performance had difficulties on simple temporal gap-detection tasks, such as detecting a silent gap in a continuous sound of moderate intensity. However, cochlear implant users with better language skills exhibited better gap-detection ability; some exhibited gap-detection thresholds similar to normal hearing listeners (Fu et al., 2001).

Maternal smoking, which could expose prenatal children to large doses of nicotine, has been found to be harmful to fetal brain development (Oncken and Kranzler, 2003) which may lead to auditory cognitive dysfunction (Dwyer et al., 2008). Maternal smoking has also been shown to affect the development of speech processing in infants. Key et al. investigated vowel-sound evoked event-related potentials in human two day old infants, whose mothers did or did not smoke during pregnancy (Key et al., 2007). The infants belonging to non-smoking mothers exhibited different event-related potentials to different vowel-sounds. In contrast, infants of smoking mothers exhibited no difference in event-related

Abbreviations: ABR, auditory brainstem response; AC, auditory cortex; gap-PPI, gap-induced prepulse inhibition; MGB, medial geniculate body; nAChR, nicotinic acetylcholine receptors; NB-PPI, narrow band noise induced prepulse inhibition; P8, postnatal day 8; RMS, root mean square; s.c., subcutaneously; SPL, sound pressure level; STg, startle response measured in a gap trial; STnb, startle response measured in a noise-burst trial; STng, startle response measured in a no-gap trial; STq, startle response measured in a no-prepulse stimulus trial

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potentials to different vowel-sounds. Since the infants from both groups experienced no differences in postnatal environment, the event-related potential differences between them are suggested to be a result of maternal smoking during pregnancy. Given that maternal smoking and exposure of newborns to second-hand smoke are not uncommon, understanding the risk of prenatal and neonatal nicotine exposure on central auditory system development could provide important and useful information about prenatal care and child education.

In the present study, we investigated the effects of neonatal nicotine exposure on the normal development of gap-detection acuity, an established behavioral testing procedure of auditory temporal resolution in rats (Friedman et al., 2004). The gap-detection acuity was measured as the level and threshold of gap-induced prepulse inhibition (gap-PPI) of the acoustic startle reflex (Friedman et al., 2004; Ison et al., 2002; Walton et al., 1997). This test does not require any auditory training and allows assessment of rapid developmental changes in neonatal rats. Nicotine was given to neonatal rat pups before the onset of hearing on postnatal days 8–12 (P8–P12), the critical period of central auditory system development (Chang and Merzenich, 2003). Since the hearing of rats develops two weeks after the birth, the auditory development in the second postnatal week in rats is equivalent to the third trimester of human fetal development (Bayer et al., 1993).

2. Materials and methods

2.1. Animals and nicotine exposure

Neonatal Sprague-Dawley rats (Harlan, Indianapolis, IN), both male and female, were randomly assigned into three groups: rats in the low dose nicotine group (N-1 mg, n = 11) were injected with 1 mg/kg nicotine, rats in the high dose nicotine group (N-5 mg, n = 10) were injected with 5 mg/kg nicotine, and control rats (n = 16) were injected with saline (10 ml/kg). Nicotine (#72290, Fluka) was diluted in saline to 0.1 mg/ml (pH 8.6). Nicotine or saline was given twice per day (one injection at 9 am and one injection at 5 pm) subcutaneously (s.c.) from P8 to P12. For acute nicotine treatment to adult rats, nicotine was diluted in saline (1 mg/ml) and injected once in a dose of 1 mg/kg (s.c.).

All procedures used in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of State University of New York at Buffalo.

2.2. Acoustic startle reflex

The prepulse inhibition of acoustic startle reflex (PPI) test was utilized to monitor temporal processing development from P20 to P60. The testing procedure has been described previously (Yang et al., 2007). Each rat was positioned in a wire mesh cage mounted on an acrylic glass base that rested on a sensitive piezoelectric transducer (Radio Shack Corp.). Three different sizes of wire mesh cages were used for rats at different age (P20–P60) to restrict the rat's movement within the calibrated sound field (824 audiometer, Larson Davis). The output of the piezoelectric transducer was sent to a low-pass filter set at 1000 Hz (LPF-300, World Precision Instruments) and then connected to an analog-digital converter (RP2.1, Tucker–Davis Technologies (TDT), FL). The root mean square (RMS) amplitude of the first 100 ms response after the onset of the sound stimulus was measured using custom software (Matlab, The MathWorks Inc.). Sound signals were generated by a second digital signal processor (RP2.1, TDT) controlled by custom software, amplified (SA1; TDT), and presented by a high frequency dome tweeter (FT28D, Fostex, NJ) located 25 cm above the rat. The startle eliciting stimulus consisted of a broadband noise-burst presented at 100 dB SPL (1–30 kHz bandwidth, 20 ms duration, 0.1 ms rise/fall time, 100 kHz sampling rate).

The amplitude of acoustic startle responses were measured for all three groups of rats at P20, P28, P35, P45 and P60 using two different testing procedures: gap-PPI and narrow band prepulse inhibition (NB-PPI). For the gap-PPI test, the startle stimulus was embedded in a white noise background (60 dB SPL) with or without a silent gap immediately prior to the startle stimulus. The duration of the silent gap varied from 1 to 100 ms and the offset of the gap preceded the onset of the startle stimulus by 60 ms. The RMS amplitude of the acoustic startle was measured in the presence of continuous noise with no gap (STng) (Fig. 1A), or continuous noise containing a gap (STg) (Fig. 1B). When the gap precedes the startle stimulus, it reduces the amplitude of the startle response in normal hearing rats (Fig. 1A vs. B), i.e. inhibits the startle response (Ison et al., 2002; Yang et al., 2007). Extent of gap-PPI in% was defined as: $(STng - STg)/STng \times 100\%$. Ten trials for each condition contained either no gap or gaps with durations of 1, 2, 4, 6, 8, 10, 15, 25, 50 and 100 ms (semilogarithmic scale), presented in random order. The inter-trial interval varied from 17 to 23 s. Startle responses in the gap condition were compared to the response in the no-gap condition using the Student's t-test and significance level was set to $P < 0.05$. The gap-detection threshold was defined as minimum duration of gap needed to induce significant inhibition of the startle response.

In the NB-PPI test, the startle stimulus was either presented alone (Fig. 1C) or preceded by a 60 dB SPL narrowband (1000 Hz bandwidth) noise-burst (50 ms, 5 ms rise/fall time) centered at 6, 12, 16, 20 and 24 kHz (Fig. 1D). The onset of noise-burst prepulse preceded the onset of the startle stimulus by 60 ms. The RMS of the acoustic startle was measured in quiet (STq) or with the noise-burst preceding the startle stimulus (STnb). When the noise-burst prepulse precedes the startle stimulus, it reduces the amplitude of the startle response (Fig. 1C vs. D), i.e. the startle is inhibited. STnb and STq were presented in pairs in randomized order. Extent of NB-PPI in% was defined as: $(STq - STnb)/STq \times 100\%$. Ten stimulus pairs (20 trials) were presented at each frequency with random inter-trial interval (17–23 s).

2.3. Auditory brainstem response (ABR) recordings

Hearing thresholds were evaluated by ABR tests. During the tests, rats were anesthetized under isoflurane (1.5%). The vertex was used as the non-inverted recording site, the ipsilateral pinna was used as the inverted recording site and contralateral pinna as the ground. The lead of the active electrode was connected to a headstage (RA4LI, TDT) using a flexible, low noise cable. The headstage was connected to a preamplifier (RA16PA), the output of which was routed to a digital signal processing module (RX5-2, Pentusa Base Station, TDT) before being further processed with software (BioSigRP version 4.4, TDT; digital band pass filter:100–3000 Hz) for the ABR recording. The sound stimulus was a tone-burst (duration was 5 ms duration, 1 ms rise/fall time) at 4, 8, 12, 16 and 20 kHz generated with a real-time digital-analog converter (RP2.1, TDT). The repetition rate was 21 times/s. Sound stimuli were presented through a high frequency dome tweeter (FT28D, Fostex). Sound levels were calibrated using free-field microphones (1/2" 2540 and 1/4" 2520, Larson Davis) sound level meter (824, Larson Davis).

2.4. Data analysis

Graphs and statistic analyses were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). The Student's t-test and one-way ANOVA test were used to