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Acoustic alterations of ultrasonic vocalization in rat pups induced by perinatal hypothyroidism

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ABSTRACT

Perinatal hypothyroidism causes serious damage to auditory functions that are essential for vocalization development. In rat pups, perinatal hypothyroidism potentially affects the development of ultrasonic vocalization (USV) as a result of hearing deficits. This study examined the effect of perinatal hypothyroidism on the development of USVs in rat pups. Twelve pregnant rats were divided into three groups and treated with the anti-thyroid drug methimazole (MMI) via drinking water, from gestational day 15 to postnatal day (PND) 21. The MMI concentration (w/v) was 0% (control group), 0.01% (low-dose group), or 0.015% (high-dose group). After birth, the pups were individually separated from the dam and littermates on PNDs 5, 10, 15, and 20, and their USVs were recorded for 5 min. On PNDs 5 and 10, compared with the control group, the low- and high-dose groups exhibited reductions of both frequency-modulated and downward USVs. On PND 15, however, the low- and high-dose groups displayed increases in number, duration, and amplitude of USVs compared with those in the control group. Lower body weights were observed for the low- and high-dose groups than for the control group. Total thyroxine concentrations in plasma were dose-dependently reduced. The onset of auditory functions appeared on PNDs 11–14. Thus, the rat pups were unable to hear externally produced USVs before PND 11. USVs emitted on PNDs 5 and 10 might have been spontaneous and independent of the pups' own or littermate-emitted USVs. The developmental retardation of vocalization-related organs or muscles might underlie the acoustic alterations of USVs on PNDs 5 and 10. The greater number, duration, and amplitude of USVs on PND 15, after which the hearing onset occurred, suggested that the elevation of auditory thresholds occurred as a result of hearing deficits in the low- and high-dose groups. Perinatal hypothyroidism appears to have caused acoustic alterations in the USV development.

1. Introduction

Auditory functions in rats normally appear on postnatal days (PNDs) 12–14 (Brunjes and Alberts, 1981; Gabriele et al., 2000). The external acoustic meatus opens and auditory startle responses are observed on PNDs 11–14 for the first time (Brunjes and Alberts, 1981). However, perinatal hypothyroidism appears to delay the onset of auditory startle responses (Comer and Norton, 1982; Schneider and Golden, 1986). In addition, the amplitude of startle responses is reduced (Goldey et al., 1995; Henck et al., 1996). Irreversible damage to auditory functions was demonstrated (Wada et al., 2013); severe hearing deficits were detected over a wide range of tone frequencies from 1 to 40 kHz (Goldey et al., 1995). Hearing deficits make it difficult for rats to hear their own vocalizations and those of their conspecifics. Auditory functions are indispensable for vocalization development.

Vocalization has been investigated, particularly in rodents such as rats and mice, and the communicative functions of vocalization have been increasingly elucidated upon (Brudzynski, 2010). For example, rat pups emit ultrasonic vocalizations (USV) when they are separated from the dam (Portfors, 2007; Ise and Ohta, 2009; Schwarting and Wöhr, 2012). The dam approaches the pups in response to their USVs, retrieves them, and returns them to the nest (Schwarting and Wöhr, 2012). In contrast to this, the pups suppress USVs upon contact with an unknown male adult rat (Takahashi, 1992; Shair et al., 1997; Wiedenmayer et al., 2003), possibly because adult male rats often attack and kill pups in order to mate with their dam (Hofer, 2010).

USVs emitted by rat pups were shown to increase on PNDs 3–5 and reach a maximum on PNDs 5–10. Subsequently, USVs decreased and then completely disappeared around PND 21, when pups were able to wean (Schwarting and Wöhr, 2012). USVs in rat

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pups have been shown to be at frequencies of approximately 40 kHz and durations of approximately 80–150 ms (Portfors, 2007; Ise and Ohta, 2009; Schwarting and Wöhr, 2012). Because 40-kHz USVs in rat pups were demonstrated to be emitted upon maternal separation, USVs were considered to express the pups' distress state (Brudzynski et al., 1999; Portfors, 2007).

Rat pups seem to hear 40-kHz USVs emitted by themselves and their littermates after PNDs 13–15 because auditory startle responses can be induced by means of a 40-kHz ultrasonic tone on PNDs 13–15 (Brunjes and Alberts, 1981). However, perinatal hypothyroidism has been shown to cause morphological abnormalities in the cochlea (Uziel et al., 1980, 1981, 1985), and hearing deficits may be extended to the ultrasonic frequency band of 40 kHz (Goldey et al., 1995). Therefore, perinatal hypothyroidism potentially affects the development of rat pup's USVs as a result of hearing deficits. Nevertheless, little is known about the effects of hypothyroidism on the development of USVs.

In this study, pregnant rats were treated with the anti-thyroid drug methimazole (MMI), and USVs emitted by the pups from control and treated mothers were recorded upon the separation from the dam. The acoustic characteristics of the USVs were analyzed and examined to determine whether perinatal hypothyroidism affected the development of USVs during the lactation period. Acoustic alterations were predicted to be caused in hypothyroid pups as a result of hearing deficits.

2. Materials and methods

2.1. Subject

Twelve pregnant Wistar rats at gestational day (GD) 12 were purchased from Japan SLC Inc. (Hamamatsu, Japan). The animals were housed in individual cages; supplied with the certified rat chow MF (Oriental Yeast Ltd., Sapporo, Japan) and tap water ad libitum; and randomly assigned to either a control group (n = 4), a low-dose group (n = 4), or a high-dose group (n = 4). MMI (Sigma Aldrich Co., Mo, USA) was dissolved in distilled water and administered to the animals via drinking water starting on GD 15 and extending to PND 21. The following concentrations of MMI (w/v) were used: 1) 0% (control), 2) 0.01% (low-dose), and 3) 0.015% (high-dose). These concentrations were selected for two reasons. First, the 0.01% MMI was the lowest concentration that causes hearing deficits in rats (Comer and Norton, 1982; Albee et al., 1989). Second, the 0.01% and 0.015% MMI treatments induced dose-dependent reduction of T4 (shown in Fig. 4). Dose-dependent alterations were expected in the acoustic parameters of the USVs. Because fetal thyroid functions in rats begin at approximately GD 17 (Gilbert and Zoeller, 2010), the MMI administration started on GD 15. MMI is able to pass through the placenta and reach the fetuses during gestation (Marchant et al., 1977; De Escobar et al., 1988; Sack et al., 1995), and it is excreted into breast milk and taken by pups on PNDs (Johansen et al., 1982; Cooper, 1984). The date of birth was designated PND 0. On PND 4, each litter was culled to four males and four females. Two pups (one male and one female) were randomly sampled from each litter as subjects. Thus, four male and four female pups were chosen from each MMI treatment group. The same pups were repeatedly tested for USV recording at each age.

The temperature of the breeding room was maintained at 22 °C ± 2 °C with a relative humidity of 50% ± 10%. The dams and pups were subjected to a 12-h light/dark cycle (light: 20:00–08:00 h; dark: 08:00–20:00 h). This experimental protocol was approved by the Animal Ethics Committee of Hokkaido University; all experimental conditions were compliant with the Guide for the Care and Use of Laboratory Animals, Hokkaido University.

2.2. Apparatus

USVs were recorded and analyzed using an ultrasonic microphone and the Sonotrack system version 2.1.5 (Metris, Hoofddorp, the Netherlands). The software of the Sonotrack system was installed on a personal computer and run on MS Windows XP Professional. The ultrasonic microphone was positioned at a height of 16 cm from the bottom of a translucent cup with a 13-cm bottom diameter, 15-cm top diameter, and 15-cm height. The ultrasonic microphone and translucent cup were placed in a sound-insulated box in order to attenuate external sound and light.

2.3. Recording of USVs

USVs were recorded on PNDs 5, 10, 15, and 20 as described in this section. A pup was individually separated from the dam and littermates in the breeding room, put into the translucent cup described above and brought to the experimental room. The pup was left alone in the sound-insulated box for a 5-min period of habituation, followed by 5 min of USV recording. Thus, the total duration of separation from the dam was 10 min. After the recording, the body weight of the pup was measured, and the pup was returned to the dam and littermates. The temperature of the experimental room was 20 °C–23 °C, and the relative humidity was 50%–70%. USVs were recorded during the dark period. The translucent cup was cleaned with ethanol and water.

2.4. Determination of thyroid hormone concentrations

Additional 24 pregnant Wistar rats were prepared for either a control group (n = 8), a low-dose group (n = 8), or a high-dose group (n = 8) and treated with MMI using the same procedure. Two pups (one male and one female) were sampled from each litter, and eight male pups and eight female pups per group were served for thyroid hormone determinations. Whole blood was collected from the abdominal aorta of ether-anesthetized pups on PNDs 20–21, centrifuged at 3000 rpm for 10 min, and the plasma was stored in a micro-tube. Both the total triiodothyronine (T3) and thyroxine (T4) concentrations were determined using the ACS-FT3 II and LKFT41 kits, respectively (Siemens Healthcare Diagnostics Co., Tokyo, Japan). Thyroid-stimulating hormone (TSH) concentrations were determined using the rat TSH ELISA kit (R-type) (Shibayagi Co., Shibukawa, Japan). All assays were performed at Mitsubishi Chemical Medience (Tokyo, Japan).

2.5. Data analyses

USVs were analyzed with the automatic selection mode of the Sonotrack system. The time resolution was 1 ms, and to reduce background noise, the low and high cut-off frequencies for the recording were set to 30 kHz and 90 kHz, respectively. The Sonotrack system calculated the lowest frequency in a periodic waveform of USV at every 1 ms time step and obtained fundamental frequencies. If the fundamental frequency at either the start or end point of an USV was out of the 30–70 kHz range, the USV was re-analyzed with the manual selection mode. USVs that satisfied all of the following criteria were selected for statistical analyses (Reno et al., 2013).

(i) The fundamental frequencies at both the start and end points of the USV were ≥30 and <70 kHz.
(ii) The mean fundamental frequency of the USV was <90 kHz.
(iii) The bandwidth between the maximum and minimum fundamental frequencies of the USV was <60 kHz.
(iv) The duration of the USV was ≥20 ms.