# THYROID HORMONE DEFICIENCY AND DEVELOPMENT OF ULTRASONIC VOCALIZATION RESPONSES IN RAT PUPS

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**Abstract**

Perinatal thyroid hormone deficiency is known to impair auditory system functions, which are crucial for the development of vocalization. Therefore, auditory system dysfunctions resulting from thyroid hormone deficiency have the potential to affect the development of ultrasonic vocalization responses in rat pups; this effect of perinatal thyroid hormone deficiency was examined in the present study. Pregnant rats were administered Methimazole, an antithyroid drug, via drinking water from gestational day 15 to postnatal day 21 at a concentration (w/v) of 0% (control group), 0.01% (low-dose group), or 0.015% (high-dose group). Ultrasonic vocalizations were recorded for 3 min under conditions of maternal separation on postnatal day 5, 10, 15, and 20. On postnatal day 15, both the low- and high-dose groups exhibited elevated ultrasonic vocalization responses, and also, a greater root mean square of ultrasonic vocalization amplitudes than the control group. Furthermore, the high-dose group exhibited a reduced percentage of frequency-modulated calls on postnatal day 5. We conclude that thyroid hormone deficiency affects the development of ultrasonic vocalization responses in rat pups as a consequence of auditory system dysfunctions.

**Introduction**

Polychlorinated biphenyls (PCBs) and dioxins affect the thyroid hormone system, which plays a crucial role in brain development. One of the consequences of thyroid hormone deficiency is irreversible damage to the auditory system functions[10], which are indispensable for the development of vocalization. Therefore, thyroid hormone deficiency resulting from exposure to PCBs and dioxins potentially affects the development of vocalization because of auditory system dysfunctions.

Rodents emit ultrasonic vocalizations (USVs) for communication. For example, rat pups often emit USVs of approximately 40 kHz when separated from the dam[3, 6, 7]; this allows the dam to approach and retrieve the pups to the nest[7]. However, auditory system dysfunctions make it difficult for rodents to hear their own USVs as well as those emitted by other rodents and potentially affect the development of USVs. In this study, we examined whether perinatal thyroid hormone deficiency affects the development of USVs emitted by rat pups upon separation from the dam.

**Materials and Methods**

Thirty pregnant Wistar rats at gestational day (GD) 12 were purchased. These rats were housed in individual cages, and ten rats each were randomly assigned to the control, low-dose, or high-dose groups. Methimazole (MMI), an antithyroid drug, was dissolved in distilled water and administered to the pregnant rats via drinking water from GD 15 to postnatal day (PND) 21 at the following concentrations (w/v): 0% (control group), 0.01% (low-dose group), or 0.015% (high-dose group). On PND 4, the pups were culled to four males and four females per litter. USV recording was performed on PND 5, 10, 15, and 20 using the Sonotrack system (Metris, The Netherlands) with a sample of four male and female pups from each group, as follows. The pups were individually separated from the dam for 5 min, following which USVs were recorded for 3 min. The pups were subsequently returned to the dam.

The temperature of the room was maintained between 20°C and 23°C, and the relative humidity at 50%–70%. 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), and USVs were recorded during the dark period. The dams were supplied with rat chow and tap water *ad libitum*. The protocol was approved by the Animal Ethics Committee of Hokkaido University, and all experimental conditions were compliant with the Guide for the Care and Use of Laboratory Animals, Hokkaido University.

Two-factor analysis of variance (ANOVA) was performed for examining effects of MMI dose and sex of the pups on the number, duration, fundamental frequency, amplitude, and subtype of USVs. USVs of rat pups could be classified into various subtypes[5]; in the present study, they were classified into two subtypes on the basis of changes in the fundamental frequency of USVs. These subtypes include the flat frequency (FF) and frequency-modulated (FM) calls, where the differences between the maximum and minimum fundamental frequencies of USVs were <3 kHz[5] or ≥3 kHz, respectively. Three-factor ANOVA was employed for examining effects of variables of MMI dose, sex of the pups, and PND on the body weight of the pups. When a primary effect was found to be significant, multiple comparisons were performed using Ryan's method. These statistical analyses were executed using ANOVA 4 (http://www.hju.ac.jp/~kiriki/anova4/about.html). The USVs obtained on PND 20 were excluded from data analyses because low numbers of USVs were obtained regardless of the group.

**Results and Discussion**

The number of USVs obtained on PND 5, 10, and 15 for each group is shown in Figure 1. Age-dependent decrease in USVs was observed in the control and low-dose group. No significant differences among the various groups were observed on PND 5; however, MMI dose-dependent elevation in USVs was observed on PND 10 [F (2, 17) = 5.436, $p < 0.05$], and significant differences were observed on PND 15 [F (2, 18) = 12.119, $p < 0.0005$]; both low- and high-dose groups exhibiting an elevated number of USVs compared with the control group [ps < 0.05]. On the other hand, no significant differences were observed in the duration and fundamental frequency of USVs between the various groups on PND 5, 10, or 15. Moreover, sex of the pups failed to show correlation with the number, duration, and fundamental frequency of USVs on PND 5, 10, and 15.

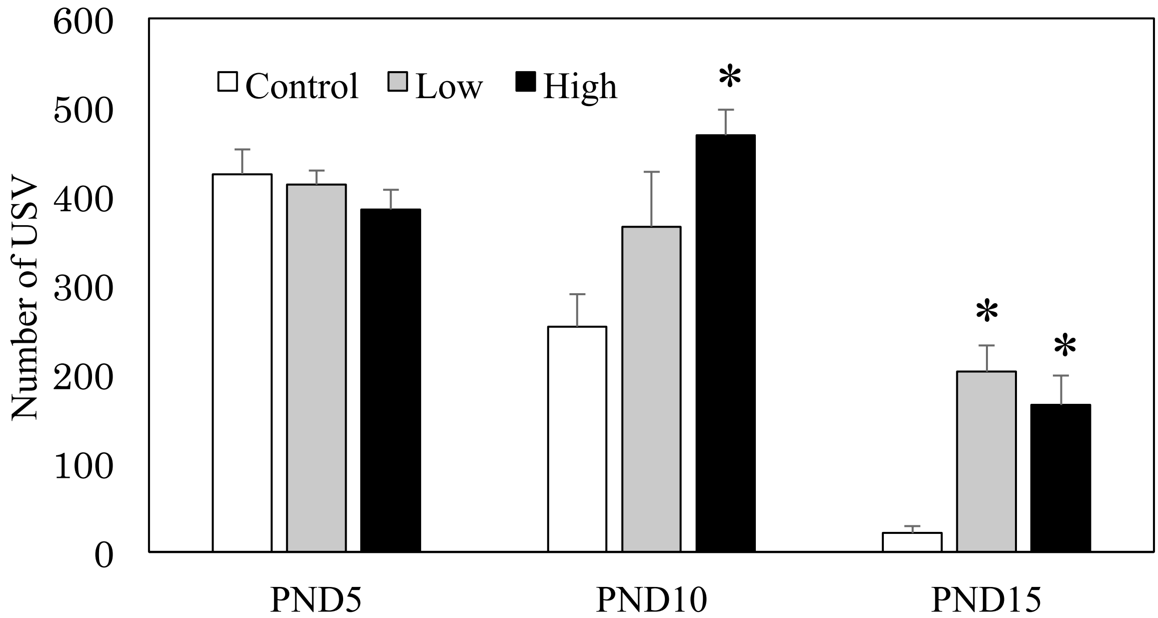


Fig. 1. Number of USVs recorded in a 3-min period upon separation of pups from the dam on PND 5, 10, and 15. Data represents mean ± SEM. *, $p < 0.05$ compared with the control group.

Figure 2 shows the root mean square (RMS) values of USV amplitudes on PND 5, 10, and 15. No significant differences were observed among the various groups on PND 5 and 10; however, significant differences were obtained on PND 15 [F (2, 17) = 7.518, $p < 0.005$]; both the low- and high-dose groups exhibited a greater RMS of USV amplitude than the control group (ps < 0.05). Sex of the pups did not influence the RMS values.

Figure 3 shows the percentage of FM calls on PND 5, 10, and 15. Age-dependent increase in FM calls was observed, and the various groups exhibited significant differences on PND 5 [F (2, 18) = 3.794, $p < 0.05$]; the high-dose group exhibited lower percentage of FM calls than the control group, with a significant difference ($p < 0.05$). Sex of the pups did not exert any effect on percentages of any of the USV subtypes.

Body weight changes are shown in Figure 4. The various groups exhibited differences in body weight gains [F (2, 18) = 45.869, $p < 0.001$]; both the low- and high-dose groups showed lower body weight gain than the control group (ps < 0.05). Significant interaction was obtained between MMI dose, age of the pups, and body weight gain [F (6, 54) = 47.768, $p < 0.001$]. Both the low- and high-dose groups exhibited lower body weight than the control group on PND 10 and 15 (ps < 0.05), whereas on PND 20, MMI dose-dependent decrease in body weight was observed (ps < 0.05). Sex of the pups did not exert any effect on body weight gain.

Studies employing animal models revealed an irreversible impairment of auditory system functions as a consequence of thyroid hormone deficiency[10], including distortion of the tectorial membrane in cochlea[8], loss of outer hair cells[9], and severe hearing deficits corresponding to the frequency range of 1–40 kHz[2]. Rat pups emit

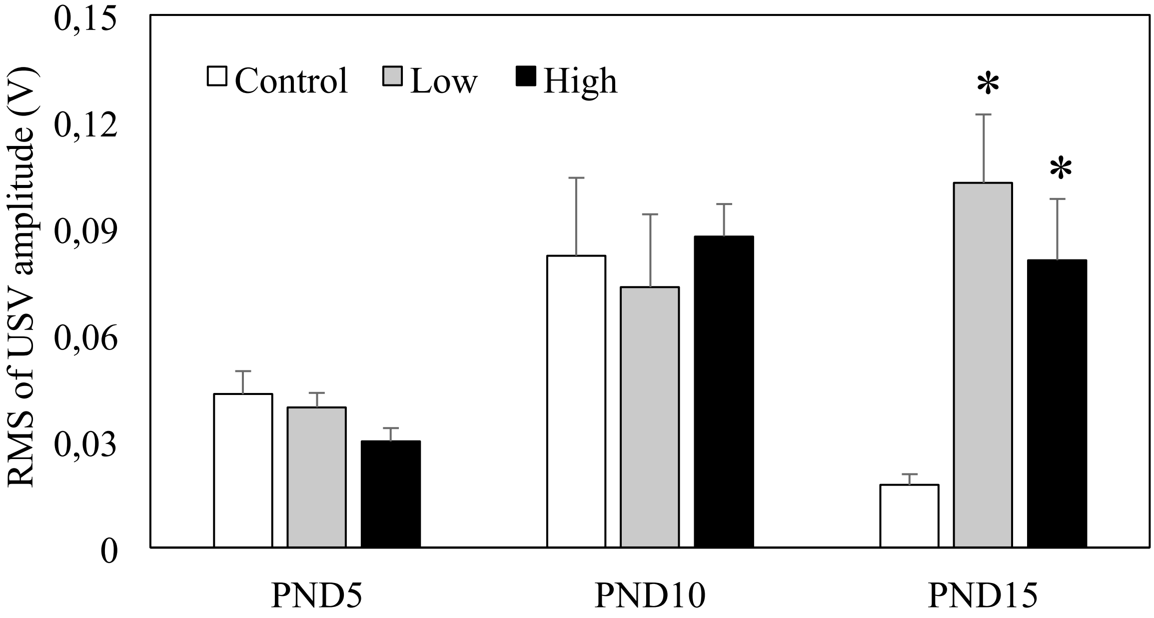


Fig. 2. RMS of USV amplitudes on PND 5, 10, and 15. Data represents mean ± SEM. *, $p < 0.05$ compared with the control group.

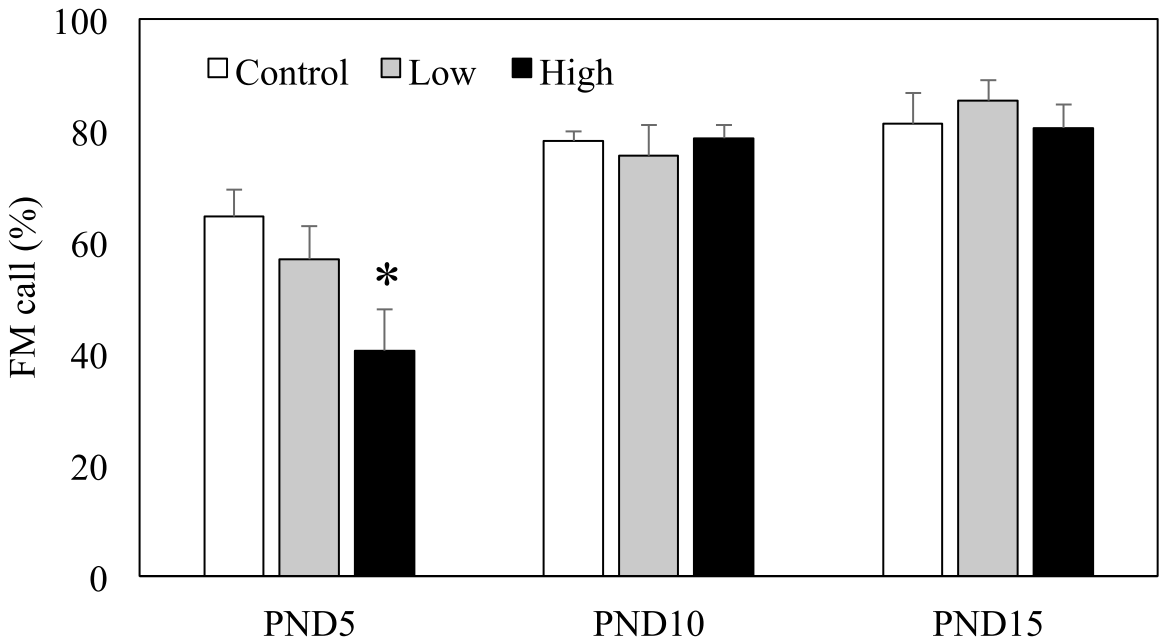


Fig. 3. Percentage of FM calls on PND 5, 10, and 15. Data represents mean ± SEM. *, $p < 0.05$ compared with the control group.

USVs of approximately 40-kHz frequency upon separation from the dam. Therefore, auditory system dysfunctions could potentially affect the development of USVs in rat pups.

In the present study, the control group was observed to exhibit age-dependent decrease in USVs, with the near-complete lack of USVs on PND 15. In contrast, as compared with the controls, both the low- and high-dose groups exhibited high number of USVs on PND 10 and significantly elevated USVs on PND 15. Furthermore, the

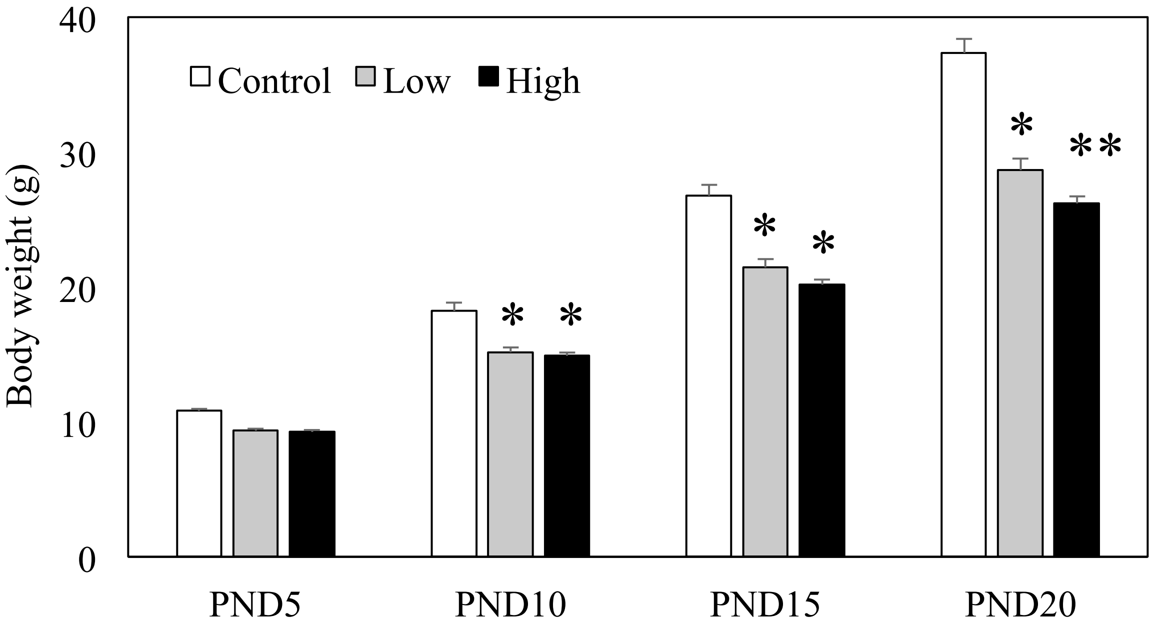


Fig. 4. Body weight changes on PND 5, 10, 15, and 20. Data represents mean ± SEM. *, $p < 0.05$ compared with the control group. **, $p < 0.05$ compared with both the control and low-dose groups.

RMS of USV amplitudes in the control group was greater on PND 10 than that on PND 5, and a decline was observed on PND 15. However, both the low- and high-dose groups exhibited greater RMS of amplitudes on PND 10 than that on PND 5, which was maintained on PND 15. Both the low- and high-dose groups emitted higher number of USVs with stronger amplitudes on PND 15 than the control group, suggesting the occurrence of hearing loss as a consequence of hypothyroidism.

Rat pups emit USVs of various subtypes, including flat, upward, downward, complex, chevron, harmonic, and so forth[5]. These subtypes could be broadly classified into two categories, the FF and FM calls, with frequency changes of <3 kHz and ≥3 kHz, respectively. Little is known about the communicative functions of the various USV subtypes, but studies suggest that the FM calls are likely to be crucial for communication between pups and their dam because they are more easily detectable compared with the FF calls[1]. In the present study, the high-dose group showed a reduction in the percentage of FM calls on PND 5 compared with both the control and low-dose groups. The inability of rat pups to move and regulate their body temperature in the absence of the dam on PND 5 suggests higher dependence on maternal care during the early lactation period[4]. Reduced emittance of the easily detectable FM calls by pups in the high-dose group suggests that the inability of the high-dose group to hear their own USVs because of auditory system dysfunctions could result in an impaired development of FM calls.


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