

## Perinatal Propylthiouracil-Induced Hypothyroidism Impaired Motor Coordination in Adult Female Offspring

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### Abstract

Thyroid hormone (TH) play crucial roles in the growth and development of brain. Efficiency of TH during the perinatal period results in severe mental and physical retardation, known as cretinism in humans. Animal models have largely focused on mild and severe hypothyroidism associated with deficits in body weight, developmental delays, and cognitive deficits. Although various behavioral analyses have been reported, the effect of perinatal hypothyroidism in adult female mice has not been clarified. The aim of this study was to examine whether propylthiouracil (PTU)-induced hypothyroidism could impair motor coordination in female offspring. We used C57BL/6j mice and divided them into three groups based on the dose of PTU which was applied during perinatal period (embryonic day-14 to postnatal day-14); control, 5 ppm, and 50 ppm groups. We observed motor coordination function and additional nociceptive test in female offspring. We found that motor coordination and nociceptive threshold were affected in 50 ppm groups. We concluded that the moderate hypothyroidism could impair motor coordination in adult offspring.

**Keywords:** moderate hypothyroidism, motor coordination

### Introduction

Thyroid hormone insufficiency during fetal and neonatal period impairs brain development.<sup>1,2</sup> TH is produced in the thyroid gland, through the iodination of tyrosine residues and coupling of iodotyrosine in the glycoprotein thyroglobulin. Secretion of TH is regulated by thyroid stimulating hormone (TSH) from the anterior pituitary.<sup>3</sup>

Maternal TH is the only source for fetus during the first trimester of gestation until birth. Severe reduction of TH during gestation is commonly associated with deficiency of iodine, which is an essential compound to synthesize TH. Reduced TH supply to fetus may cause mental retardation, deaf-mutism, motor dysfunction and cognitive impairment,

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which are sometimes irreversible even with early TH treatment after birth.<sup>4,5,7,8</sup>

Hypothyroid animal models, which are induced by administration of anti-thyroid drug such as PTU, are commonly used to study the role of TH on brain development. This drug inhibits TH synthesis by inhibiting thyroid peroxidase (TPO) activity. PTU treatment in perinatal period dramatically affects cerebellar development in rodent.<sup>6</sup> In the present study, we examined whether PTU-induced hypothyroidism could impair motor coordination in female offspring by conducted rotarod test and von Frey hair test as additional examination.

## Methods

### Animals

The animal experimentation in this study was approved by the Animal Care and Experimentation Committee of Gunma University. Mice (C57BL/6 strain) used in this study were bred in the Bioresource Center of Gunma University Graduate School of Medicine.

### Treatment Schedule

Animal treatment schedule is shown in Figure 1. Adult male and female mice were mated and the pregnancy was confirmed as embryonic day (ED) 1. Dams were administered 5 or 50 ppm of PTU in drinking water from ED 14 to postnatal day (PND)

21. Control mice were administered distilled water. Dams and all female offspring were separated at weaning day (PND 28). Motor coordination and nociceptive threshold test were conducted at PND 70.

### Weight Measurement

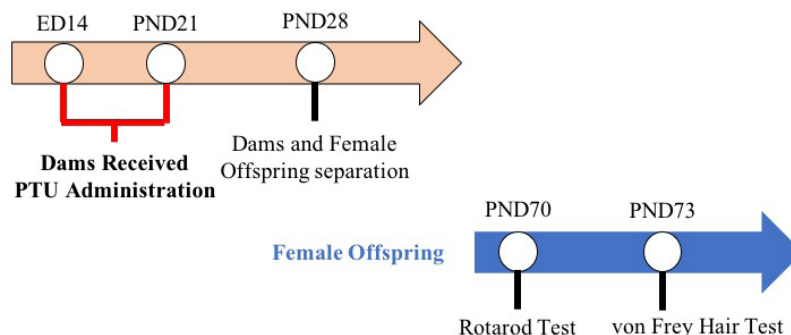
Female offspring were weighed at PND 7, PND 14, PND 21, and PND 28.

### Rotarod Test

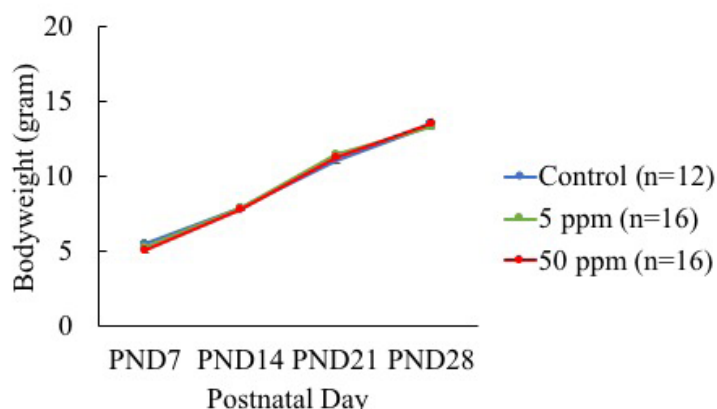
Rotarod test was performed to determine motor coordination and motor learning. Mice were placed on the cylinder drum of a rotarod apparatus (LE 8300, LSI Letica Scientific Instruments, Barcelona, Spain). Before the test, mice were habituated for 1 minute. The apparatus was started at a speed of 4 rpm and was accelerated to 40 rpm. The mice performed 5 trials per day. To evaluate motor learning, the test was performed for 3 consecutive days.

### von Frey hair test

von Frey hair test was conducted to determine nociceptive threshold. Mechanical force was applied using von Frey hairs with different buckle forces (0.2–4.0 g). Mice were individually placed on the platform, and filaments were applied to the hind paw. We performed 5 trials with 30 seconds intervals. The mechanical nociceptive threshold was defined as the lowest force that evoked paw withdrawal in at least 50% of the trials.



**Figure 1. Animal Treatment Schedule**



**Figure 2. Bodyweight Measurement**

#### *Statistical Analysis*

Statistical comparisons were performed by one- way ANOVA with Bonferroni post hoc test using SPSS Software version 22.0 (IBM SPSS, Armonk, NY, USA). Differences were considered significant at  $p < 0.05$ . All values are presented as the mean  $\pm$  SEM

#### **Results and Discussion**

This study is part of the author's research project in which previous results have been published.<sup>2</sup> TH status has been measured and TH levels on 5 ppm groups tend to decrease, although not statistically significant. On the other hand, 50 ppm groups showed low levels. Thus, we classified 5 ppm as "mild" and 50ppm as "moderate" hypothyroid mice model.

#### *Bodyweight Measurement*

TH plays an essential role in bodyweight regulation through regulating energy expenditure<sup>9,10</sup> Study in human, thyroid dysfunction cause significant changes in body weight and resting metabolic rate.<sup>10,11</sup> Study in rat showed that PTU at 3 ppm dose altered bodyweight of pups<sup>12</sup>, whereas no significant decrease have found in mice.<sup>1</sup> In present study, PTU administration in perinatal period did not decrease offspring's bodyweight (Figure 2),

which is consistent with the previous study in mice.<sup>1</sup> However, the mechanism underlying TH and bodyweight changes are not yet fully understood.

#### *Perinatal Hypothyroidism Impaired Motor Coordination*

As shown in Figure 3, the time spent on the rotarod was significantly decrease in PTU-treated groups in three consecutive days. Several brain regions are involved in motor coordination, but cerebellum plays the major role. TH regulates cerebellum function. Perinatal hypothyroidism reduced the growth and branching of Purkinje cell dendrites and the number of synapses between Purkinje cell dendrite and granule cell axons.<sup>13</sup> Abnormal cerebellar function may induce aberrant motor coordination.

#### *Perinatal Hypothyroidism Decreased Nociceptive Threshold*

Hypothyroidism decreased nociceptive threshold in 50ppm groups (Figure 4). vonFrey test reflects somatosensory cortex function. Sufficient maternal thyroid hormones may be necessary to determine a normal nociceptive function of offspring.<sup>14</sup> Previous study showed that hypersensitivity may alter motor coordination, and avoidance behaviour.<sup>15</sup>

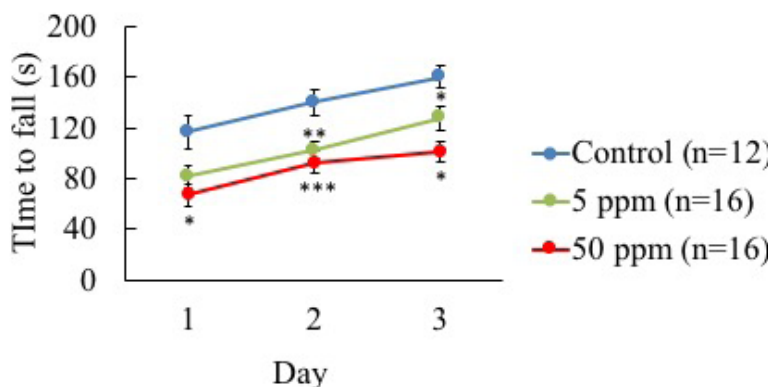


Figure 3. Rotarod Test

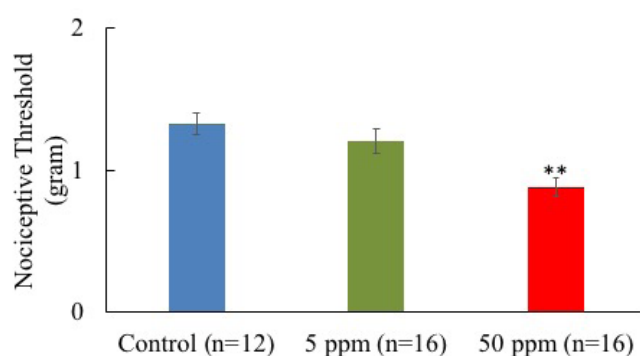


Figure 4. von Frey Hair Test

Regarding to the result, further study is fully required to link motor coordination, abnormal cerebellar development, and hypersensitivity.

### Conclusion

Perinatal PTU-induced hypothyroid impaired motor coordination in adult female offspring. Additionally, we also found that hypothyroidism induced hypersensitivity that may contribute in motor coordination impairment. However, to clarify these issues, additional study is required.

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### Conflict of Interest

None declared.

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