



In Utero and Postnatal Propylthiouracil-Induced Mild Hypothyroidism Impairs Maternal Behavior in Mice

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Thyroid hormones (THs) play crucial roles in general and brain development. Even if the hypothyroidism is mild, it may alter brain function, resulting in irreversible behavioral alterations. Although various behavioral analyses have been conducted, the effects of propylthiouracil (PTU) treatment during *in utero* and postnatal periods on maternal behavior have not yet been studied. The present study examined in mice whether THs insufficiency during development induce behavioral changes. Pregnant C57BL/6j mice were divided into three groups, and each group was administered different dosages of PTU (0, 5, or 50 ppm) in drinking water during *in utero* and postnatal periods (from gestational day 14 to postnatal day 21). First, locomotor activity and cognitive function were assessed in the offspring at 10 weeks. Next, female offspring were mated with normal mice and they and their offspring were used to assess several aspects of maternal behavior (identifying first pup, returning all pups to nest, time spent nursing, and licking pups). As expected, locomotor and cognitive functions in these mice were disrupted in a PTU dose-dependent manner. On postpartum day 2, dams who had been exposed 50 ppm PTU during *in utero* and postnatal periods displayed a significantly longer time identifying the first pup and returning all three pups back to the nest, less time nursing, and decreased licking behavior. The decrease in maternal behavior was significantly correlated with a decrease in cognition. These results indicate that insufficiency of THs during *in utero* and postnatal periods impairs maternal behavior, which may be partly induced by impaired cognitive function.

Keywords: maternal behavior, mild hypothyroidism, cognition, thyroid hormones, hippocampus

INTRODUCTION

Thyroid hormones (THs) (L-triiodothyronine, T3; thyroxine, T4) play crucial roles in the development and functional maintenance of the central nervous system. During development, THs regulate the growth and morphogenesis of brain and neuronal cells. In the cerebellum, for example, THs affect the dendritic growth of cerebellar Purkinje cells, the proliferation and migration of granule cells, and the synaptogenesis of cerebellar neurons (1). As a consequence, insufficiency of THs during development leads to disrupted motor coordination in adulthood (2, 3). Similarly in the hippocampus, the migration of granule cells and dendritic growth of pyramidal cells are also disrupted by hypothyroidism (4, 5), inducing aberrant synaptic function and learning (6). Increased anxiety- and depression-like behaviors in mice with hypothyroidism have also been reported (7–12). Overall,

these results indicate that THs act in multiple regions of the brain during development and adulthood, affecting various behaviors throughout life.

Maternal behavior is one of the social behaviors between mother and offspring. Several hormones, including estrogen (13, 14), prolactin (15), vasopressin, and oxytocin (15–17), are involved in regulating maternal behavior. In rats, maternal behaviors, particularly licking and grooming, regulate the development of the offspring's endocrine, emotional, and cognitive responses to stress (18). Maternal behavior tests have been used extensively as a parameter for brain development and stress responses (19). THs have also been implicated in maternal behavior by regulating hippocampal serotonin activity and glucocorticoid expression (20, 21). Recent studies have also shown that type 3 deiodinase (D3), which is a critical factor in regulating TH availability in the brain, may be also involved in controlling maternal behavior (12). D3 knockout mice showed impaired maternal behavior, increased aggression toward their offspring, and low levels of oxytocin and vasopressin with altered expression of their receptors (12). Although these results indicate that THs play an important role in controlling maternal behavior, the mechanisms of TH action on maternal behavior remain unclear.

In this study, we examined whether *in utero* and postnatal propylthiouracil (PTU)-induced mild hypothyroidism impairs maternal behavior in mice. To achieve this aim, we rendered pregnant mice hypothyroid by administering PTU in drinking water during pregnancy and the lactating period. Female offspring were used to examine the locomotor and cognitive functions using open field test, object recognition test (ORT), and object-in-location recognition test (OLT). This was followed by mating them with normal male mice and after delivery, assessing maternal behavior by examining pup retrieval characteristics, nursing, and licking times. Our study showed that even mild hypothyroidism disrupted maternal behavior and that the magnitude of disruption of maternal behavior was correlated with anxiety-like behavior and impaired memory function.

MATERIALS AND METHODS

Animals

The animal experimentation protocol in this study was approved by the Animal Care and Experimentation Committee of Gunma

University. All efforts were made to minimize the number of animals used in this study and their suffering. Mice (C57BL/6 strain) used in this study were bred in the Bioresource Center of Gunma University Graduate School of Medicine. They were kept at 24°C under a 12-h light/12-h dark cycle (light 7:00 a.m.–7:00 p.m.) and 50–70% relative humidity, with food available *ad libitum*.

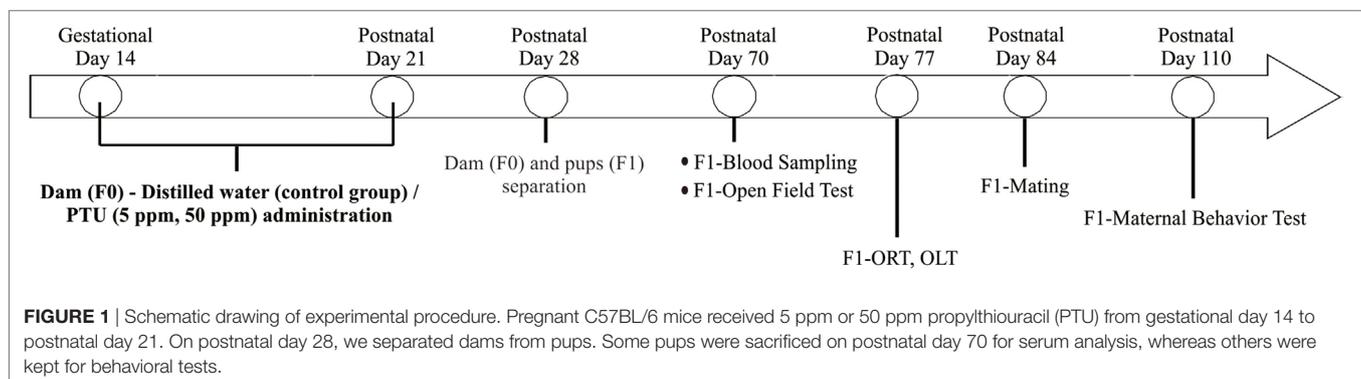
Treatment Schedule

A schematic figure of the animal treatment schedule is shown in **Figure 1**. Adult male and female mice were mated over 1 day and the pregnancy was confirmed as gestational day 1. Dams (termed F0) were rendered hypothyroid by administering either 5 or 50 ppm of PTU in drinking water from gestational day 14 to postpartum day 21 (22). Control mice were administered distilled water. After weaning on postpartum day 28, dams (control = 4 mice, 5 ppm = 6 mice, 50 ppm = 6 mice) and all female offspring (termed F1) (control = 12 mice, 5 ppm = 16 mice, 50 ppm = 16 mice) were separated and given distilled water only. Behavioral testing of F1 female offspring started at postnatal day 70. Treatment and individual housing continued throughout the behavioral testing period.

After behavioral tests, control and PTU-exposed female F1 mice were mated with normal male mice. Once pregnancy was suspected by abdominal enlargement, female mice were housed individually and checked every morning until delivery. Maternal behavior tests of F1 were performed at postnatal day 110. The number of mice in each experiment was described in Table S2 in Supplementary Material.

Blood Collection and TH Measurement

Blood was collected from a subset of F1 mice at postnatal day 70 under an intraperitoneal anesthetic (i.p.) injection of ketamine/xylazine (22.5 mg/ml ketamine and 1 mg/ml xylazine in 0.9% NaCl; 5 ml/kg). Ten F1 mice from five different F0 dams were used for measurement of TH levels in each group, while the remainder participated in the behavioral studies. Blood was withdrawn from the heart. Blood samples were then centrifuged (14,000 rpm) to separate serum. Serum was kept at –80°C until use. Free T4 (fT4) and free T3 (fT3) concentrations in serum were measured by chemiluminescent micro particle immunoassay (CMIA) on a single automatic analyzer using the architect SYSTEM (Abbott ARCHITECT i2000; Abbott Laboratories, Maidenhead,



United Kingdom). The lowest detectable limits were 1.00 pg/ml and 0.40 ng/dl for fT3 and fT4, respectively (23). Five tube samples were used for measurement, and serum samples from two mice in the hypothyroidism group were collected in each tube. Sensitivity threshold values were fitted when the concentration was below the sensitivity limits.

Behavioral Tests Before Mating

As shown in **Figure 1**, behavioral tests for F1 were performed from postnatal day 70 to 110.

Open Field Test

As described in previous studies (23, 24) and shown in **Figure 3A**, each mouse was individually placed in the center of a 45 cm × 45 cm × 20 cm (width × length × height) open-field chamber containing 16 × 16 crossed infrared beams set along the sides at intervals of 2.5 cm (**Figure 3A**; LE 8811, Panlab, S.L.U. Barcelona, Spain) (23). Locomotion was recorded for 30 min by tracking the position of the mouse and the number of times that beams were crossed. Data were analyzed using the Acti-Track program (Panlab, S.L.U.). The total distance traveled and time spent in the central area (23 cm × 23 cm in the middle of the field) was measured. Additionally, data were presented in three different times to define the onset time of anxiety-like-behavior.

ORT and Object-in-Location Recognition Test (OLT) in Rodents

As shown in **Figure 4A**, ORT/OLT were performed in a 30 cm × 30 cm × 39 cm white, rectangular open field as reported in previous studies (22, 25). Mice were exposed to the experimental apparatus in the open field for 15 min/day for 3 days to habituate them to the environment. The recognition tests (ORT/OLT) are consisted of a training phase and a test phase individually. During the training phase (5 min), two identical objects were placed in the field. After a 10-min retention interval, mice were placed back in the field to do the test phase (3 min). To examine object recognition memory, one of the two objects was replaced by a novel object in a test phase (ORT) (22, 25). After ORT session, we performed a training phase for OLT, and changed the location of one object in a test phase to examine object-in-location memory (OLT) (**Figure 4B**) (25). Previous studies showed mice tended to spend a longer time exploring the novel object/location if they remembered the previous object (22, 25). Mice were performed the tests in one-time trial. All sessions were recorded on video (Sony DVR 4 camera, recording resolutions: HD 352 × 480; 330,000-pixel, frame rates: 10 frames per second) and exploration time was measured manually with a stopwatch. The discrimination ratio was determined by dividing the time spent exploring the novel object by the total time exploring both objects during the test session.

Maternal Behavior Tests

Pup Retrieval Behavior

In order to assess maternal behavior in F1 mice, we first measured the length of time required to retrieve the pups that had been removed from the nest using a slightly modified protocol from a previous study (15). The test was performed during the dark

phase of F1 at postnatal day 110. The experimental procedure was recorded with a video camera. Initially, dams and pups were briefly removed from their home cage. Then, three pups were placed in different corners of the cage, away from the nest. The dam was then reintroduced into the cage. Time of first pup identification was defined as the time that the dam took to touch a pup with her nose. Such behavior was followed by picking up the pup in her mouth to bring it back to the nest. The time required for first pup identification and for replacing all three pups in the nest was measured manually using a stopwatch. If the mice could not retrieve all pups within 15 min, the test was interrupted and remaining pups were replaced in the nest.

Nursing and Pup Licking

F1 dam-pup interaction was also measured by observing arched-back nursing and pup-licking behaviors (26). Time spent nursing was defined as the time that mice spent in the nest in an arched-back posture. Behaviors not related to dam-pup interaction (leaving the nest, eating, and drinking) were also measured. These behaviors were observed for two 1-h periods per day (in both the light phase, 9:00–10:00, and the dark phase, 1:00–2:00). The behavior of mice was recorded with a video camera, and time was measured manually with a stopwatch.

Statistical Analysis

Statistical comparisons were performed by one- or two-way ANOVA followed by the 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 ± SEM.

To analyze the relationship between cognitive function and maternal behavior (**Figure 7**), the correlations between discrimination ratio of object recognition or object-in-location and maternal behavioral parameters were calculated using the correlation graph simple linear regression method by SPSS.

RESULTS

The Effect of PTU Treatment on the Number of Pups Delivered

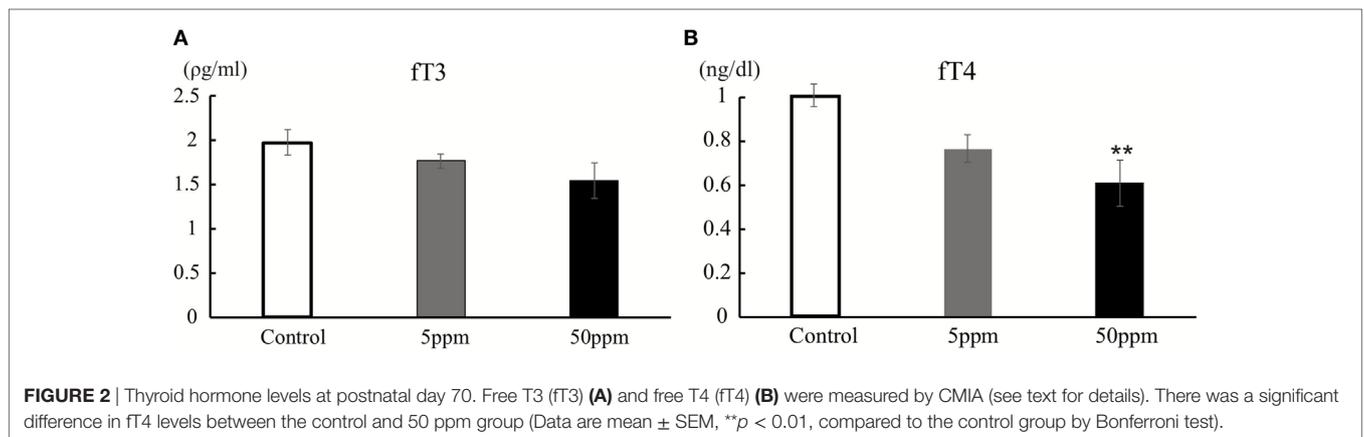
Propylthiouracil treatment did not induce failure of delivery or abandonment of offspring in F0 mice (data not shown), whereas PTU treatment during *in utero* and postnatal periods did induce such tendencies of F1 mice in dose-dependent manner (Table S1 in Supplementary Material). In F1, one mouse treated with 5 ppm PTU did not deliver pups at all, although the pregnancy was confirmed. Furthermore, two mice treated with 50 ppm PTU did not deliver and four mice abandoned their offspring. After excluding these mice, the number of delivered pups was counted (**Table 1**). There was no significant difference in the number of delivered mice in F0 groups, whereas in F1 mice, PTU treatment during *in utero* and postnatal periods significantly affected the number of pups delivered. While control mice delivered 6.25 ± 0.372 mice, mice treated with 5 or 50 ppm only delivered 4.87 ± 0.274 mice or 3.6 ± 0.267 mice, respectively (by Bonferroni test; $p = 0.007$, control vs. 5 ppm group; $p = 0.008$, control vs. 50 ppm group).

TABLE 1 | Number of offspring from individual mothers.

Dam	Control			5 ppm			50 ppm		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
F0 Generation									
F0-1	3	4	7	2	5	7	3	6	9
F0-2	2	4	6	1	6	7	1	4	5
F0-3	3	3	6	2	4	6	2	3	5
F0-4	4	5	9	2	3	5	1	2	3
F0-5	–	–	–	4	3	7	2	3	5
F0-6	–	–	–	0	2	2	3	5	8
F0-6	–	–	–	0	2	2	3	5	8
Average			6.67			5.67			5.83
SEM			0.494			0.802			0.901
F1 Generation									
F1-1	2	5	7	4	2	6	2	3	5
F1-2	3	4	7	3	3	6	2	1	3
F1-3	1	5	6	2	3	5	2	0	2
F1-4	2	3	5	2	2	4	1	3	4
F1-5	3	2	5	2	3	5	1	2	3
F1-6	4	2	6	3	4	7	2	2	4
F1-7	3	2	5	1	3	4	3	1	4
F1-8	3	5	8	2	2	4	2	2	4
F1-9	5	1	6	3	1	4	1	2	3
F1-10	3	6	9	2	3	5	1	3	4
F1-11	2	3	5	3	1	4	–	–	–
F1-12	4	2	6	4	2	6	–	–	–
F1-13	–	–	–	3	2	5	–	–	–
F1-14	–	–	–	1	4	5	–	–	–
F1-15	–	–	–	2	1	3	–	–	–
Average			6.25			4.87**			3.60***
SEM			0.372			0.274			0.267

** $p < 0.01$, *** $p < 0.001$.

The failure number of F1 dams of giving birth and taking care of pups were omitted.

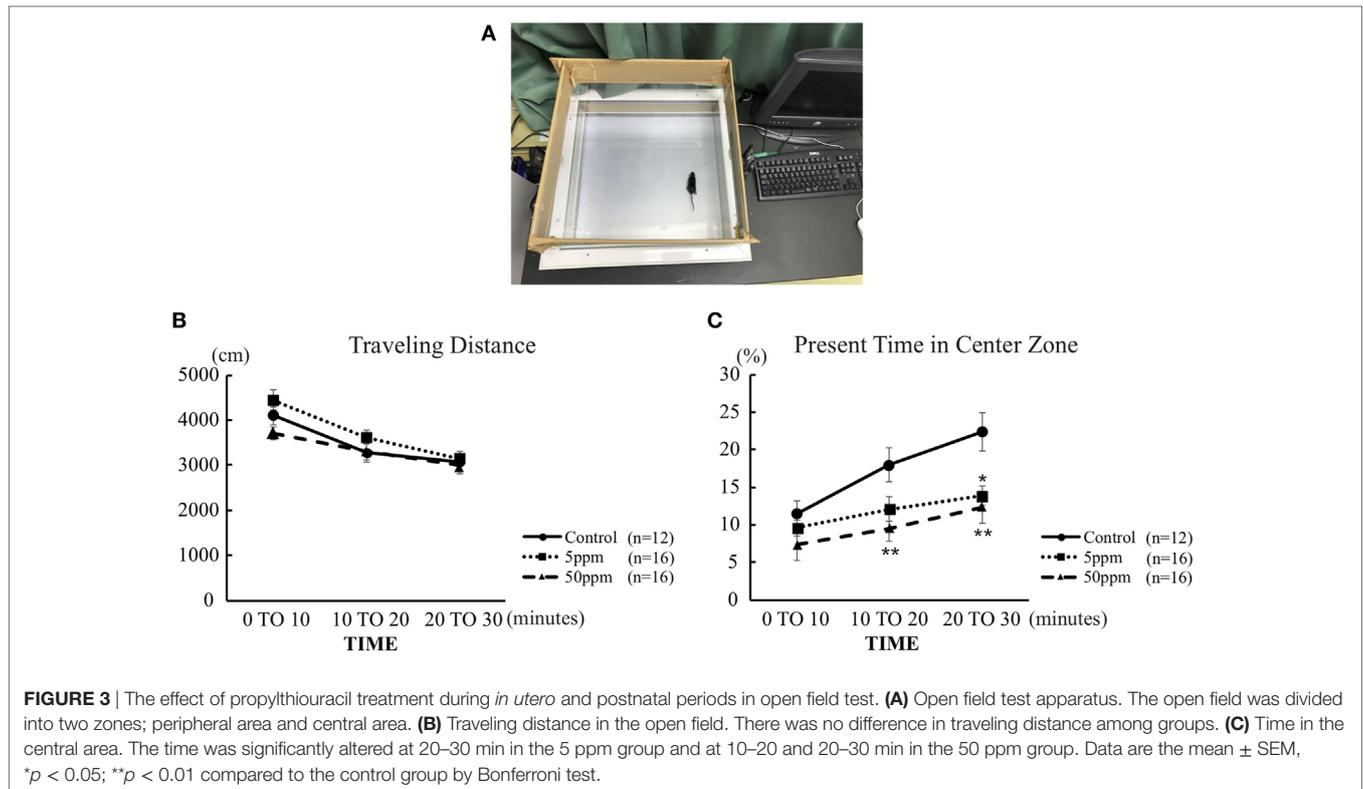


Change in TH Concentrations in F1 Mice

As shown in **Figure 2**, there was no significant difference in ft3 levels among F1 groups (by ANOVA $F_{(2,12)} = 2.051$; $p = 0.171$). However, ft4 levels were significantly altered by PTU treatment during *in utero* and postnatal periods in F1 mice [by ANOVA; $F_{(2,12)} = 6.790$; $p = 0.011$]. *Post hoc* analysis showed that ft4 levels in the 50 ppm group were lower than those of the control group (by Bonferroni test; $p = 0.008$), signifying PTU exposure leads to mild hypothyroidism in the 50 ppm group, even as late as postnatal day 70.

The Effect of PTU Treatment During *in Utero* and Postnatal Periods on Locomotor Activity and Cognition Changes in Locomotor Activity

We first performed the open field test on F1 mice. Activity in the open field was monitored for 30 min. As shown in **Figure 3B**, there was no significant difference in traveling distance among groups. However, as shown in **Figure 3C**, 5 ppm group spent less time in the central area over 20–30 min compared with the control group



(by Bonferroni test; *p* = 0.032), whereas 50 ppm group spent less time in the central area over 10–20 and 20–30 min (by Bonferroni test; *p* = 0.010 at 10 min, *p* = 0.007 at 20 min). These results indicate an increase in anxiety induced by mild and moderate hypothyroidism without altering locomotor activity.

Aberrant Cognitive Function

In the ORT, the discrimination ratio was significantly decreased in the 50 ppm group compared with the control group (by Bonferroni test; *p* = 0.042) (Figure 4C). On the other hand, the 5 ppm group did not show any significant difference compared with the control group. In the OLT, the discrimination ratio decreased significantly in both the 5 and 50 ppm groups compared with the control group (*p* = 0.035) (Figure 4D). These results indicate that mild hypothyroidism induces cognitive impairment. These results are essentially the same as those reported in previous studies (9, 27).

Alteration of Maternal Behavior Induced by PTU Treatment During in Utero and Postnatal Periods

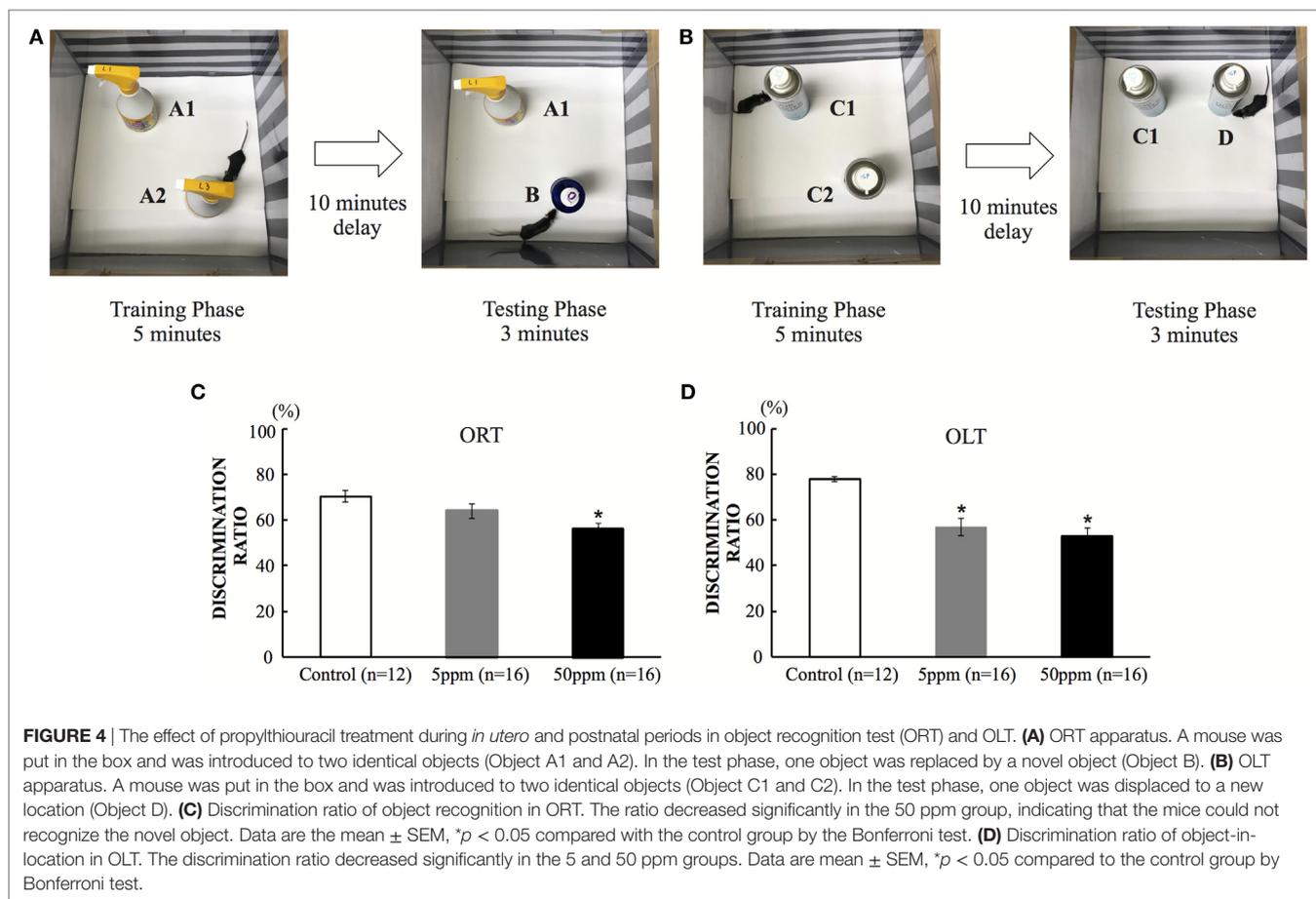
Disruption of Pup Retrieval

To examine the effect of hypothyroidism on maternal behavior, we first examined the pup retrieval behavior of F1 mice on postnatal day 110. Dams were transiently removed from the cage. Three pups were placed in a different corner of the cage, outside of the nest. Immediately after the dam was returned to the cage, the dam identified the first pup within a few seconds, picked it up and replaced in the nest. As shown in Figure 5B, ANOVA

showed a significant treatment effect ($F_{(2, 34)} = 6.482; p = 0.004$), indicating that PTU treatment during *in utero* and postnatal periods altered the time required to identify the first pup. As shown in Figures 5B,C, mice in the 50 ppm group required a significantly longer time to identify the first pup (control mice 2.13 ± 0.235 s; 5 ppm 3.04 ± 0.472 s; 50 ppm, 4.89 ± 1.081 s; *p* = 0.028, by Bonferroni test). They also took longer to place all three pups back in the nest (by Bonferroni test; *p* = 0.0001 vs. control). Some mice in the 50 ppm group required longer than 15 min to finish retrieval. However, the time to finish retrieval in the 5 ppm group was not different from that of control mice.

Decrease in Nursing and Pup Licking

We evaluated two aspects of F1 dams' behavior on postnatal day 2. One aspect is related to mother–pup interactions, such as taking a nursing posture (arched-back nursing) in the nest and licking the pups. The other aspect examined behaviors not related to mother–pup interaction, such as time spent outside of the nest, and time spent eating and drinking. The behaviors were observed for 1 h, twice a day (in the light phase and in the dark phase). A significant treatment effect was observed by ANOVA for nursing behavior [light phase, $F_{(2, 34)} = 152.973, p < 0.001$; dark phase, $F_{(2, 34)} = 61.637, p = 0.0001$], and pup licking [light phase, $F_{(2, 34)} = 30.048, p = 0.0001$; dark phase, $F_{(2, 34)} = 120.655, p = 0.0001$], indicating that PTU treatment during *in utero* and postnatal periods disrupted behaviors related to mother–pup interaction (Figures 6A,B). *Post hoc* analyses revealed mice in the 50 ppm group showed significantly decreased nursing in both



the light and dark phases (light phase, $p = 0.037$; dark phase, $p = 0.003$) and pup licking in the dark phase ($p = 0.002$), whereas no significant difference in 5 ppm group compared to control group. We also found that PTU treatment during *in utero* and postnatal periods altered the time spent outside of the nest in both groups [light phase, $F_{(2,34)} = 91.519$; 5 ppm, $p = 0.035$; and 50 ppm, $p = 0.000$. dark phase, $F_{(2,34)} = 91.519$; 5 ppm, $p = 0.01$ and 50 ppm, $p = 0.0001$] (**Figure 6C**). *Post hoc* analysis showed a significant increase in the time spent outside of the nest in both the 5 ppm ($p = 0.028$) and 50 ppm ($p = 0.003$) groups. Because the mouse is a nocturnal animal, they usually spend longer eating and drinking during the night (dark phase). Control mice showed a normal circadian rhythm by spending longer time eating and drinking during the dark phase, whereas PTU-treated mice lacked this circadian rhythm (**Figure 6D**). These results indicate that *in utero* and postnatal PTU-induced mild/moderate hypothyroidism disrupted maternal behavior.

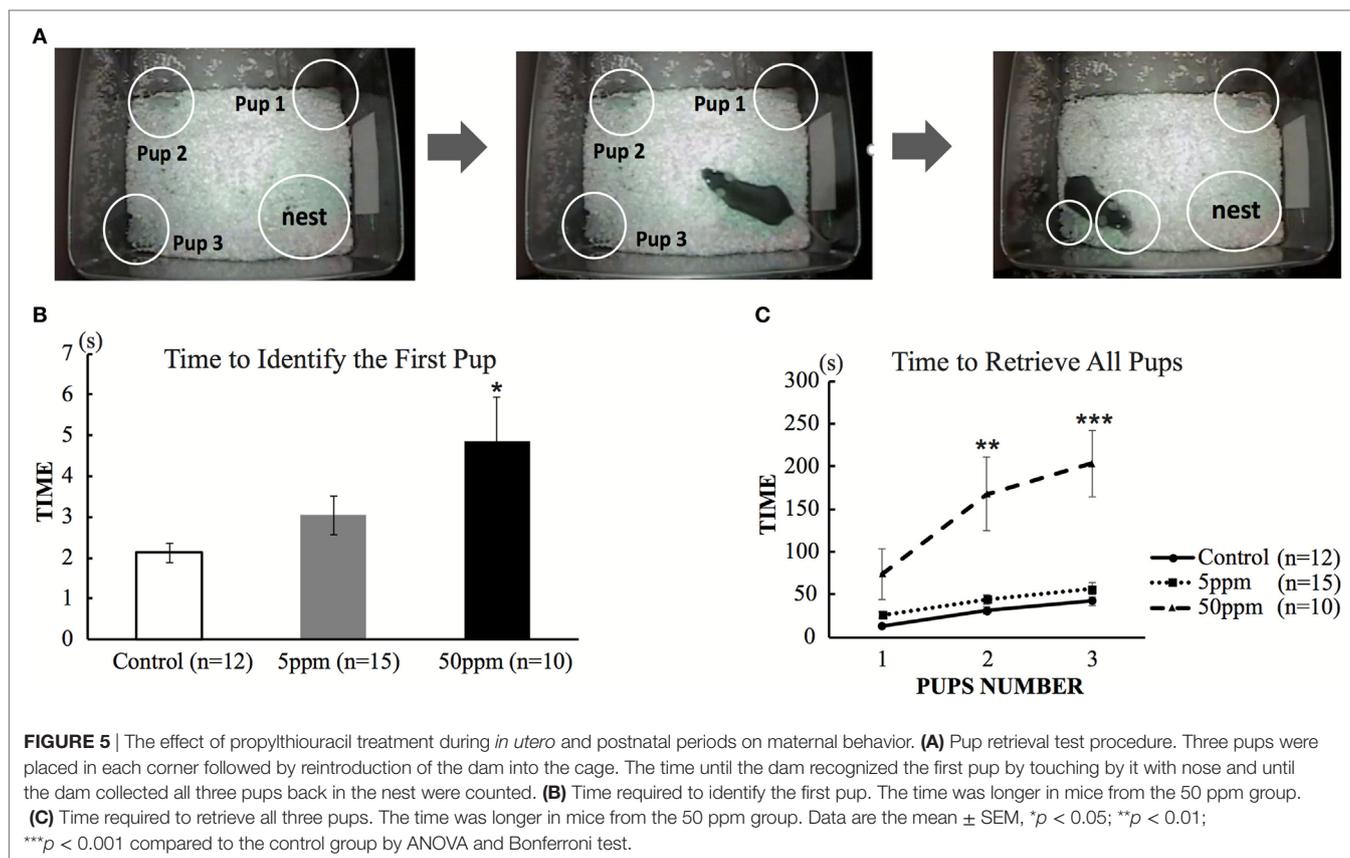
Correlation Between Discrimination Ratio of Object Recognition or Object-in-Location and Maternal Behaviors

In light of previous findings showing maternal behavior is associated with cognitive function (26, 28), we correlated the maternal behavior parameters with the discrimination ratios for the memory function of Object Recognition or Object-in-Location measured

in the present study. There were significant negative correlations between discrimination ratio of object recognition and first pup identification time ($R = -0.58$; $p = 0.0001$) (**Figure 7A**), as well as three pup retrieval time ($R = -0.43$; $p = 0.003$) (**Figure 7B**). There were no statistical significances between the discrimination ratio of object recognition and either nursing time or pups licking time (Figure S1 in Supplementary Material). In addition, there were significant positive correlations between the discrimination ratio of object-in-location and both nursing time and pup-licking time ($R = 0.53$; $p = 0.0001$ and $R = 0.64$; $p = 0.0001$) (**Figures 7C,D**). In contrast, no significant correlations were observed between the discrimination ratio of object-in-location and both time required for identification of the first pup and three-pup retrieval time (Figure S1 in Supplementary Material). There was no significant correlation between open field test and any of the maternal behavioral parameters (data not shown). Overall, these results indicate that cognitive impairment caused by PTU treatment during *in utero* and postnatal periods may be partly involved in generating aberrant maternal behavior.

DISCUSSION

In the present study, we examined the effect of PTU treatment during *in utero* and postnatal periods on maternal behavior in mice. We measured THs of F1 mice in adulthood and found a



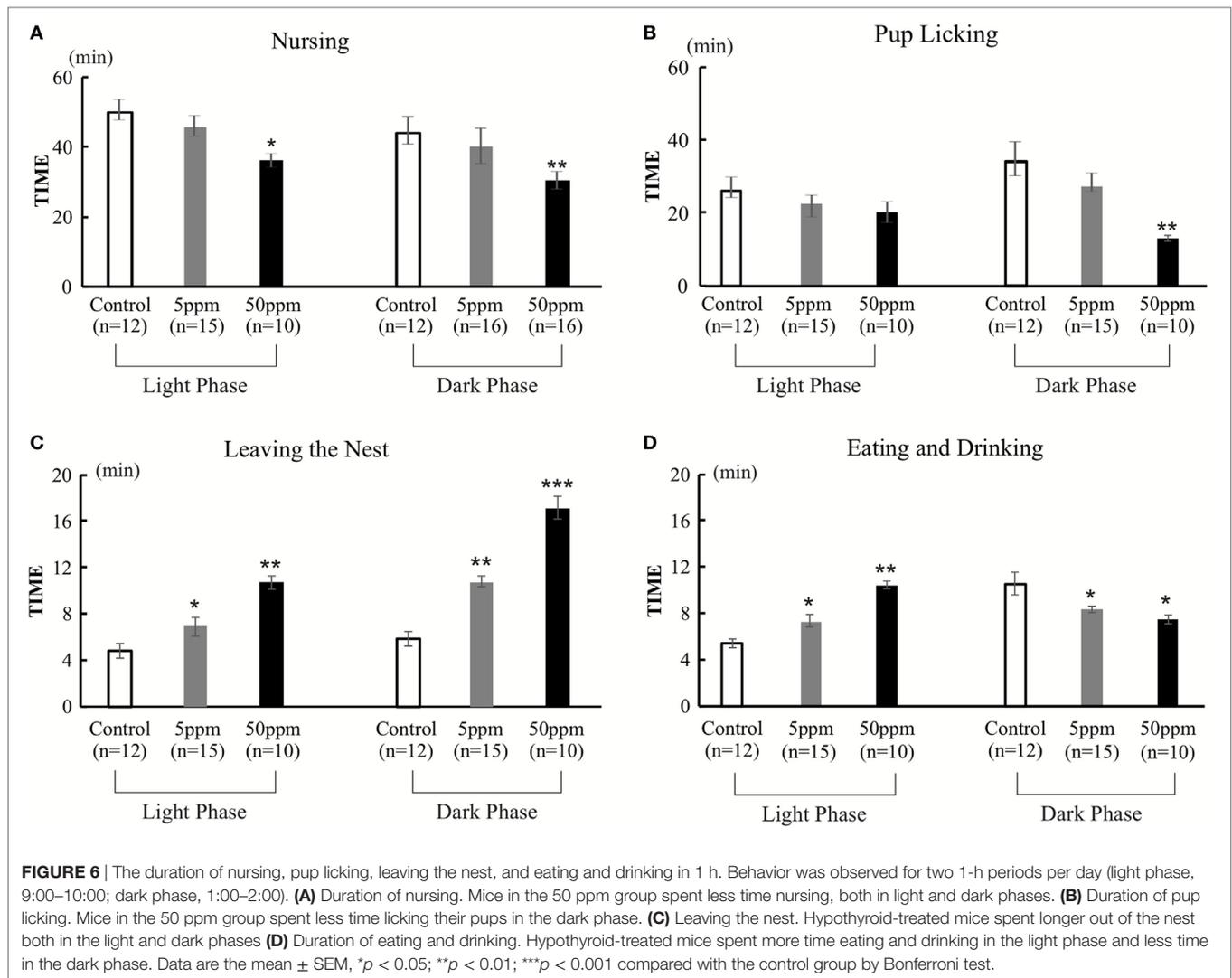
PTU dose-dependent weak decrease in fT4 levels, indicating that, although hypothyroidism during brain development may be mainly responsible for altering behavior, a continuous decrease in TH levels may have also contributed to behavioral alteration. As reported previously in male rats (29), *in utero* and postnatal PTU-induced mild/moderate hypothyroidism increased anxiety (Figure 3), impaired cognitive function (Figure 4), disrupted maternal behavior, such as increased time to identify and retrieve pups (Figure 5), decreased time for nursing and pup licking, increased time for being away from the nest, and disrupted circadian rhythm in eating and drinking (Figure 6). The magnitude of cognitive deficit was correlated with the level of observed aberrant maternal behavior (Figure 7). Moreover, we found PTU treatment during *in utero* and postnatal periods significantly affected the number of F1 offspring. These results, therefore, indicate that *in utero* and postnatal PTU-induced mild/moderate hypothyroidism disrupted maternal behavior at least in part through impaired cognitive function.

Previous studies have shown that low dose (1 ppm) PTU treatment during *in utero* and postnatal periods in rats caused a decrease in total T4 levels, and 3 ppm treatment elevated thyroid-stimulating hormone levels in postnatal day 14 (30) or postnatal day 21 (31), but not in postnatal day 90. Another study in rats showed that 3 ppm PTU treatment did not alter total T3 (tT3) on postnatal day 3, but decreased on postnatal day 15, whereas tT3 returned to be normal on postnatal day 22 (6). In the present study, we also measured fT3 and fT4 levels to investigate whether

the exposure of PTU treatment during *in utero* and postnatal periods caused the changes of the TH status at postnatal day 70. We found both fT3 and fT4 levels on 5 ppm-treated group tend to decrease, even though not statistically significant. On the other hand, 50 ppm-treated group showed low TH levels (Figure 2). Based on these results, we categorized 5 ppm group as “mild” and the 50 ppm group as “moderate” hypothyroid mice model. Although the mechanisms causing this continuous decrease in TH levels are not yet clear, PTU treatment during *in utero* and postnatal periods may have permanently disrupted the function of the thyroid gland, as well as exposed the pups to lower levels of maternal THs.

Thyroid hormones have direct effects on granulosa cells, luteal cells, and oocytes, indicating a direct interference with normal ovarian function in animal (32) and human cases (33, 34). Therefore, hypothyroid condition may cause disruption of reproductive function and development (35–37). In animal study, abnormality of THs level impaired the development of ovarian cells and caused infertility (32). In this study, we found that 50 ppm F1 generation showed lower number of pups (Table 1). This result indicates *in utero* and postnatal PTU exposure may disrupt placental and ovarian development and consequently decrease fecundity.

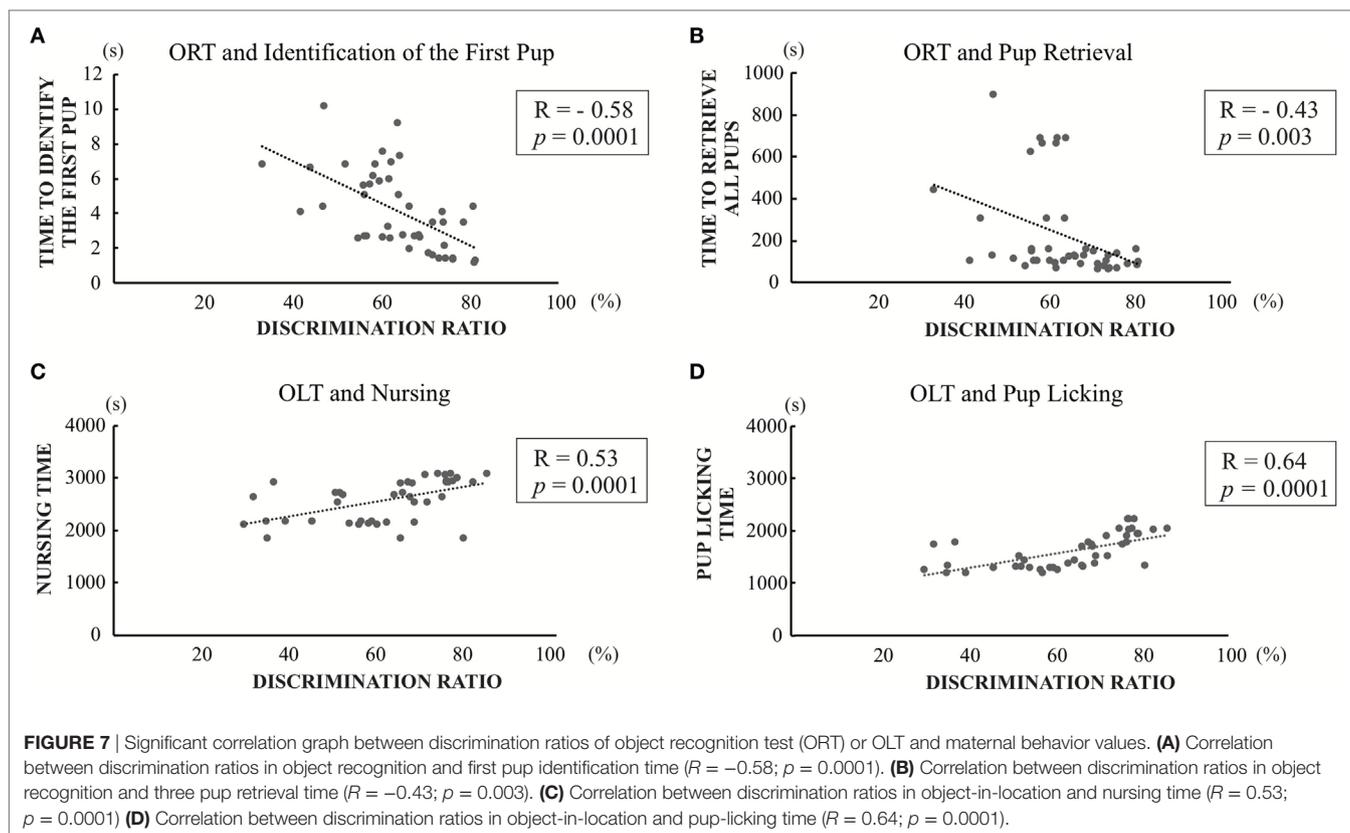
In this study, we evaluated the maternal behavior of F1 offspring, and found that the behaviors were affected by PTU exposed during *in utero* and postnatal periods (Figures 5 and 6). Responses or behaviors shown by mammalian females are



important for the physiological and behavioral development of their offspring (38). A study of an *in utero* maternal process showed that the fetal stage is a crucial period for behavioral development (15). In rodents, maternal behaviors are exhibited in several ways, including pup retrieval, licking, nursing, and crouching over pups to keep them warm (39). The expression of maternal behaviors during gestation and postpartum period is regulated by dynamic changes in hormonal secretion (39, 40), such as prolactin, that can affect the nurturing behaviors of both mothers and their female offspring (15). Prolactin acts in the medial preoptic area (39, 41) to promote the activation of neural circuitry that is associated with nurturing behavior in adulthood. On the other hand, variations in maternal licking/grooming and arched-back nursing have been associated with the development of neural systems that mediate hypothalamic–pituitary–adrenal and behavioral responses to stress as well as certain forms of learning and memory (20, 26, 42). Furthermore, multiple sensory inputs such as visual, auditory, olfactory, and tactile stimuli trigger maternal behavior (43). According to a study in rats, perinatal hypothyroidism results in irreversible damage to

auditory function resulting in hearing loss (44); consequently, F1 mothers may not be able to hear ultrasonic vocalization or calls produced by pups that should have immediately induced retrieval (45). Because THs are responsible for the development of various neuronal and neuroendocrinological functions, PTU treatment during *in utero* and postnatal periods may have disrupted functional development of such systems even if it is mild or moderate.

In the open field test, we found no significant difference in traveling distance among groups (Figure 3A), although the decreased time spent in the center zone by the PTU-treated group may indicate an increase in anxiety (Figure 3B). Increased anxiety due to *in utero* and postnatal hypothyroidism (9) and in thyroid receptor knockout mouse models (11) has been reported previously. Moreover, previous studies have shown that TH manipulation in early life affects hippocampal glucocorticoid receptor protein levels (20, 46). In the present study, we also found that PTU treatment during *in utero* and postnatal periods induced anxiety-like behavior in female mice. Thus, increased anxiety may alter maternal behavior.



We evaluated the disruption of cognitive function in mice by conducting Object Recognition and Object-in-Location Tests. Previous studies have shown that developmental TH insufficiency impairs spatial learning memory in rodent models (29). Our results showed that the discrimination ratio of object recognition of the 50 ppm group decreased significantly (Figure 4C) and considered to be influenced by either the hippocampal or cortical lesions (47). Among such brain regions, the perirhinal cortex may play a major role in controlling object recognition memory (48). When this region is damaged, object recognition performance is impaired (49). On the other hand, the result for OLT showed that the discrimination ratio of object-in-location in both PTU-treated groups decreased significantly (Figure 4D), suggesting that the mice may not be able to discriminate the object in the new location due to decreased spatial memory acquisition (50). Such impairments may be caused by aberrant function of the hippocampus (51, 52). Previous studies have shown that maternal care is associated with spatial learning and memory (26, 28). This possibility has been confirmed further in the present study, showing correlations between discrimination ratio of object recognition or object-in-location and maternal behavior values (Figure 7). Although additional analysis may be required, *in utero* and postnatal PTU-induced mild/moderate hypothyroidism disrupted maternal behavior, which may be caused in part by impairment of cognition and anxiety.

We previously reported the effects of PTU administration on the TH status and cognitive function in male mouse offspring,

using the same administration schedule as for the present study (either 5 ppm or 50 ppm) (22). Unlike the rat model, which showed a significant reduction of serum T3 and T4 levels and growth retardation by 10 ppm PTU, and decreased T3 levels with increased TSH levels by 3 ppm (6), our mouse model with 5 ppm did not show any increase in TSH levels at postpartum day 21 in both dams and pups. Instead, only a weak decrease in ft4 levels was observed only in dams by 5 ppm PTU. With 50 ppm treatment, on the other hand, although dams showed an elevation of TSH levels with decreased ft3 and ft4 levels, pups showed only a weak decrease in ft4 levels without a corresponding increase in TSH levels. Despite such a small change in TH status, offspring of both groups showed a disrupted cognitive function in a PTU dose-dependent manner in both male (22) and female (present study) offspring. Furthermore, 50 ppm-treated offspring showed decreased levels of NMDA receptor subunits in the hippocampus, whereas such decrease was not observed in 5 ppm-treated group. Taken together with these findings, we defined 5 ppm PTU group as “mild” and 50 ppm group as “moderate.” However, these definitions may be rather subjective as we do not have clear criteria to discriminate among mild, moderate, and severe hypothyroidism in mouse model. Further study with finer titration of PTU dose may be required to clarify such difference. Nevertheless, our present study clearly demonstrated that gestational and postpartum PTU treatment that induced only a little alteration of TH status in both dams and pups caused an alteration of maternal behavior of offspring, indicating an important role of TH on regulating such behavior.

There are some limitations in this study. First, although the decrease in TH levels in PTU-treated F1 mice at postnatal day 70 was observed, it is not clear whether the behavioral alterations seen in F1 rodents are due to prenatal or postnatal hypothyroidism or to maternal hypothyroidism during gestation. TH may regulate target gene expression differently during prenatal and postnatal period. Maternal hypothyroidism may alter the expression of maternal factor that may play an important role in fetal development. Second, our behavioral results indicated that the effect of hypothyroidism is different among the brain functions and some of them are critically affected even under small TH changes. However, no report has shown the lowest PTU dose that causes adverse effect. To clarify these issues, additional study including gene expression analysis is required.

In summary, mild or moderate hypothyroidism due to PTU treatment during *in utero* and postnatal periods were seen to disrupt maternal behavior by inducing anxiety and memory impairment. Additionally, we found that a decrease in fecundity of offspring may be caused by abnormal TH status. Our series of studies have provided useful information for further understanding the role of THs on the construction in pregnancy and mother-offspring interactions. More careful attention may be required to support hypothyroidism patients when they want to have children, even if their TH status is within normal ranges. Furthermore, precise control of thyroid function during pregnancy and the lactating periods may be important for both maternal care and cognitive function development in offspring.

ETHICS STATEMENT

The animal experimentation protocol in this study was approved by the Animal Care and Experimentation Committee of Gunma University. All efforts were made to minimize the number of animals used in this study and their suffering.

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AUTHOR CONTRIBUTIONS

MAK, YT, and IA conducted the complete experiment and prepared the data and manuscript. WM and NK had responsibility for the whole experiment, earned the grant, made the strategy, and prepared the manuscript. AH and MK contributed by experimental support.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at <https://www.frontiersin.org/articles/10.3389/fendo.2018.00228/full#supplementary-material>.

FIGURE S1 | Insignificant correlation graph between discrimination ratios of object recognition or object-in-location and maternal behavior values. **(A)** Correlation between discrimination ratios of object recognition and nursing time ($R = 0.28$). **(B)** Correlation between discrimination ratios of object recognition and pup licking ($R = 0.36$). **(C)** Correlation between discrimination ratios of object-in-location and identification of the first pup ($R = -0.25$). **(D)** Correlation between discrimination ratios of object-in-location and three pup retrieval time ($R = -0.38$).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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