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1 **Title:** Developmental hypothyroidism disrupts visual signal detection performance in rats

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1 **Abstract**

2 Thyroid hormones (THs) are essential for proper brain development in mammals. TH insufficiency  
3 during early development causes structural and functional abnormalities in brain leading to cognitive  
4 dysfunction. The specific effects of developmental hypothyroidism on attention have not been well  
5 characterized in animal models. The present study was conducted to characterize the effects of  
6 developmental hypothyroidism on attention in rats, and tested the hypothesis that the hypothyroidism has  
7 adverse impacts on attention by means of a visual signal detection task. Pregnant rats were exposed to the  
8 anti-thyroid drug, methimazole (0.02% w/v) via drinking water from gestational day 15 through postnatal  
9 day (PND) 21 to induce maternal and neonatal hypothyroidism. Male offspring served as subjects for the  
10 task started on PND 90. A light stimulus (500ms, 250ms or 50ms) was presented in signal trials and not  
11 in blank trials. The offspring were required to discriminate these signal events, and subsequently press the  
12 correct lever. The correct response for signal and non-signal events was considered as hit and correct  
13 rejection, respectively. The hypothyroid offspring exhibited a decreased hit response for short signals  
14 (250ms and 50ms) which requires the higher attentional demand. The total number of lever responses  
15 during inter-trial interval (ITI) was also increased in the hypothyroid group. The number of lever  
16 responses was negatively correlated with a hit response at 50ms, not at 250ms. These results suggest that  
17 developmental hypothyroidism disrupts signal detection performance via impairment of visual attention  
18 and the altered lever response behavior.

19

20 **Keyword**

21 attention; thyroid hormone; developmental hypothyroidism; signal detection task; rat

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## 1 **1. Introduction**

2 Thyroid hormones (THs) are essential factors for proper development of the central nervous system  
3 (CNS) in mammals. Severe hypothyroidism in early development results in impairments of brain  
4 development, and neurological cretinism [1]. Although profound adverse outcomes can be prevented by  
5 TH replacement, subtle neurocognitive dysfunctions, including IQ deficits, impaired motor skills and  
6 lower scores on neuropsychological tests were observed in the children with congenital hypothyroidism  
7 (CH) [2-4]. Moreover, deficits in childhood seems to persist into the adulthood [5, 6], suggesting that the  
8 transient disruption of TH system during CNS development would lead to the permanent adverse  
9 outcomes.

10 Laboratory studies using animal models, mostly rats, have also examined the adverse effects of  
11 hypothyroidism during CNS development on brain morphology and function. Developmental  
12 hypothyroidism disturbs a broad range of the neurodevelopmental events, including dendritic arborization,  
13 neurite outgrowth, myelination, synaptogenesis, as well as cell differentiation and migration [7-9].  
14 Perturbance of these neurodevelopmental events results in structural [10-13] and functional [14, 15]  
15 abnormalities in brain, which culminates in behavioral alterations and cognitive dysfunctions in rats that  
16 experience developmental hypothyroidism. Developmental hypothyroidism has been shown to increase  
17 locomotor activity, and this increase persisted into adulthood [16-18]. Memory and spatial learning are  
18 also affected by the hypothyroidism. Perinatally-hypothyroid rats took longer time to find a hidden  
19 platform in a water maze task, even as adults following recovery of TH concentrations [19, 20]. Even  
20 when the duration of hypothyroid state was restricted to a few days during gestation, the water maze  
21 performance was impaired in adult rats [21]. Further, previous studies using operant tasks demonstrated  
22 performances of animals that had developmental hypothyroidism were disrupted. Perinatal  
23 hypothyroidism altered adaptive behavior of rats in an alternative cyclic ratio (ALCR) schedule; the rats  
24 were unable to change response requirement, and perseverated on the incorrect lever [22, 23]. In a  
25 reversal learning paradigm, hypothyroid rats also showed a delay in adapting to changes in response  
26 requirement [24]. These behavioral tests were conducted when the developmentally hypothyroid rat had  
27 reached adolescence or adulthood and a euthyroid status. Therefore, these findings in rats support clinical  
28 studies in humans, and suggest that developmental hypothyroidism may have permanent adverse impacts  
29 on cognitive function, even if the hypothyroid period was transient.

30 Attention deficits are one of the common features reported in children with congenital  
31 hypothyroidism, but these deficits have not been well characterized in rodent models. Children with CH  
32 have exhibited altered task performances in a continuous performance task (CPT), which could be  
33 interpreted as an impairment of sustained attention [25]. Rovet & Hepworth [26] showed that several  
34 aspects of attention could be disrupted in adolescence following CH. Furthermore, these attentional  
35 problems persisted into adulthood [6]. These clinical studies suggest that TH insufficiency during CNS

1 development may impair cognitive processes associated with attention. However, animal models to  
2 characterize the effects of TH insufficiency during CNS development on attention are currently lacking.

3         The present study was conducted to characterize the effects of developmental hypothyroidism  
4 on attention in rats. We operationally defined attention as an ability to detect a brief and temporally  
5 unpredictable sensory stimulus in the present study. To measure this type of attention, a signal detection  
6 task has been designed in rodent studies [27]. Animals were required to discriminate whether a signal (e.g.  
7 light or noise) had been presented or not in each trial, and subsequently to press the correct lever in  
8 accordance with the trial type. The signal was brief and temporally unpredictable, requiring animals to  
9 attend to the signal presentation. Previous studies have demonstrated that signal detection performance  
10 was altered by signal qualities such as the signal duration [28-33]. Signal detection performance  
11 decreased as the signal duration shortened, indicating that the attentional demand increases to detect  
12 shorter signal. Based on these findings, we hypothesized that developmental hypothyroidism would result  
13 in the selective and significant decrease of the detection performance for shorter signal if the  
14 hypothyroidism adversely affects attention in rats.

## 16 **2. Materials and methods**

### 17 **2.1. Animals**

18 Pregnant Wistar rats (n=16) were obtained on gestational day (GD) 8. These rats were individually  
19 housed and randomly assigned to either the control or experimental group (8 per group). The  
20 experimental group was treated with the anti-thyroid drug, methimazole (MMI, 2-mercapto-1-methyl  
21 imidazole), at concentrations of 0.02% (w/v) via drinking water from GD15 to postnatal day (PND) 21 to  
22 induce perinatal hypothyroidism. MMI blocks biosynthesis of THs [34] and can cross the placenta,  
23 reaching fetal to maternal serum ratio of approximately 1:1[35]. The MMI concentration in the present  
24 study has been employed in previous studies, and is known to result in the marked reduction of THs in  
25 offspring [36, 37]. All litters were culled to eight pups on PND 7 with an equal number of males and  
26 females when possible. At weaning (PND 21), one male rat was selected from each litter and housed  
27 individually. The offspring from the MMI-treated group were weaned on PND 28 due to the retardation in  
28 somatic development. From PND 21 to PND 28, dams from the treated group were given distilled water  
29 without MMI in order to prevent the offspring from ingesting additional MMI. Offspring had free access  
30 to food through 12 weeks of age. Following this period, offspring were placed under food restriction and  
31 maintained at 85% of their free-feeding body weights. Water was available *ad libitum* in the home cages.  
32 Behavioral tests were initiated on PND 90 after the offspring treated with MMI would achieve the  
33 euthyroid status [18]. The room temperature was maintained at 22±2°C and the relative humidity was  
34 50±10% under a 12-h light/dark cycle (dark, 07:00-19:00 h). The behavioral tests were conducted in the  
35 dark period. This research was carried out with the approval of The Center for Advanced Science and

1 Technology (Hokkaido University). All animal procedures complied with the NIH Animal Care  
2 Guidelines and The Guide for the Care and Use of Laboratory Animals (Hokkaido University).

## 3 4 **2.2. Apparatus**

5 Five standard operant chambers were used. The chambers were arranged as follows: a signal light (white  
6 LED, 0.34 W), a food cup, and two response levers were installed on the front panel of the chamber. The  
7 signal light was mounted on the center of the front panel 11 cm above the floor. The food cup was 10 cm  
8 below the signal light and a food pellet (50 mg) was delivered as a reward from a pellet dispenser. Two  
9 response levers protruded from the panel at a position 3 cm above the floor and 8 cm to the left and right  
10 of the food cup. A house light (white LED, 0.34 W) and pure tone generator (3.3 kHz, 85dB) were fixed  
11 on the ceiling. A speaker with a diameter of 17 cm was placed outside of the chamber, and white noise  
12 (70dB) was presented to mask external sounds. The chamber was set in an isolation box designed to  
13 attenuate external light and sound. Experiment and data recording were controlled by a personal  
14 computer.

## 15 16 **2.3. Behavioral testing**

### 17 **2.3.1. Training**

18 Animals were initially trained to press either left or right lever under a continuous reinforcement (CRF)  
19 schedule. In this schedule, one lever response yielded one reward (a 50 mg food pellet). Both the house  
20 light and the signal light were turned off in this training step. The house light remained off till the third  
21 step of a signal detection task (see 2.3.2. Signal detection task). After learning the contingency, in the  
22 next training step, animals were required to press the right lever in a signal session and the left lever in a  
23 blank session. A signal light was always on in a signal session, and the light was off in a blank session.  
24 Hereafter, the right lever was associated with the signal. When rats obtained 50 rewards, the session  
25 ended. Signal and blank sessions were alternated every other day. This training was conducted for six  
26 days (three days for each session). In the third step, one session was 50 trials, and a session consisted of  
27 signal and blank trials. These trials were presented in a pseudo-random order with an equal number within  
28 a session. While the signal light was on in a signal trial, it was off in a blank trial. When rats pressed the  
29 correct lever, a trial ended with a reward and the next trial started. This training continued for three days.  
30 In the final training step, the forced discrimination training was done. Either a signal or a blank trial  
31 began after a pure tone was presented for 500ms. In a signal trial, 3s after presentation of the tone, signal  
32 light was illuminated for 1s. After the signal light was turned off, a limited hold (LH) period, i.e. the time  
33 window that a lever response yields a reward, began. In a blank trial, 3s after presentation of the tone, the  
34 LH period started. LH period was 8s, and the tone was presented during this LH period. If animals  
35 pressed the correct lever, the reward was delivered, and the tone was turned off. Even if rats pressed the  
36 incorrect lever, the trial continued until they pressed the correct lever. After the correct response or the

1 elapse of the LH, an inter-trial interval (ITI) began. The ITI was 10s, and lever pressing during the ITI did  
2 not yield any rewards. After the ITI elapsed, the next trial began. The order of signal and blank trials was  
3 pseudo-randomized, and each trial type consisted of 45 trials. This training was conducted for four days.  
4

### 5 **2.3.2. Signal detection task**

6 An outline of the task procedure for the signal detection task is shown in Table 1. The task procedure of  
7 the first step of the signal detection task was the same as that of the forced discrimination training, except  
8 that the LH period was shortened to 4s, ITI was lengthened to 15s, and the lever pressing during LH  
9 period was defined as hit, miss, correct rejection or false alarm according to the trial type and response  
10 levers. In a signal trial, right and left lever responses were defined as a hit and a miss, respectively. In a  
11 blank trial, right and left lever responses were defined as a false alarm and a correct rejection, respectively.  
12 For a hit or a correct rejection, the reward was given. No response during LH period was defined as  
13 omission. The learning criteria for this step were that relative hit and correct rejection were more than  
14 75%, and that omission was less than 20%, for three consecutive days. The relative hit and correct  
15 rejection, and the omission (%) in a session were calculated as follows:  
16

17  $P(\text{HIT}) = \text{number of hit} / (\text{number of hit} + \text{number of miss})$

18  $P(\text{CR}) = \text{number of correct rejection} / (\text{number of correct rejection} + \text{number of false alarm})$

19  $\text{Omission} (\%) = \text{number of omission} / \text{total trial}$   
20

21 In the next step, the pure tone previously used as a cue for signal presentation was eliminated, and the ITI  
22 was changed to  $18 \pm 3\text{s}$ . Other parameters and the procedure of the task were the same as those of the first  
23 step. The learning criteria of this step were that the  $P(\text{HIT})$  and  $P(\text{CR})$  were more than 80%, and that  
24 omission (%) was less than 20%, for three consecutive days. In the third step, a house light was turned on  
25 throughout the experiment to decrease a salience of the signal light and not to animals detect the signal  
26 light without paying attention to it. Hereafter, the house light was turned on throughout experiments.

27 Other task parameters and procedures were the same as those of the second step. The learning criteria of  
28 this step were that  $P(\text{HIT})$  and  $P(\text{CR})$  were more than 80%, and that omission (%) was less than 20%, for  
29 five consecutive days. In the final step of the task, the durations of the signal light were shortened to  
30 500ms, 250ms or 50ms. An equal number of signal and blank trials (45 trials each) were presented  
31 pseudo-randomly. Equal numbers of signal trials for respective signal durations were also presented in a  
32 pseudo-random order. The criteria of a stable performance for the final step were as follows: (1) for the  
33 signal trials with 500ms signal light and blank trials,  $P(\text{HIT})$  and  $P(\text{CR})$  were more than 80%, the  
34 variation of  $P(\text{HIT})$  and  $P(\text{CR})$  were within 20%, and omission (%) was less than 20%; (2) for signal  
35 trials with 250ms signal light,  $P(\text{HIT})$  was more than 80%, the variation of  $P(\text{HIT})$  was within 20%, and  
36 omission (%) was less than 20%; and (3) for signal trials with 50ms signal light, the variation of  $P(\text{HIT})$

1 was within 20% and omission (%) was less than 20%. In the case that the task performances did not  
2 satisfy all of the criteria for five consecutive days within 30 sessions, the data which satisfied at least  
3 criterion (1) were used for data analyses.

#### 4 5 **2.4. Statistical analysis**

6 An analysis of variance (ANOVA) was used to analyze the behavioral measures from the signal detection  
7 task. The behavioral measures were as follows: P (HIT) at 500ms, 250ms and 50ms signal duration, P  
8 (CR), the reaction time for hit response at each signal duration and correct rejection response, and the  
9 number of the blank and signal lever responses during the ITI. The reaction time was defined as the time  
10 between the initiation of the LH period and a hit or correct rejection response. The number of the blank  
11 and signal lever responses during ITI for each signal event (signal or non-signal) and each signal duration  
12 (500ms, 250ms and 50ms) were collapsed. This is because rats could not predict which signal event and  
13 signal duration came in next trial during ITI, it is plausible that the signal event and duration do not affect  
14 the number of the lever responses during ITI. For analyses of the behavioral measures, the averaged data  
15 of five consecutive sessions that satisfied the criteria of the stable performance at the final step of the  
16 signal detection task were used. P (HIT) and P (CR) were arc sine-transformed and the reaction time was  
17 subjected to log-transformation for statistical analyses. P (HIT) and reaction time for hit response were  
18 analyzed by a two-way ANOVA with MMI treatment as a between-subject factor and signal duration as a  
19 within-subject factor. The number of blank and signal lever response during ITI were analyzed by a  
20 two-way ANOVA with MMI treatment as a between-subject factor and a lever type as a within-subject  
21 factor. P (CR), reaction time for correct rejection response, and body weight were analyzed by a one-way  
22 ANOVA with MMI treatment as a between-subject factor. Post-hoc test was conducted using Ryan's  
23 method with an adjusted significance level.

24 One MMI-treated rat did not reach the criterion (3) in the final step of the signal detection task.  
25 The behavioral data of this rat satisfied criteria (1) and (2) were used for the statistical analyses. Another  
26 MMI-treated rat failed to reach criteria (2) and (3) in the final step. The behavioral data of this rat  
27 satisfied criterion (1) were included in the statistical analyses. Since two MMI-treated rats did not reach  
28 the final step of the signal detection task, the data of eight control rats and six MMI-treated rats was  
29 analyzed.

30 In the case that the significant differences between groups were found in the signal detection  
31 performance and the number of lever response during ITI, the Pearson product-moment coefficient  
32 correlation was calculated between these measures. This is because altered lever response behavior, i.e.  
33 the decrease or increase of the number of lever response during ITI, may have affected the signal  
34 detection performance.

### 35 36 **3. Results**



### 3.1. Body weight

MMI treatment significantly decreased the body weight of the offspring at PND84 compared with the control group ( $F(1,12) = 7.63, p < 0.05$ ). This result showing the body weight of the MMI-treated group did not recover to control level is consistent with previous studies that employed the same dosing regimen of MMI [22, 23].

### 3.2. Signal detection performance

MMI treatment caused a significant decrease in P (HIT) and increase in P (CR), respectively (Fig.1). For P (HIT), a two-way ANOVA revealed the significant main effects of MMI treatment ( $F(1, 12) = 9.55, p < 0.01$ ) and the signal duration ( $F(2, 24) = 48.54, p < 0.001$ ). There was also a significant interaction of the treatment and signal duration ( $F(2, 24) = 5.19, p < 0.05$ ). Post-hoc comparison demonstrated that the P (HIT) for the signal durations of 250ms and 50ms in the MMI-treated group was significantly lower than that of the control group. On the other hand, P (CR) of the MMI-treated group was significantly higher than that of the control ( $F(1, 12) = 5.31, p < 0.05$ ).

No significant effect of MMI treatment was observed on reaction time for both hit and correct rejection response (Fig.2). A two-way ANOVA for reaction time of hit response showed a significant main effect of signal duration ( $F(2, 24) = 40.03, p < 0.001$ ), and an interaction of treatment and signal duration ( $F(2, 24) = 3.62, p < 0.05$ ). The post-hoc comparison showed that the reaction time was increased in a signal duration-dependent manner in both control and MMI-treated groups although there were no significant differences between groups in reaction time at any signal duration.

MMI treatment significantly increased the number of blank lever responses during ITI (Fig. 3). A two-way ANOVA for the number of lever responses during the ITI revealed that the effects of MMI treatment and lever type were significant ( $F(1,12) = 17.60, p < 0.005, F(1,12) = 38.65, p < 0.001$ ), and that there was an interaction of treatment and lever type ( $F(1,12) = 22.96, p < 0.001$ ). The post-hoc comparison showed that the number of the blank lever responses in the MMI group was higher than that of the control group.

### 3.3. Correlation between the lever response and signal detection performance

Significant correlations were observed between the number of the blank lever responses and P (HIT) at 50ms signal duration ( $r = -0.79, p < 0.01$ ) and P (CR) ( $r = 0.63, p < 0.05$ ) while the correlation between the number of the blank lever response and the P (HIT) at 250ms was not significant. It should be noted that we separated the collapsed number of the blank lever response into the number of the blank lever response for each signal type (signal or blank) and each duration (250ms and 50ms) in correlation analyses to correctly assess the relationship between the lever response and the signal detection performance.

#### 1 **4. Discussion**

2 The present study examined the effects of developmental hypothyroidism on attention in rats by means of  
3 a visual signal detection task. We tested the hypothesis that developmental hypothyroidism disrupts signal  
4 detection performance for a short signal, which requires higher attentional demand. Perinatal MMI  
5 treatment causing maternal and neonatal hypothyroidism impaired accuracy of signal detection for short  
6 signals. MMI treatment also increased the number of blank lever responses during the ITI. The number of  
7 blank lever responses was partly correlated with signal detection performance. These data suggest that  
8 developmental hypothyroidism disrupts signal detection performance via a deficit in visual attention and  
9 alteration of lever response behavior.

10 MMI treatment impaired accuracy of signal detection mainly due to decrease of P (HIT) for  
11 short signals (Fig. 1). The reaction time for hit and correct rejection was not different between groups  
12 (Fig.2). This decrease in P (HIT) indicates that MMI treatment adversely affected visual attention to brief  
13 signals. Previous reports of signal detection tasks have shown that P (HIT) was altered in a  
14 duration-dependent manner in intact animals [28-33]. This result indicates that detection of short signals  
15 requires greater attentional demand and thus experimental manipulations that selectively decrease P (HIT)  
16 for short signal can be interpreted as impairing attention [29]. As Echevaria et al. [29] proposed, this view  
17 is supported by the findings demonstrating that drug treatment that disturbs attentional processing in  
18 humans decreased P (HIT) for short signals in rats [38, 39]. Therefore, the decrease of P (HIT) in the  
19 MMI-treated group reflects impairments of visual attention required for signal detection.

20 Alternatively, it is possible that MMI exposure may have mediated developmental defects in  
21 visual systems, e, g. malformation of retina [40] and/or visual cortex [41], and this could have disrupted  
22 signal detection performance. However, it is unlikely that possible developmental visual deficits  
23 contributed to the impairments of signal detection performance in the present study, as the P (HIT) for the  
24 long signal duration (500ms) between control and MMI-treated groups was comparable. This suggests  
25 that the MMI-treated group had no obvious visual deficits. Moreover, no significant differences were  
26 observed in reaction time between the groups for all signal durations, but delayed response to the correct  
27 lever would be expected if visual deficits had disturbed the signal detection. Thus, the potential visual  
28 deficits likely did not affect the signal detection performance.

29 MMI treatment significantly increased the number of blank lever responses during ITI. This  
30 increased unnecessary lever responses may reflect a perseverative tendency, which has been observed  
31 previously in rats that have experienced developmental hypothyroidism. For example, Shalock et al. [42]  
32 documented that the learning of the differential reinforcement of low rates (DRL) schedule was not  
33 impaired by perinatal hypothyroidism induced by the anti-thyroid drug, propylthiouracil (PTU). However,  
34 while the response rate of the control group decreased in DRL schedule across training sessions, the rate  
35 of the PTU-treated rats (0.3% w/w food and 0.001% drinking water from birth until PND30) remained  
36 constantly higher. They regarded this response behavior of the hypothyroid rats as the perseverative

1 tendency. Again, adult rats rendered perinatally-hypothyroid induced by MMI (0.025% in the dam's  
2 drinking water from GD16 until PND25) also showed perseverative lever responses in the ALCR  
3 schedule [22, 23]. This schedule required rats to press one lever for a fixed ratio, and next press another  
4 lever, alternately. The hypothyroid rat completed this schedule when only one lever was extended in an  
5 operant chamber (forced trial). However, the hypothyroid rat failed to complete the schedule when two  
6 levers, active and inactive levers, were extended (choice trial). This was because the hypothyroid rat  
7 perseverated to one lever and could not switch the response levers appropriately. Considering these  
8 previous findings, the increased number of the blank lever response observed in the present study may  
9 also be considered a perseverative tendency; however, in the present study, the MMI-treated rat could  
10 switch the response lever, as shown in the P (HIT) that was over the chance level even at 50ms signal  
11 duration. This means that the MMI-treated rat can switch the lever response appropriately if a  
12 discriminative stimulus of sufficient duration and strength is presented.

13           One possible explanation as to why the MMI-treated group perseverated to the blank lever,  
14 rather than the signal lever, may be that the MMI-treated group adopted a “positional strategy” in the task.  
15 Bushnell et al. [43] reported a bias toward pressing the blank lever in the signal detection task. According  
16 to the observations, rats positioned themselves near the blank lever during ITI and then moved to a signal  
17 lever when a signal was presented. A computational model of this behavior suggests that rats adopt this  
18 strategy because the light conditions during the pre-signal interval match the conditions for which press  
19 on the blank lever yields reward [44]. It is assumed that the combination of a perseverative tendency of  
20 the MMI-treated group with a positional strategy may result in the increased number of blank lever  
21 responses observed.

22           Alternatively, the tendency of the MMI-treated group to press the blank lever may have  
23 resulted from the auditory dysfunction caused by developmental hypothyroidism. TH insufficiency during  
24 the early postnatal period leads to malformation of the cochlea [45], which results in hearing loss [37, 46]  
25 in developmentally-hypothyroid rat models. The present study employed pure tone as a cue of LH period.  
26 However, due to auditory dysfunction caused by MMI-induced developmental hypothyroidism, the MMI  
27 treated group may perceive the tone with lower intensity compared with the control group. If so, it would  
28 be more difficult for the MMI-treated group to notice when the blank trial started because no visual cue  
29 was presented in blank trial before initiation of LH period. To address this problem, the MMI-treated  
30 group may tend to press the blank lever during ITI and switch the response to the signal lever when the  
31 signal light was turned on.

32           The correlation analyses revealed that significant positive and negative correlations of number  
33 of blank lever responses with P (CR) and P (HIT) at 50ms, respectively, but not for P (HIT) at 250ms.  
34 These results indicate that the number of blank lever responses during the ITI would be associated with  
35 signal detection performance. The increased number of blank lever responses in the MMI-treated group  
36 would affect their signal detection performance. A blank lever response yielded correct rejection or miss

1 in the present study. Tendency to press blank lever during the ITI was expected to increase these  
2 consequences. Since miss was inversely proportional to hit, possible increase of miss would decrease P  
3 (HIT). This speculation is consistent with the increased P (CR) and decreased P (HIT) in the MMI-treated  
4 group. Thus, the altered lever response behavior during ITI would decrease P (HIT) in the MMI-treated  
5 group. However, since the significant correlation was limited to P (HIT) at 50ms, the altered lever  
6 response behavior cannot account for decrease in P (HIT) at 250ms. It is assumed that the decrease in P  
7 (HIT) at 250ms would not be attributed to the increased blank lever response, but rather to impairments of  
8 attentional process required for signal detection. At the same time, the decrease in P (HIT) at 50ms may  
9 be attributed to the combination of impairments of attention and the altered lever response behavior.  
10 Taken together, disruption of signal detection performance in the MMI-treated group would be derived  
11 from a deficit in attention and altered lever response behavior.

12 In summary, the present study provides evidence that developmental hypothyroidism disrupts  
13 visual signal detection performance in rats. The selective decrease of P (HIT) for short signals indicates  
14 that impairments of attentional process for signal detection results in the disrupted performance. The  
15 correlation analyses also suggest that the alteration of lever response behavior contribute to the disruption  
16 of signal detection performance. Currently, there is little information regarding the relationship between  
17 developmental hypothyroidism and attention in animal models. Further studies need to assess several  
18 aspects of attention in rats that have experienced developmental hypothyroidism. Additionally, although  
19 the present study as well as previous studies adopted a dosing regimen that dramatically reduced the TH  
20 concentrations in the offspring from fetal through postnatal period, several examples in the literature [47,  
21 48] underscore the importance of examining the impacts of modest TH insufficiency on the developing  
22 organism to appropriately evaluate its potential risk. Recent findings show that the even modest TH  
23 insufficiency can disturb proper CNS development; both clinical [49, 50] and experimental studies  
24 [51-53] support this view. Thus, future research also needs to be conducted to characterize the impacts of  
25 modest as well as severe TH insufficiency during CNS development on cognitive function, including  
26 attention.

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31 fellow (M.H).

## 32 33 **Reference**

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#### 13 14 **Figure Legends**

15 Fig.1. Signal detection performance. P (HIT) is shown in a line graph and P (CR) in a bar graph. While P  
16 (HIT) for the MMI group was significantly decreased at 250ms and 50ms, P (CR) of the MMI group was  
17 higher than that of the control. \* indicates significant difference between groups ( $p < 0.05$ ). Error bar is  
18 expressed as standard error of mean (SEM).

19  
20 Fig.2. Reaction time for hit and correct rejection responses. Reaction time for hit response and correct  
21 rejection response are shown in a line graph and a bar graph, respectively. The reaction time for the hit  
22 response was increased in a signal duration-dependent manner. There were no significant differences  
23 between groups at any duration. The reaction time for correct rejection was also comparable between  
24 groups. Error bar is expressed as standard error of mean (SEM).

25  
26 Fig. 3. The number of blank and signal lever responses during ITI. The MMI-treated group exhibited an  
27 increased number of blank lever responses compared to that of the control. \* indicates a significant  
28 difference between groups ( $p < 0.05$ ). Error bar is expressed as standard error of mean (SEM).



**Table. 1. Training step of a signal detection task**

	<b>Task procedure and parameters</b>	<b>Criterion</b>
Step1	A cue tone was presented for 500ms. Three second after the cue tone presentation, a signal was presented for 1s. Limited hold (LH) period was 4s. Inter-trial interval (ITI) was 15s. The number of trials was 90trials. (45 trials for both signal and blank trials)	Both P (HIT) and P (CR) were more than 75% and omission (%) was less than 20% for three consecutive days.
Step2	No cue tone was presented before a signal presentation. A signal was presented for 1s. LH period was 4s. ITI was 18±3s. The number of trials was 90trials. (45 trials for both signal and blank trials)	Both P (HIT) and P (CR) were more than 80%, and omission (%) was less than 20% for three consecutive days.
Step3	No cue tone was presented before a signal presentation. House light was turned on. A signal was presented for 1s. LH period was 4s. ITI was 18±3s. The number of trials was 90trials. (45 trials for both signal and blank trials)	Both P (HIT) and P (CR) were more than 80%, and omission (%) was less than 20% for five consecutive days.
Final step	No cue tone was presented before signal presentation. House light was turned on. Signal duration was 500ms, 250ms or 50ms. (15 trials for each signal duration) LH period was 4s. ITI was 18±3s. The number of trials was 90trials. (45 trials for both signal and blank trials)	(1) P (HIT) at 500ms and P (CR): Accuracies were more than 80%, and variations were within 20% and omission (%) was less than 20% for five consecutive days.  (2) P (HIT) at 250ms: Accuracy was more than 80%, and variation was within 20% and omission (%) was less than 20% for five consecutive days.  (3) P (HIT) at 50ms: The variation was within 20% and omission (%) was less than 20% for five consecutive days.