Title: Developmental hypothyroidism disrupts visual signal detection performance in rats

Author names and affiliations:
Masashi Hasegawa [a *]
Hiromi Wada [a]
[a] Division of Psychology, Graduate School of Letters, Hokkaido University

***Corresponding author:** Masashi Hasegawa
Address: Division of Psychology, Graduate School of Letters, Hokkaido University, Kita10 Nishi7, Kita-ku, Sapporo, 060-0810, JAPAN
Phone: +81-11-706-3321
Fax: +81-11-706-3321
Email: m-hasegawa@let.hokudai.ac.jp

Abstract

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

Keyword

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

1. Introduction

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

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

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

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

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

2. Materials and methods

2.1. Animals

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

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

2.2. Apparatus

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

2.3. Behavioral testing

2.3.1. Training

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

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

2.3.2. Signal detection task

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

P (HIT) = number of hit / (number of hit + number of miss)
P (CR) = number of correct rejection / (number of correct rejection + number of false alarm)
Omission (%) = number of omission / total trial

In the next step, the pure tone previously used as a cue for signal presentation was eliminated, and the ITI was changed to 18±3s. Other parameters and the procedure of the task were the same as those of the first step. The learning criteria of this step were that the P (HIT) and P (CR) were more than 80%, and that omission (%) was less than 20%, for three consecutive days. In the third step, a house light was turned on throughout the experiment to decrease a salience of the signal light and not to animals detect the signal light without paying attention to it. Hereafter, the house light was turned on throughout experiments. Other task parameters and procedures were the same as those of the second step. The learning criteria of this step were that P (HIT) and P (CR) were more than 80%, and that omission (%) was less than 20%, for five consecutive days. In the final step of the task, the durations of the signal light were shortened to 500ms, 250ms or 50ms. An equal number of signal and blank trials (45 trials each) were presented pseudo-randomly. Equal numbers of signal trials for respective signal durations were also presented in a pseudo-random order. The criteria of a stable performance for the final step were as follows: (1) for the signal trials with 500ms signal light and blank trials, P (HIT) and P (CR) were more than 80%, the variation of P (HIT) and P (CR) were within 20%, and omission (%) was less than 20%; (2) for signal trials with 250ms signal light, P (HIT) was more than 80%, the variation of P (HIT) was within 20%, and omission (%) was less than 20%; and (3) for signal trials with 50ms signal light, the variation of P (HIT)

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

2.4. Statistical analysis

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

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

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

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.

4. Discussion

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

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

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

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

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

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

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

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

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

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

Acknowledgement

The authors thank Drs. Katie B. Paul, Phillip J. Bushnell and Kevin M. Crofton for their thoughtful comments on early versions of this manuscript. This research was supported by Grant-in-Aid for JSPS fellow (M.H).

Reference

[1] Porterfield SP. Vulnerability of the developing brain to thyroid abnormalities: environmental insults to the thyroid system. Environ Health Perspect 1994;102 Suppl 2:125–30.

[2] Kooistra L, Laane C, Vulsma T, Schellekens JMH, van der Meere JJ, Kalverboer AF. Motor and cognitive development in children with congenital hypothyroidism: a long-term evaluation of the effects of neonatal treatment. J Pediatr 1994;124:903-9.

[3] Rovet JF, Hepworth SL. Dissociating attention deficits in children with ADHD and congenital hypothyroidism using multiple CPTs. J Child Psychol Psychiatr 2001; 42: 1049–56.

[4] Bongers-Schokking JJ, Koot HM, Wiersma D, Verkerk PH, de Muinck Keizer-Schrama SM. Influence of timing and dose of thyroid hormone replacement on development in infants with congenital hypothyroidism. J Pediatr 2000;136:292–7.

[5] Oerbeck B, Sundet K, Kase BF, Heyerdahl S. Congenital hypothyroidism: influence of disease severity and L-thyroxine treatment on intellectual, motor, and school-associated outcomes in young adults. Pediatrics 2003;112:923 –30.

[6] Oerbeck B , Sundet KS, Kase BF, et al. Congenital hypothyroidism: no adverse effects of high dose thyroxine treatment on adult memory, attention, and behaviour. Arch Dis Child 2005;90:132–7.

[7] Bernal J, Nunez J. Thyroid hormones and brain development. Eur. J. Endocrinol 1995;133:390–8.

[8] Oppenheimer JH, Schwartz HL. Molecular basis of thyroid hormonedependent brain development. Endocr Rev 1997;18:462–75

[9] Thompson CC, Potter GB. Thyroid hormone action in neural development. Cereb Cortex 2000;10: 939–45.

[10] Madeira MD, Cadete-Leite A, Andrade JP, Paula-Barbosa MM. Effects of hypothyroidism upon the granular layer of the dentate gyrus in male and female adult rats: a morphometric study. J Comp Neurol. 1994; 314:171–86.

[111 Madeira MD, Sousa N, Lima-Andrade MT, Calheiros F, Cadete-Leite A, Paula-Barbosa MM. Selective vulnerability of the hippocampal pyramidal neurons to hypothyroidism in male and female rats. J Comp Neurol 1992;322:501–18.

[12] Madeira MD, Paula-Barbosa MM. Reorganization of mossy fiber synapses in male and female hypothyroid rats: a stereological study. J Comp Neurol 1993;337:334-52.

[13] Hasegawa M, Kida I, Wada H. A volumetric analysis of the brain and hippocampus of rats rendered perinatal hypothyroid. Neurosci Lett 2010;479:240–4.

[14] M.E. Gilbert, C. Paczkowski, Propylthiouracil, (PTU)-induced hypothyroidism in the developing rat impairs synaptic transmission and plasticity in the dentate gyrus of the adult hippocampus. Dev Brain Res 2003;145:19–29.

[15] M.E. Gilbert, Alterations in synaptic transmission and plasticity in area CA1 of adult hippocampus following developmental hypothyroidism. Dev Brain Res 2004;148:11–8.

[16] Darbra S, Balada F, Garau A, Gatell P, Sala J & Marti-Carbonell MA. Perinatal alterations of thyroid hormones and behaviour in adult rats. Behav Brain Res 1995; 68: 159−64.

[17] Kobayashi K, Tsuji R, Yoshioka T, Kushida M, Yabushita S, Sasaki M, et al. Effects of hypothyroidism induced by perinatal exposure to PTU on rat behavior and synaptic gene expression. Toxicology 2005;212:135–47.

[18] Tamasy V, Meisami E, Vallerga A, Timiras PS. Rehabilitation from neonatal hypothyroidism: spontaneous motor activity, exploratory behavior, avoidance learning and responses of pituitary–thyroid axis to stress in male rats. Psychoneuroendocrinol 1986;11:91–103.

[19] Gilbert ME, Sui L. Dose-dependent reductions in spatial learning and synaptic function in the dentate gyrus in adult rats following developmental thyroid hormone insufficiency. Brain Res 2006;1069:10–22.

[20] Reid RE, Kim EM, Page D, O'Mara SM, O'Hare E. Thyroxine replacement in an animal model of congenital hypothyroidism. Physiol Behav 2007;91:299–303.

[21] Opazo MC, Gianini A, Pancetti F, Azkcona G, Alarco´ n L, Lizana R, et al. Maternal hypothyroxinemia impairs spatial learning and synaptic nature and function in the offspring. Endocrinology 2008;149: 5097-106.

[22] MacNabb C, O'Hare E, Cleary J, Georgopoulos AP. Congenital hypothyroidism impairs response alternation discrimination behavior. Brain Res 1999;847:231-39.

[23] MacNabb C, O'Hare E, Cleary J, Georgopoulos AP. Varied duration of congenital hypothyroidism potentiates perseveration in a response alternation discrimination task. Neurosci Res 2000;36:121-7.

[24] Brosvic GM, Taylor JN, Dihoff RE. Influences of early thyroid hormone manipulations: delays in pup motor and exploratory behavior are evident in adult operant performance. Physiol Behav 2002;75:697-715.

[25] Kooistra L, Van Der Meere JJ, Vulsma T, Kalverboer AF. Sustained attention problems in children with early treated congenital hypothyroidism. Acta Paediatr 1996; 85: 425–429.

[26] Rovet JF, Hepworth SL. Attention problems in adolescents with congenital hypothyroidism: a multicomponential analysis. J Int Neuropsychol Soc 2001; **7**: 734-44.

[27] Bushnell P (1998) Behavioral approaches to the assessment of attention in animals. Psychopharmacology 146:348-61

[28] Burk JA. Introduction of a retention interval in a sustained attention task in rats: effects of a visual distracter and increasing the inter-trial interval. Behav Processes 2004;67:521-31.

[29] Echevarria DJ, Brewer A, Burk JA, Brown SN, Manuzon H, Robinson JK. Construct validity of an operant signal detection task for rats. Behav Brain Res 2005;157:283-90.

[30] Himmelheber AM., Sarter M, Bruno JP. Increases in cortical acetylcholine release during sustained attention performance in rats. Cognitive Brain Res 2000; 9:313-25.

[31] Kozak R, Bruno JP, Sarter M. Augmented prefrontal acetylcholine release during challenged attentional performance. Cereb Cortex 2006;16: 9-17.

[32] McGaughy J, Sarter M. Behavioral vigilance in rats: task validation and effects of age, amphetamine, and benzodiazepine receptor ligands. Psychopharmacology 1995;117:340-57.

[33] Woolfrey KM, Hunt PS, Burk JA. Postnatal ethanol exposure disrupts signal detection in adult rats. Neurotoxicol Teratol 2005;27:815-23.

[34] Cooper DS. Antithyroid drug, N Engl J Med 2005;352: 905-17.

[35] Marchant B, Brownlie BE, McKay-Hart D, Horton PW, Alexander WD. The placental transfer of propylthiouracil, methimazole and carbimazole. J Clin Endocrinol Metab 1977;45:1187-93.

[36] Calvo R, Obregon MJ, Ruiz de Ona C, Escobar del Rey F, Morreale de Escobar G. Congenital hypothyroidism, as studied in rats. Crucial role of maternal thyroxine but not of 3,5,3′-triiodothyronine in the protection of the fetal brain. J Clin Invest 1990;86:889-99.

[37] Knipper M, Gestwa L, TenCate WJ, Lautermann J, Brugger H, Maier H et al. Distinct thyroid hormone-dependent expression of TrKB and p75NGFR in nonneuronal cells during the critical TH-dependent period of the cochlea. J Neurobiol 1999;38:338-56.

[38] Givens B. Effect of ethanol on sustained attention in rats. Psychopharmacology 1997;129:135-40.

[39] Presburger G, Robinson JK. Spatial signal detection task in rats is differentially disrupted by Δ-9-tetrahydroxycannabinol, scopolamine, and MK-801. Behav Brain Res 1999; 68:27-34.

[40] Harpavat S, Cepko CL. Thyroid Hormone and Retinal Development: An Emerging Field. Thyroid 2003;13:1013-19.

[41] Ipina SL, Ruiz-Marcos A. Dendritic structure alterations induced by hypothyroidism in pyramidal neurons of the rat visual cortex. Dev Brain Res 1986:394;61–7.

[42] Schalock RL, Brown WJ, Smith RL. Long-term effects of propylthiouracil-induced neonatal hypothyroidism. Dev Psychobiol 1979;12:187-99.

[43] Bushnell PJ, Kelly KL, Crofton KM. Effects of toluene inhalation on detection of auditory signals in rats. Neurotoxicol Teratol 1994;16:149-60.

[44] Schmajuk NA, Bushnell PJ. A computational model reveals classical conditioning mechanisms underlying visual signal detection in rats. Behavioural Processes 2009;82: 340-51.

[45] Uziel A. Periods of sensitivity to thyroid hormone during development of the organ of Corti. Acta Otolaryngol (stockh) 1986;429:23-7

[46] Goldey ES, Kehn LS, Rehnberg GL, Crofton KM. Effects of developmental hypothyroidism on auditory and motor function in the rat. Toxicol Appl Pharmacol 1995;135(1):67-76.

[47] Crofton KM. Developmental disruption of thyroid hormone: correlations with hearing dysfunction in rats. Risk Anal 2004;24:1665-71.

[48] Crofton KM. Thyroid disrupting chemicals: mechanisms and mixtures. Int J Androl 2008;31:209-23

[49] Vermiglio F, Presti VP Lo, Moleti M, et al. Attention deficit and hyperactivity disorders in the offspring of mothers exposed to mild-moderate iodine deficiency: a possible novel iodine deficiency disorder in developed countries. J Clin Endocr Metab 2004;89:6054-60.

[50] Berbel P, Mestre JL, Santamaria A, Palazon I, Franco A, Graells M, et al. Delayed neurobehavioral development in children born to pregnant women with mild hypothyroxinemia during the first month of gestation: the importance of early iodine supplementation. Thyroid 2009; 19:511-19.

[51] Auso E, Lavado-Autric R, Cuevas E, Escobar del Rey F, Morreale de Escobar G, P. Berbel. A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. Endocrinology 2004;145:4037-44.

[52] Goodman JH, Gilbert ME. Modest thyroid hormone insufficiency during development induces a cellular malformation in the corpus callosum: a model of cortical dysplasia. Endocrinology 2007;148:2593-7.

[53] Berbel P, Navarro D, Ausó E, Varea E, Rodríguez AE, Ballesta JJ, et al. Role of late maternal thyroid hormones in cerebral cortex development: an experimental model for human prematurity. Cereb Cortex 2009;20:1462-75.

Figure Legends

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

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

Fig. 3. The number of blank and signal lever responses during ITI. The MMI-treated group exhibited an increased number of blank lever responses compared to that of the control. * indicates a significant 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

	Task procedure and parameters	Criterion
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.