HYPOTHYROIDISM DISRUPTS PREPULSE INHIBITION OF AUDITORY STARTLE RESPONSE IN RATS

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Abstract
Mother rats were exposed to the anti-thyroid drug methimazole at concentrations of 0, 0.002, and 0.02% (w/v) in drinking water from gestational day 15 to postnatal day 21. At 10 months of age, offspring received behavioral tests of auditory startle responses and prepulse inhibition under conditions of a 115 dB startle stimulus with a 75, 85, or 95 dB prepulse. The group treated with 0.02% methimazole displayed disruptions of prepulse inhibition at a prepulse intensity of 75 dB. Greater disruptions were obtained for female hypothyroid rats. We propose that hypothyroidism impairs the sensory gating mechanisms that protect the central nervous systems from flooding of sensory stimuli, which could result in attention deficits.

Introduction
Attention-deficit hyperactivity disorder (ADHD) is characterized by attention deficits, hyperactivity, and impulsiveness. One risk factor for ADHD is the hypothyroidism induced by environmental endocrine disrupters such as polychlorinated biphenyls (PCBs) and dioxins. These chemicals are thought to disrupt thyroid hormone systems and affect the normal development of the central nervous system (CNS).

Recently, disruptions of prepulse inhibition (PPI) were identified in children with ADHD.[2,3] PPI refers to the inhibition of a startle response when the high-intensity startle stimulus is preceded by a non-startling low-intensity stimulus. PPI is explained by the sensory gating mechanisms in which the preceding non-startle stimulus activates an inhibitory network that reduces the reaction to the startle stimulus. This mechanism likely protects the CNS from stimuli that might otherwise result in cognitive fragmentation and disturbance.[1]

In this work, we introduced PPI and examined if hypothyroid animals display PPI disruptions. The presence of PPI disruptions in hypothyroid animals suggests that these animals are a suitable model system of ADHD.

Materials and Methods
Six pregnant Wistar rats were purchased on gestational day 8. Animals were housed in individual cages and randomly assigned to a control group (n = 2), a low-dose group (n = 2), or a high-dose group (n = 2). The anti-thyroid drug methimazole, dissolved in distilled water, was administered to pregnant rats via drinking water from gestational day 15 to postnatal day 21 at concentrations (w/v) of 0% (control), 0.002% (low-dose), and 0.02% (high-dose). After weaning, two male and two female offspring were sampled from each dam. Four male and four female offspring were assigned to each dose group, designated MC (male control), FC (female control), ML (male low-dose), FL (female low-dose), MH (male high-dose), and FH (female high-dose). These animals were individually housed under *ad libitum* feeding conditions. Tap water was constantly available in the home cages. Behavioral tests began at 43 weeks of age. One rat from each of the MH and FH groups died before experiments could begin; therefore, the sample size of the MH and FH groups was three.

The room temperature was maintained at 22 ± 2°C with a relative humidity of 50 ± 10% under a 12-h light/dark cycle (light, 19:00-07:00 h; dark, 07:00-19:00 h). Behavioral tests were performed during the dark period. This research was carried out with the approval of the Center for Advanced Science and Technology (Hokkaido University). All environmental conditions complied with the Guide for the Care and Use of Laboratory Animals (Hokkaido University).

A clear acrylic cage (15cm×22cm×12cm) with an aluminum mesh wall on both sides was used as an experimental chamber. A piezoelectric accelerometer (GH313A, GA-245SO; KEYENCE, Osaka, Japan) was attached underneath the cage to detect startle responses. Voltage outputs from the accelerometer were digitized at

1 kHz and recorded on a personal computer through a 60Hz low-pass filter. White noise was used for both the auditory startle stimulus and prepulse. White noise was amplified by a speaker adjacent to the experimental chamber. The chamber and speaker were placed in a sound insulation box to attenuate external light and sound. Throughout testing, background noise was maintained at a constant level (70 dB).

After a 5 min habituation to the experimental chamber in the presence of continuous background noise, all rats received a behavioral test to evaluate startle responses and PPI. The startle response test was comprised of 10 trials in which animals were exposed to a startle stimulus of 115 dB with a duration of 40 ms. The PPI test consisted of 10 startle trials with prepulse and eight startle trials without prepulse. In the startle trial with prepulse, prepulses at 75, 85, or 95 dB with a 20 ms duration was presented 30 ms before the startle stimulus. In the startle trials without prepulse, the startle stimulus was presented alone. The startle trials with and without prepulse were presented in random order. The inter-trial interval was varied with a mean of 20 s. The startle response was defined as the difference between the maximum and minimum peak amplitudes of the voltage output within 200 ms after the onset of the startle stimulus. The percentage of PPI was calculated as $100\times(P-PP)/P$, where P is the average voltage of startle responses in the eight startle trials without prepulse and PP is that seen in the 10 startle trials with prepulse. The behavioral test was performed on three days. The intensity of the prepulse was elevated every day beginning at 75, then increasing the dose to 85, and 95 dB for the half of the animals and descending order (95 to 85 to 75 dB) for the remaining animals.

Results and Discussion

The effect of methimazole dose on the startle response was significant [$F(2,16)=41.04$, $p<0.001$] (Fig. 1). According to the multiple comparison tests of Ryan's method, the high-dose group displayed greater reactions to the startle stimulus than either the control or low-dose groups [$t=8.26$, $df=16$, $p<0.001$; $t=6.86$, $df=16$, $p<0.001$]. The effects of day and trial to reduce startle responses were also significant [$F(2,32)=5.57$, $p<0.008$; $F(4,64)=10.48$, $p<0.001$]. The startle response amplitude declined for all of the groups as the days and trials progressed. There was a significant effect of sex [$F(1,16)=5.83$, $p<0.028$]; male rats displayed higher amplitude startle responses than the female rats. None of the interactions between these factors were significant.

In the PPI test, we discovered a significant effect of the prepulse intensity [[$F(2,32)=10.41$, $p<0.001$]. PPI percentages obtained using the 75 dB prepulse were compared to the 85 dB and 95 dB prepulses using the multiple comparison tests [$t=3.45$, $df=32$, $p<0.001$; $t=4.19$, $df=32$, $p<0.001$]. The interaction between dose and prepulse intensity was also significant [$F(4,32)=3.12$, $p<0.028$], with significant interactions found between sex, dose, and prepulse intensity [$F(4,32)=4.31$, $p<0.007$]. The multiple comparison tests revealed that the high-dose group exhibited a decreased percentage of PPI in comparison to the control group with a 75dB prepulse [$t=3.65$, $df=48$, $p<0.001$] (Fig. 2). Especially in female rats, FH exhibited greater reductions of PPI percentages than the FC and FL rats [$t=4.59$, $df=48$, $p<0.001$; $t=2.59$, $df=48$, $p<0.013$]. In addition, the PPI percentages for the high-dose group were significantly decreased at the 75dB prepulse in comparison to both the 85 dB and the 95 dB prepulses [$t=3.39$, $df=32$, $p<0.002$; $t=4.40$, $df=32$, $p<0.001$]. The reductions in PPI percentages for FH animals were inversely proportional to the intensity of the prepulse [75 dB vs 85 dB, $t=2.82$, $df=32$, $p<0.008$; 85 dB vs 95 dB, $t=2.61$, $df=32$, $p<0.014$; 75 dB vs 95 dB, $t=5.42$, $df=32$, $p<0.001$].

The percentage of PPI was decreased for the high-dose group, indicating that clear disruptions of PPI were displayed in the hypothyroid rats. Braff and Geller[1] proposed the sensory gating mechanisms within the CNS that protect it from stimulus flooding. A non-startle stimulus first sets up an inhibitory network that reduces the reaction to the following startle stimulus; our work suggests that hypothyroidism impairs these mechanisms. In the startle response test, the high-dose group elevated the amplitude of the startle response. The elevation could be explained by the impairments of the sensory gating mechanisms, which did not activate the inhibitory network.

Severer disruptions of PPI are found for the female animals in the current experiments. Some roles of estrogen are reported in PPI studies[5] but we have no definite information on the relationships between the thyroid and estrogen functions.

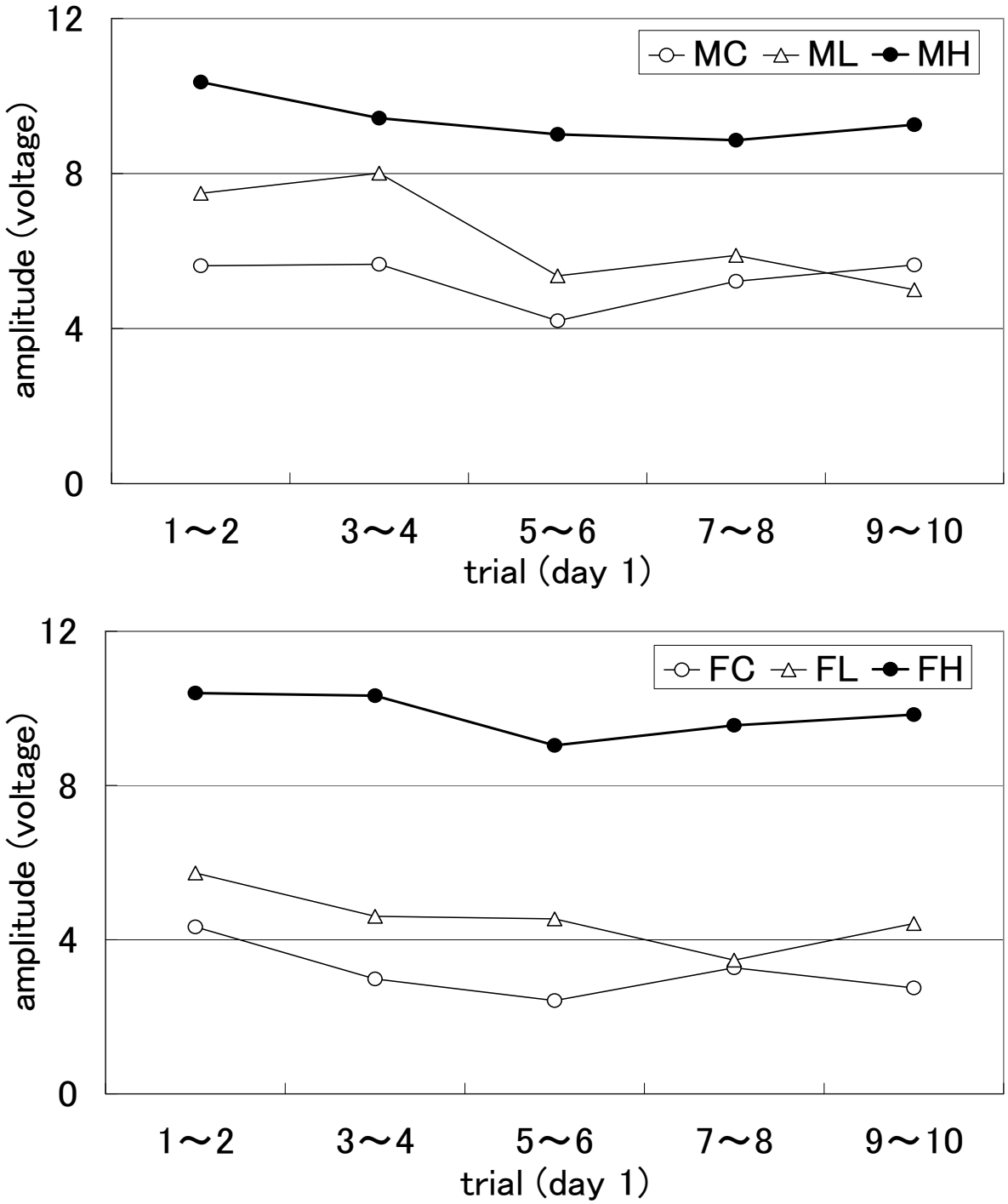

Fig. 1. Effects of methimazole treatments on the amplitude of startle responses on day 1. The intensity of the startle stimulus was 115 dB. Effects of dose were significant; the high-dose group animals (MH and FH) displayed greater reactions to startle stimuli than either the controls (MC and FC) or the low-dose group (ML and FL). The effects of the trial were significant; the amplitude of the startle response declined for all groups as trials progressed. Significant effects of sex were also observed. Male rats exhibited higher amplitude startle responses than female rats.

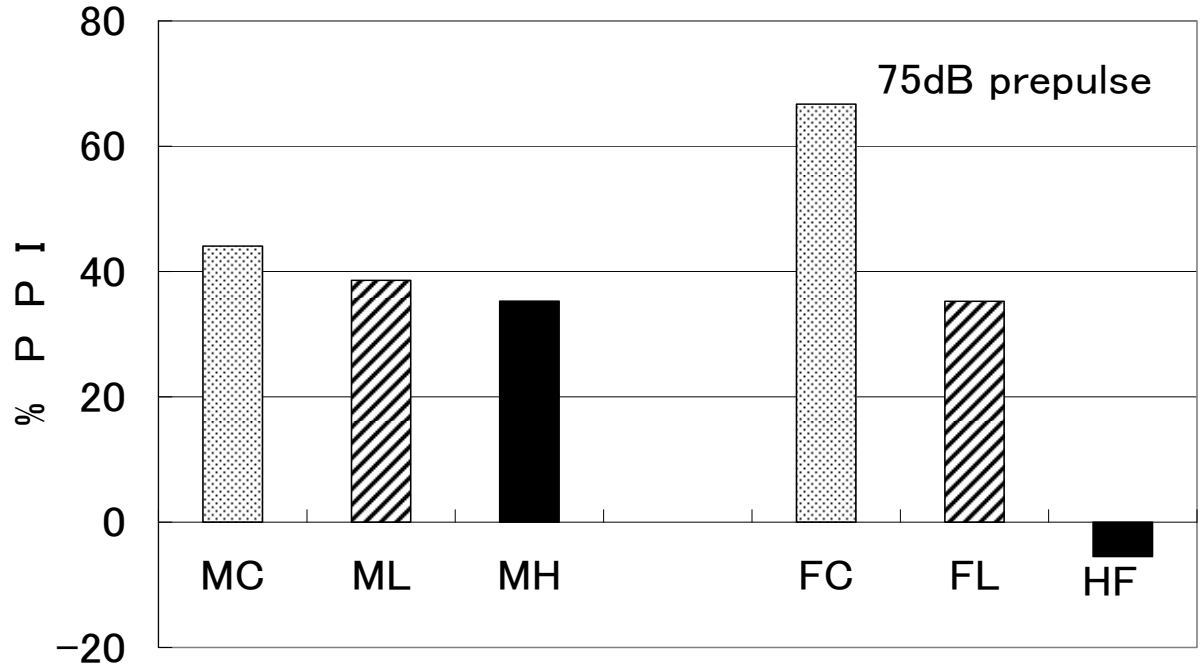

Fig. 2. Effects of methimazole treatments on the percentage of PPI. A startle stimulus of 115 dB with a prepulse of 75 dB reveals that the high-dose group animals (MH and FH) decreased PPI percentages from the control levels (MC and FC). FH demonstrates a greater reduction in PPI percentages than those seen for either FC or FL. PPI percentages are listed below 0.

The disruptions of PPI are observed in children with ADHD.[2,3] Furthermore we report attention deficits in hypothyroid animals using a target detection task.[6] The ability to shift attention to a new target is disturbed because the hit response percentages are decreased when the target is presented soon after the previous trial. Hypothyroid animals increase locomotion and rearing in an open field test.[4] Impulsive responses with inter-response times less than 0.5 s are obtained in a differential reinforcement of long latency schedule.[7] These behavioral deficits are similar to those in ADHD children and therefore, it serves as evidence of the validity of hypothyroid-animal model systems of ADHD.

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