HYPOTHYROIDISM GIVES RISE TO PANIC TENDENCIES IN RESPONSE TO AUDITORY STARTLE STIMULI IN THE RAT: NEUROTOXICOLOGICAL POTENTIAL OF PERSISTENT ORGANIC POLLUTANTS

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Abstract
Pregnant 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. Offspring received behavioral testing of an auditory startle response and prepulse inhibition at four and 26 weeks of age. The group treated with 0.02% methimazole displayed enhanced reactions to 40-ms auditory startle stimuli of 115dB. We did not, however, observe any effect of hypothyroidism on prepulse inhibition. We did discern a developmental delay in body weight gain for the 0.02% methimazole-treated group at four weeks of age, which recovered to normal levels by 26 weeks of age. We have concluded that perinatal hypothyroidism results in the panic tendencies in early childhood, which persists into adulthood. Several persistent organic pollutants disrupt the thyroid hormonal axis, which may result in neurotoxicological outcomes.

Introduction
Developmental disorders pose a serious problem worldwide. Persistent organic pollutants (POPs) are thought to be a risk factor for developmental disorders because of their inhibitory effects on the thyroid. The thyroid produces key hormones necessary for the regulation of normal brain development.[6]

Prepulse inhibition (PPI), the inhibition of a startle response when a high-intensity startle stimulus is preceded by a non-startling low-intensity stimulus, has been employed to attention studies. As PPI is thought to reflect information processing in the early automatic/preattentive stages,.[2] PPI is a useful measure to study attention processing. Recently, disruptions of prepulse inhibition (PPI) have been reported in children with attention-deficit hyperactivity disorder (ADHD)[1,3] characterized by hyperactivity, impulsivity, and attention deficits. These children did not inhibit a startle response when a startle stimulus is preceded by a non-startling stimulus.

We have applied PPI to studies of animal attention to examine if hypothyroid animals display disruptions in PPI.[7] Hypothyroid animals do not display attention deficits, but do exhibit sensory deficits in response to auditory stimuli as well as increased reactions to auditory startle stimuli, indicating panic tendencies.

These experiments, however, were conducted after the animals had reached adulthood at 43 weeks of age. Little attention has been paid to the developmental effects of hypothyroidism. In this study, we examined PPI in hypothyroid animals at four and 26 weeks of age to identify long-lasting effects of perinatal hypothyroidism.

Materials and Methods
Twenty-four pregnant Wistar rats were purchased on gestational day 8. Animals were housed in individual cages and randomly assigned to a control group (n=8), a low-dose group (n=8), or a high-dose group (n=8). The anti-thyroid drug methimazole, dissolved in distilled water, was administered to animals via drinking water beginning on gestational day 15 until postnatal day 21 at concentrations (w/v) of 0% (control), 0.002% (low-dose), or 0.02% (high-dose). After weaning at 21 days of age, one male and one female offspring were sampled from each dam. Eight male and eight female offspring were assigned to

each group, designated male control (MC), female control (FC), male low-dose (ML), female low-dose (FL), male high-dose (MH), and female high-dose (FH) groups. These animals were individually housed with chow and water *ad libitum*. Behavioral tests were conducted at four and 26 weeks of age.

Test chambers were clear acrylic cages (15 cm × 22 cm × 12 cm) with aluminum mesh walls on both sides. A piezoelectric accelerometer (GH313A, GA-245SO; KEYENCE, Osaka, Japan) attached underneath the cage detected the vibrations induced by a startle response. Voltage outputs from the accelerometer digitized at 1000 Hz were recorded on a personal computer through a 60 Hz low-pass filter. White noise was used for both the auditory startle stimulus and prepulse. White noise was amplified by a speaker placed adjacent to the test chamber. Both the chamber and the speaker were placed within a sound insulation box to attenuate external light and sound. Throughout testing, background noise was maintained at a constant level of 70 dB.

After a 5-min habituation to the test chamber in the presence of continuous background noise, all of the rats received behavioral tests to evaluate a startle response and PPI. To test startle responses, rats were exposed to a startle stimulus of 115 dB with a duration of 40 ms. Testing involved 10 trials in a day for three days. Inter-trial intervals were varied with a mean of 20 s. To test PPI, rats were exposed to either the startle stimulus alone (pulse trial: P trial) or the startle stimulus with a preceding prepulse (prepulse trial: PP trial). A 20-ms prepulse of 75, 85, or 95 dB intensity was presented 30 ms before the startle stimulus. PPI testing consisted of eight P trials and 10 PP trials per day for three days. The P and PP trials were presented randomly. The intensities of the prepulses changed every day in ascending order of 75, 85, and 95 dB for one-half of the rats and in descending order (95, 85, and 75 dB) for the remaining rats.

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 testing was performed during the dark period. This research was conducted with the approval of the Center for Advanced Science and Technology of Hokkaido University. All environmental conditions complied with the Guide for the Care and Use of Laboratory Animals for Hokkaido University.

Body weights were analyzed by two-factor ANOVA for sex and methimazole dose (between subjects). Startle response data were analyzed by three-factor ANOVA for sex, methimazole dose (between subjects), and day (within subjects). PPI data were analyzed by three-factor ANOVA for sex, methimazole dose (between subjects), and P/PP trial (within subjects). When the primary effects were significant, we employed multiple comparison tests by Ryan's method.

Results and Discussion

Methimazole treatment significantly decreased body weight gain in high-dose animals at four weeks of age [$F(2,42)=40.84$, $p<0.01$] (Fig. 1) in comparison to the control and low-dose groups [$t=7.87$, $df=42$, $p<0.01$: $t=7.79$, $df=42$, $p<0.01$, respectively]. Sex also had a significant effect on body weight; male animals were heavier than the female rats [$F(1,42)=63.68$, $p<0.01$]. There were no significant effects of methimazole treatment on body weight at 26 weeks of age, although male animals maintained heavier body weights than the female groups [$F(1,42)=1124.87$, $p<0.01$].

Methimazole administration had a significant effect on the amplitude of startle responses at four weeks of age [$F(2,42)=5.16$, $p<0.01$] (Fig. 2). The high-dose group displayed increased amplitude startle responses than those seen for either the control or low-dose groups [$t=2.71$, $df=42$, $p<0.01$: $t=2.85$, $df=42$, $p<0.01$, respectively]. Sex also had a significant effect on startling at this early age; male animals exhibited higher amplitude startle responses than female rats [$F(1,42)=15.01$, $p<0.01$]. The day of testing was also significant, with amplitude of responses gradually decreasing over time [$F(2,84)=3.69$, $p<0.05$]. The

significant effect of methimazole on the amplitude of a startle response remained at 26 weeks of age [$F(2,42)=22.00$, $p<0.01$]. The high-dose group still exhibited greater startle responses than the control and low-dose groups [$t=5.88$, $df=42$, $p<0.01$: $t=5.66$, $df=42$, $p<0.01$, respectively]. Male animals continued to display higher amplitude startle responses than the female group [$F(1,42)=16.30$, $p<0.01$], demonstrating the persistence of a significant effect of sex. At this age, however, the day of testing did not have a significant effect on startle responses in older animals.

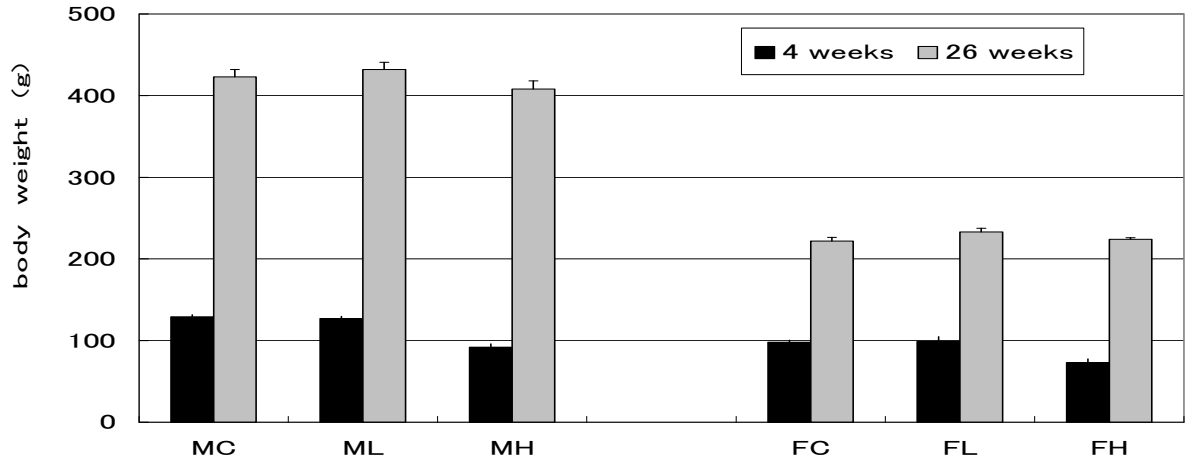

Fig. 1. Effects of methimazole on body weights. Data are means and SEM.

PPI was identified at four weeks of age for all prepulse intensities [75 dB: $F(1,42)=60.50$, $p<0.01$; 85 dB: $F(1,42)=224.75$, $p<0.01$; and 95 dB: $F(1,42)=161.75$, $p<0.01$]. PPI was also observed at 26 weeks of age for all three prepulse intensities [75 dB: $F(1,42)=236.17$, $p<0.01$; 85 dB: $F(1,42)=386.75$, $p<0.01$; and 95 dB: $F(1,42)=577.32$, $p<0.01$]. All methimazole-treated animals exhibited decreased startle responses in PP trials in comparison with P trials.

This study revealed that animals administered a high-dose of methimazole displayed greater amplitude auditory startle responses. In addition, all methimazole-treated groups exhibited PPI without any significant effect of methimazole dose. The high-dose group also exhibited decreased body weight gain at four weeks of age, which recovered to control levels by 26 weeks of age.

The high-dose group displayed greater amplitude startle responses at four weeks of age. This overreaction was long-lasting, persisting at 26 weeks of age. Negishi *et al.*[5] demonstrated that as hypothyroid rats are exceedingly fearful of electrical shock, they are unable to successfully learn the location of the hole enabling them to avoid the shock. It is suggested that hypothyroid animals easily panic which interferes with avoidance learning.[5] The results of this study suggest that panic tendencies appear in early childhood and persist into adulthood.

No effect of methimazole dose on PPI was observed. A startle response was inhibited in methimazole administrations when the startle stimulus was preceded by a prepulse. PPI was observed even when the prepulse had an intensity of 75 dB, the lowest intensity of the three prepulses used in testing. Thus, hypothyroidism does not affect either attention processes or sensory processes for auditory stimuli.

Body weight gain was decreased in the high-dose group in comparison to the control and low-dose groups. The high-dose group, however, increased in body weight with time, reaching the same levels as the control group by 26 weeks of age. Hypothyroidism has previously been reported to cause developmental delay of body weight gain,[4,8] but this delay would resolve by adulthood.

From these data, we conclude that perinatal hypothyroidism causes panic tendencies that are long-lasting. These results suggest the neurotoxicological potential of POPs to affect thyroid hormone systems.

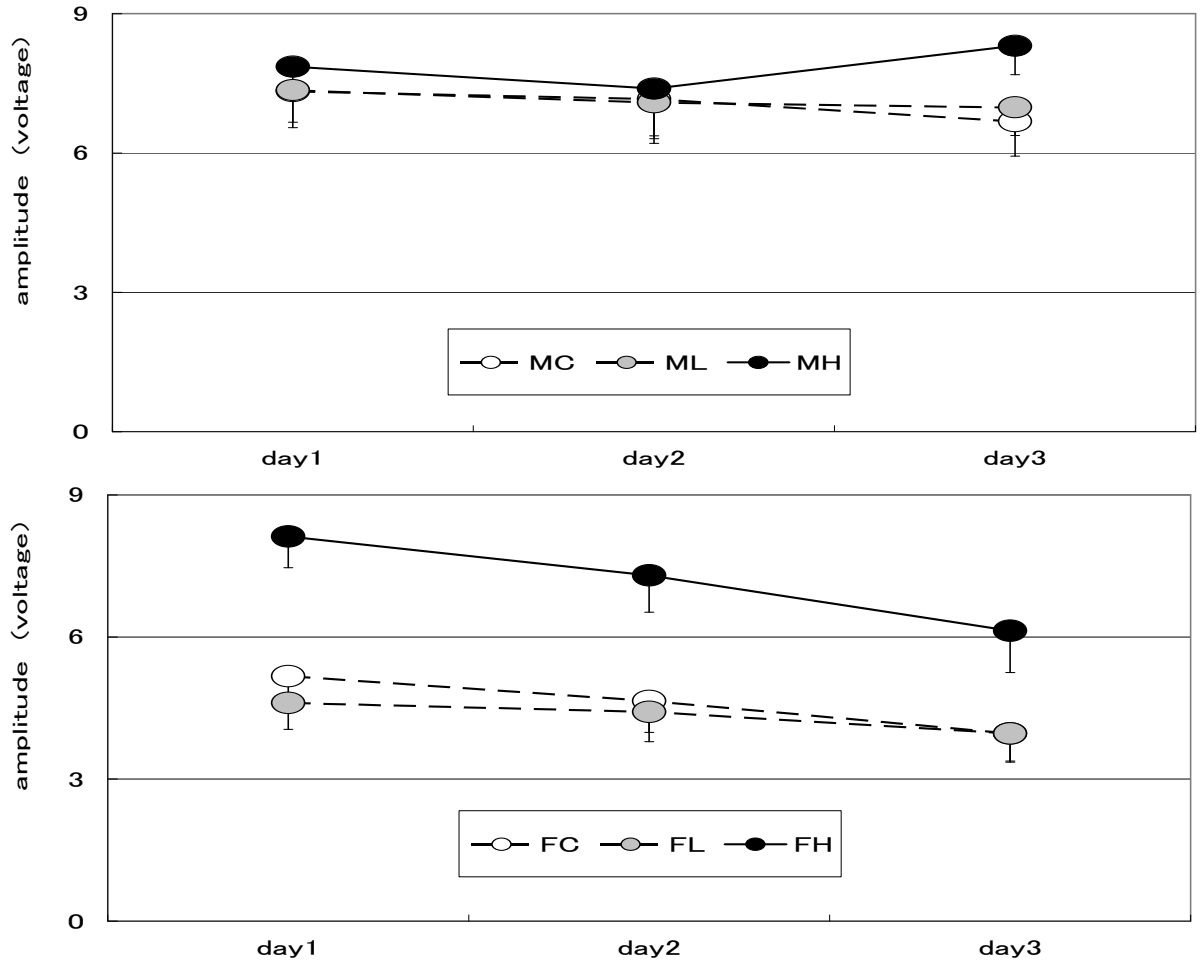

Fig. 2. Effects of methimazole on startle responses at 4 weeks of age. Data are means and SEM.

Acknowledgments

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