PERINATAL HYPOTHYROID RATS EXHIBIT LONG-LASTING IMPAIRMENTS IN SPACIAL LEARNING IN THE MORRIS WATER MAZE

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Abstract

The aim of this study is to clarify whether perinatal hypothyroidism affects spatial learning. Pregnant rats were treated with the anti-thyroid drug methimazole at concentrations of 0 or 0.02% (w/v) in drinking water from gestational day 15 to postnatal day 21. We then tested the offspring for deficits in learning ability in the Morris water maze at the age of 45 days or 15 months and found that the treated group exhibited increased swimming distance and time to reach the platform. The differences between the treated group and the control group were much greater at the age of 15 months. The swimming speed was also slower in the treated group at the age of 45 days but there was no difference at age of 15 months compared with controls. From these studies, we conclude that perinatal hypothyroidism affects spatial learning and the impairments worsen with aging.

Introduction

Persistent organic pollutants (POPs) are thought to be a risk factor for developmental disorders as evidenced by their disruption of the thyroid hormone system, which produces key hormones necessary for optimal brain development. Jacobson and Jacobson[3] surveyed children whose mothers ingested fish that were contaminated with polychlorinated biphenyls (PCBs), a class of POPs. The results showed that compared to control children, IQ scores, including memory ability, were lowered in children with prenatal PCB exposure. Furthermore, impairments in memory ability were evident at the 11-year follow-up study.[4] In light of studies such as these, we hypothesize that PCB-induced hypothyroidism affects brain development and results in learning impairment. To determine whether perinatal hypothyroidism affects learning ability, we induced perinatal hypothyroidism in experimental animals and tested their learning ability in the Morris water maze. The testing was performed at the age of 45 days or 15 months, and we identified long-lasting effects.

Materials and methods

Sixteen pregnant rats (Wistar strain) were purchased from Japan SLC Inc. on gestational day 8. The animals were randomly assigned to a control group (n=8) or a treated group (n=8). From gestational day 15 until postnatal day 21, we administered the anti-thyroid drug methimazole, dissolved in distilled water, to animals via drinking water at concentrations (w/v) of 0% (control) or 0.02% (treated). After weaning at 21 days of age, two male and two female offspring were sampled from each control dam. The offspring were assigned to the younger control (YC, eight males and eight females) or the older control (OC, eight males and eight females) group. Offspring from the treated group were weaned at 28 days of age because of developmental delays. These offspring were assigned to the younger treated (YT, eight males and eight females) or the older treated (OT, eight males and eight females) group. The dams of the treated group were just given distilled water after postnatal day 21. We behaviorally tested these animals in the Morris water maze at 45 days of age for the younger groups and 15 months of age for the older groups. Two male animals died before the behavioral testing began at the age of 15 months. Thus, the number of animals in their respective groups, OC and OT, decreased to 15. Rats in the younger groups were housed with two or three animals of the same sex, but the rats in the older groups were housed individually. All animals were supplied with chow and water *ad libitum*. 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 testing was performed during the dark period.

The water maze was a vinyl-chloride circular pool that measured 120 cm in diameter and 55 cm in depth. The pool was filled with water to a depth of 20 cm. The water was maintained at 23 ± 1°C and darkened with non-toxic black carbon ink. The pool was divided into four quadrants, and a circular platform 15 cm in diameter was submerged in the center of one of the quadrants 1 cm below the water surface. We changed the position of the platform for each animal. There were various extra-maze stimuli such as desks, shelves, and lighting fixtures

around the pool for use as spatial cues. These stimuli were fixed throughout the behavioral testing. A video camera was mounted on the ceiling above the center of the pool to record swimming images of the animals.

The trial began by placing the animal facing the wall of the pool in a quadrant that did not contain the platform. The animal was allowed to swim until it climbed onto the platform. If the animal did not reach the platform within 120 seconds, the experimenter guided the animal to the platform. The animal was left on the platform for 15 seconds and then returned to the cage. The next trial started after a 10- to 20-minute interval. All animals had trials in each of the three different quadrants each day for six days, and the order of the starting placement was changed every day. We obtained the swimming distance and time to reach the platform from the video images. The swimming speed was calculated as the the swimming distance divided by the swimming time.

The behavioral data obtained each day from the three different starting quadrants were pooled into one training block. We analyzed the data using a three-factor ANOVA with between-subjects variables of sex and methimazole dose and a within-subjects variable of training block. When the main effects were significant, we employed multiple comparison tests by Ryan's method.

This research was conducted with the approval of Hokkaido University. All environmental conditions complied with the Guide for the Care and Use of Laboratory Animals for Hokkaido University.

Results and discussion

Figure 1 shows the total swimming distance required to reach the platform from the three different starting quadrants. The total swimming distance of 45-day-old animals significantly differed by dose [$F(1,28)=6.11$, $p<0.02$] and training block [$F(5,140)=73.92$, $p<0.001$]. The YT group required a longer distance to reach the platform than the YC group. We found that the swimming distance of animals at 15 months of age also differed by dose [$F(1,26)=23.59$, $p<0.001$] and training block [$F(5,130)=32.97$, $p<0.001$]. The OT group required a much longer distance than the OC group. The interaction between dose and training block was also significant [$F(5,130)=3.74$, $p<0.005$]. The swimming distance in the OT group was longer than that in the OC group in training blocks 2 to 4 ($p<0.001$) and 5 ($p<0.005$). We did not observe an effect of sex at either age group.

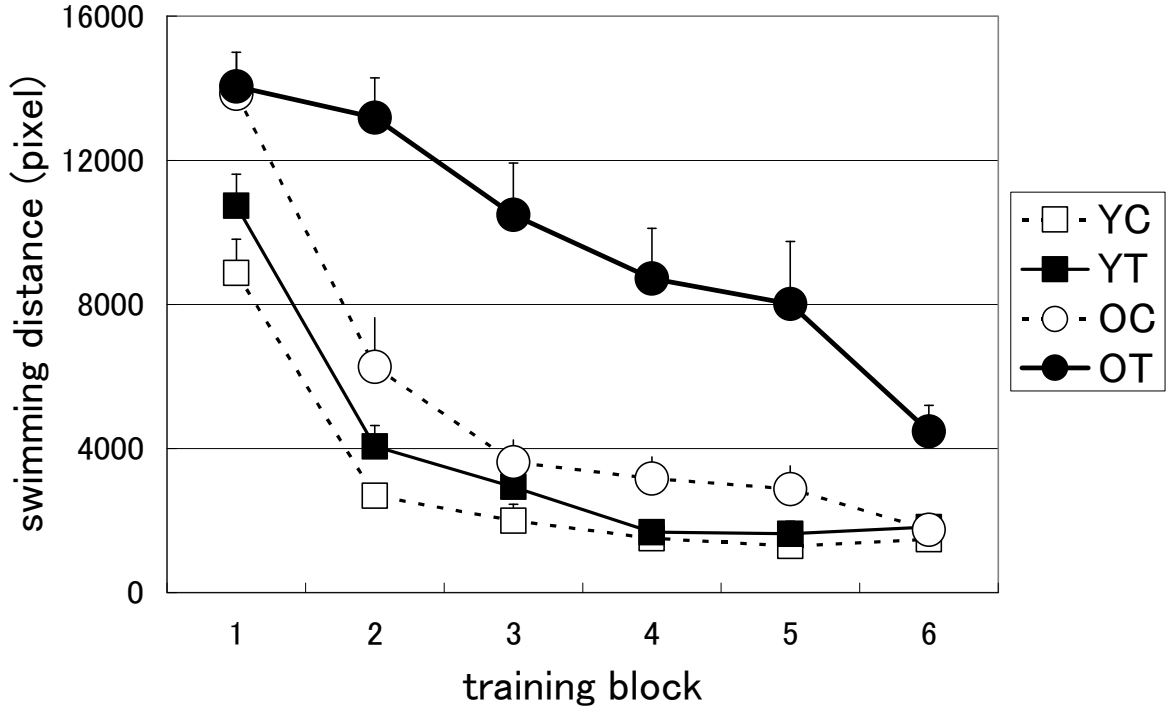

Fig. 1. Effects of methimazole on swimming distance to the platform. Since the effect of sex was not significant, the male and female data were combined and expressed as means plus SEM. YC: younger control; YT: younger treated; OC: older control; OT: older treated.

Figure 2 shows the total swimming time required to reach the platform from the three different starting quadrants. The swimming time of 45-day-old animals significantly differed by dose [$F(1,28)=19.28$, $p<0.001$] and training block [$F(5,140)=115.98$, $p<0.001$]. Similar to the swimming distance results, the YT group required a longer time to reach the platform than the YC group. The interaction between dose and training block was also significant [$F(5,140)=2.40$, $p<0.05$]. The swimming time in the YT group was longer than that in the YC group in training block 1 ($p<0.001$) and 2 ($p<0.01$). Furthermore, in animals at 15 months of age, we observed that swimming time significantly differed by dose [$F(1,26)=18.26$, $p<0.001$] and training block [$F(5,130)=27.34$, $p<0.001$]. The OT group required a much longer time to reach the platform than the OC group. Again, we did not find a significant effect of sex at either age (45 days or 15 months) for total swimming time.

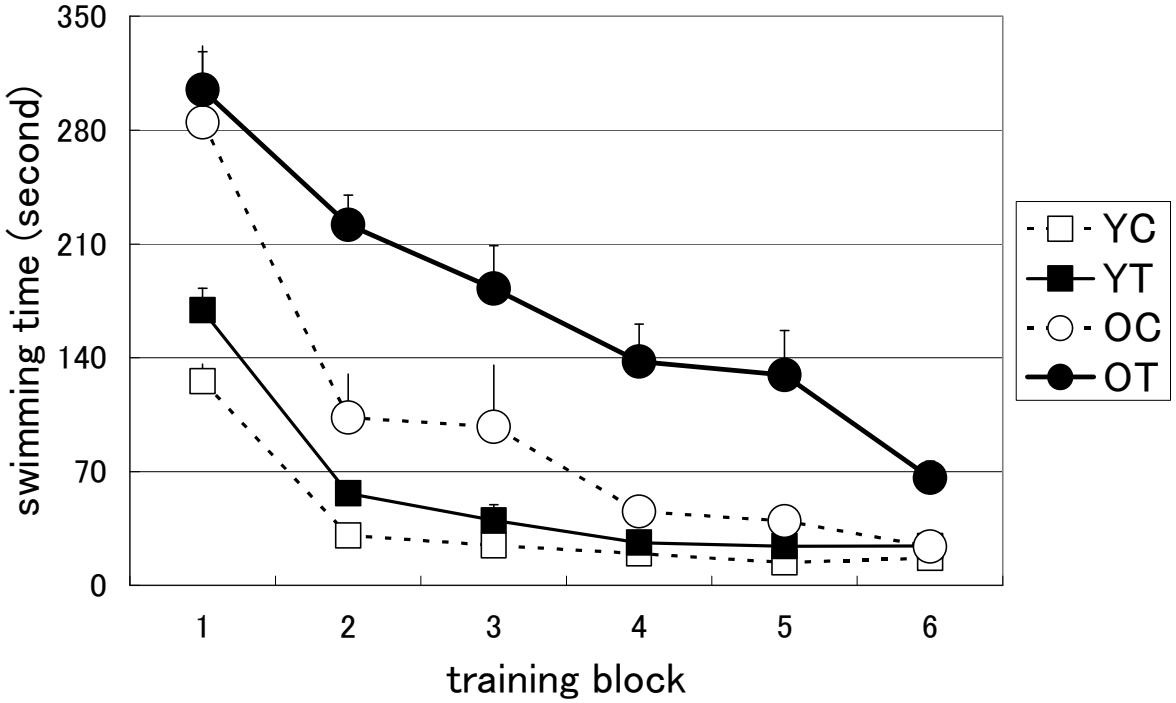

Fig. 2. Effects of methimazole on swimming time to reach the platform. Since the effect of sex was not significant, the male and female data were combined and expressed as means plus SEM. YC: younger control; YT: younger treated; OC: older control; OT: older treated.

Figure 3 shows the swimming speed as calculated by dividing the total swimming distance by the total swimming time. The swimming speed of 45-day-old animals significantly differed by dose [$F(1,28)=32.79$, $p<0.001$] and training block [$F(5,140)=9.29$, $p<0.001$]. The YT group exhibited decreased swimming speed compared to the YC group. The interaction between dose and training block was also significant [$F(5,140)=3.13$, $p<0.05$]. We found that the speed in the YT group was slower than that in the YC group in training blocks 2 and 5 ($p<0.001$), blocks 3 and 4 ($p<0.05$), and block 6 ($p<0.005$). Interestingly, the swimming speed at 15 months of age significantly differed by training block [$F(5,130)=3.84$, $p<0.005$] but not by dose. Again, we did not find a significant effect of sex at either age (45 days or 15 months).

Our study revealed that animals treated with an anti-thyroid drug displayed longer swimming distances and times to reach the platform at the age of 45 days. The differences between the treated and control groups decreased with the training blocks; thus, there were no differences between the groups in the last training block. This may suggest that the treated animals exhibited delayed spatial learning but were able to compensate over time. Furthermore, swimming speed was decreased in the treated animals, which indicates the possibility that swimming ability was reduced due to delayed physical development. The treated animals exhibited longer swimming distances and times at 15 months of age. Since the swimming speed was not different between the

treated and the control animals, the swimming ability was not compromised in the treated group. Moreover, the differences between the treated and the control animals were much greater in the older groups compared to those at 45 days of age.

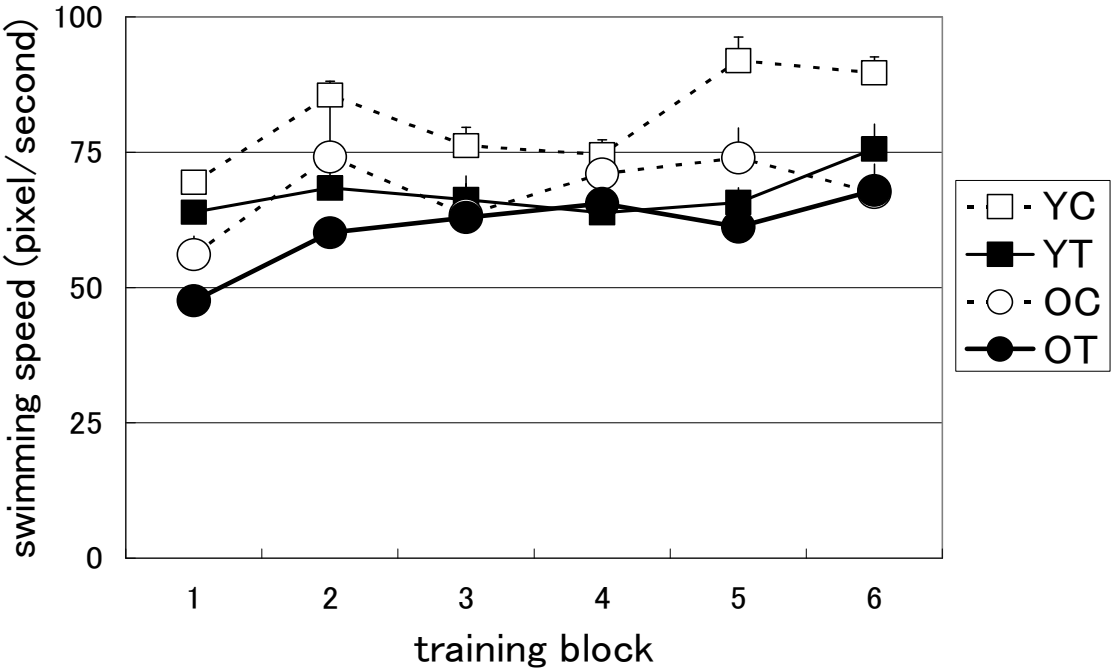

Fig. 3. Effects of methimazole on swimming speed. Since the effect of sex was not significant, the male and female data were combined and expressed as means plus SEM. YC: younger control, YT: younger treated, OC: older control, OT: older treated.

Research suggests that perinatal hypothyroidism affects gene expression related to neural network formation. For example, Barradas et al.[1] used a similar treatment paradigm as this study and found delayed expression of a myelin marker in the hippocampus of the offspring. Kobayashi et al.[2] reported that perinatal hypothyroidism can down-regulate gene expression associated with synapse plasticity or myelin formation in the cortex and hippocampus. Moreover, hypothyroid animals exhibit decreased abilities in water maze learning and reduced LTP in the hippocampus.[5] Indeed, abnormalities in gene expression are thought to affect neural network formation and result in learning and memory impairments.[2] According to these results, we conclude that perinatal hypothyroidism affects spatial learning as a result of the deterioration of neural network formation. Furthermore, we demonstrated that the spatial learning impairments worsen with aging, which suggests the necessity of neurotoxicological studies in aging research.

Acknowledgments

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References

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