

## DEVELOPMENT OF NOVEL BEHAVIOURAL TESTS FOR RODENTS TO STUDY THE HIGH-ORDER BRAIN FUNCTION ALTERED BY IN UTERO AND LACTATIONAL EXPOSURE TO DIOXINS

Kekeyama M, Endo T, Zhang Y, Miyazaki W, Tohyama C.

Laboratory of Environmental Health Sciences, Center for Disease Biology and Integrative Medicine (CDBIM), Graduate School of Medicine, The University of Tokyo, 7-3-1 Bunkyo-ku, Tokyo 113-0033, Japan.

### Abstract

We reported in the DIOXIN2007 conference that perinatal exposure to a low dose of dioxin induced anxiety-like behavior of rat offspring and perturbed a paired-associative learning performance in 'Flavor Map' test. Here, we introduce characteristic features of the three novel behavioral tests that have been recently developed (Flavor Map, KODOMO and IntelliCage) to elucidate effects of maternal exposure to dioxins on the high-order brain function in rats and mice. First, 'Flavor Map test' enables to examine the short term memory, long term memory and a paired-associative learning ability in adult rats. The task imposed to rats is presumably identical to an acquisition of city map in humans. Second, 'KODOMO' test that we have developed makes it possible to examine learning and emotional behavioral performance in juvenile rats. By this 'KODOMO' test, the attention deficit hyperactivity disorder (ADHD)-like behavior accompanied with a partial learning deficit was clearly observed in juvenile rats following maternal exposure to a polychlorinated biphenyl congener. Third, we developed our own learning test protocol in mice by using 'IntelliCage' system, that is capable of accommodating 12 mice at the same time to monitor and record their basic physical activities and learning function (Galsworthy *et al.* 2005). We could increase the efficiency of detection of the learning behavior. Using this system, male mice born to dams administered TCDD at 3.0  $\mu\text{g}/\text{kg}$  were found to have lower ability to make correct choice at the beginning of a repeated reversal task phase. These three new behavioral test batteries were found to be useful to study the high-order brain functions of rats and mice.

### Introduction

Maternal exposure to dioxins has been reported to affect the high-order brain function of offspring even when the exposure level is too low to affect their dams. On a molecular level, we have reported that a single oral dose of 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) of 200 or 800 ng/kg on gestation day (GD) 15 altered mRNA levels of glutamate NMDA receptor in Long-Evans hooded rats, indicating that such a low dose of TCDD affects molecular basis of the high-order brain function in the rat offspring<sup>1</sup>. On a behavioral level, TCDD affected the learning performance of offspring in the schedule-controlled operant behavioral task, especially in the multiple schedule of FR and DRL tasks, whereas no effect was observed when either FR or DRL only was imposed<sup>2</sup>, suggesting that TCDD affected complicated learning performance rather than simple one in the high-order brain function. Next, in collaboration with Richard Morris, we developed a novel sophisticated learning test methodology in rats, named 'Flavor Map' test, which enables to examine the paired-associative learning performance of rats using an event arena apparatus<sup>3</sup>. We applied this test to rat offspring born to dams exposed to TCDD on GD15, and found that the paired-associative learning performance was affected in the offspring (as reported in the DIOXIN2007 conference).

Here, we introduce three useful behavioral tests including 'Flavor Map' test, that have been developed recently to study possible alterations in the high-order brain functions in rats and mice. First, in order to study the more advanced function among the high-order brain function, we improved 'Flavor Map' test to study effects of maternal exposure to methylmercury. Second, to examine learning and emotional behavioral performance in juvenile rats, we have developed 'KODOMO' test, named after 'Knocking on Devil's of Mouth' and also 'children' in Japanese, and examined effects of maternal exposure to polychlorinated biphenyl (PCB) 153, a

abundant congener present in humans, since PCBs are suspected to induce the attention deficit hyperactivity disorder (ADHD)<sup>4</sup>. Third, we used IntelliCage<sup>5,6</sup> system, a commercially available apparatus that was originally developed by professor H.P. Lipp at University of Zurich<sup>5,6</sup>, to study the high-order brain function in mice. This system has an advantage over earlier behavioral tests in terms of automatic recording of physical activities and learning performance of 12 mice at a time. Unfortunately, the experimental test protocol provided by the manufacturer has a limitation of sensitivity and accuracy of detecting possible changes in behavior, we developed a new protocol to test learning performance, and applied it to study effects of maternal exposure to TCDD in mice.

## Materials and Methods

**Animals and exposure to chemicals:** In the 'Flavor Map' test, Long-Evans Hooded rats were exposed *in utero* to MeHg via maternal consumption of drinking water containing 0, 0.5, or 5 ppm of Hg as methylmercuric chloride, from 2 weeks before mating to the day of birth. In the 'KODOMO' test, pregnant Long-Evans Hooded rats were dosed by gavages with 0 (vehicle) or 2.0 mg/kg of 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153). In the 'IntelliCage' test, pregnant C57BL/6 mice were dosed by gavages with 0 (vehicle) or 3.0 µg/kg of TCDD. In all tests, one male offspring per litter (6 to 8 animals in each exposed group) was randomly selected for the behavioral experiment. Animal experiments and handling of hazardous chemicals were performed, according to the guidelines for animal experiments and environmental safety, respectively, at the University of Tokyo.

**'Flavor Map' test:** The event arena and the Flavor Map testing were described previously<sup>3</sup>. Briefly, animals were trained to learn flavor-place associations concurrently, using different flavors of food (flavor cues) and sand-wells (place cues) located within the event arena. After being given a cue flavor in a start box, the animals recall the spatial location with which it is associated, and run into the arena to that location to secure more of that flavor of food. The animals visited and sometimes dug at incorrect sand-wells, which did not contain food on that particular trial, until they found the correct one.

**'KODOMO' test:** We have developed this unique apparatus in our laboratory (Fig. 1), and this apparatus consists of three fields that range to length, and each wall between fields has three automatic gates to pass through. Habituation period of 5 days was given to 15 day-old rats until postnatal day (PND) 20, and learning task was assigned to the rats from PND 20 to 25.



**Fig. 1.** Behavioral test apparatus for juvenile rats named Knocking on Devil's of Mouth (KODOMO).

**'IntelliCage' test:** IntelliCage<sup>5,6</sup> (New Behavior AG, Zurich, Switzerland) is an automated system designed for continuous long-term recording of the home cage behavior in social groups and complex analysis of basic activities and learning in adult mice, and can be controlled by a computer with installed software that executes preprogrammed experimental schedules and registers numbers of visits to corner chambers, nose pokes to the door areas and tube lickings as parameters of behavior. Mice must make an access to correct corner chambers of IntelliCage. A simple spatial discrimination task was assessed on first 7 days. After this task, reversal tasks were assessed every 7 days.

**Statistical Analyses:** All statistical analyses were carried out by using the SPSS 15.0 data analysis with comprehensive statistics software. Data were analyzed by one-way or independently two-way ANOVA, followed by Scheffé's post-hoc test. A difference in means was considered significant at  $p < 0.05$ .

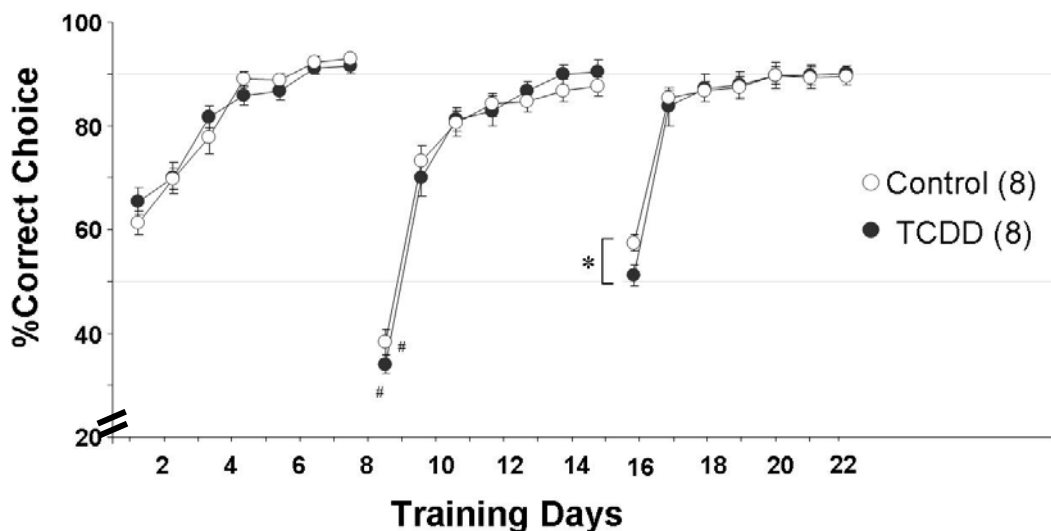
## Results and Discussion

**'Flavor Map' test:** As reported in Dioxin2007, maternal exposure to 200 ng/kg of TCDD (a single oral dose on GD 15 to their dams) decreased paired-associate learning in Flavor Map test in offspring, but 800 ng/kg exposure had no effects, suggesting that the effects of dioxin on paired-associate learning do not follow a monotonous dose response curve. In a MeHg exposed experiment, we found that maternal exposure to MeHg significantly perturbs the short term and long term memory formation in a dose-dependent manner. The present results demonstrates that Flavor Map test can be used study a more advanced function among the high-order brain function.

**'KODOMO' test:** We found that control animals succeed to learn the assigned task by PND 25. The time and distance of movement to a goal (correct gate), and number of errors before reaching a goal decreased gradually over training. In contrast, PCB153-exposed animals showed longer time and distance of movement than control animals ( $p < 0.01$ ). In addition, the percentage of active time of PCB153-exposed animals gradually increased over training, whereas that of control animals did not change throughout tests. As shown in Fig. 2, the PCB153-exposed animals seem to show impulsive and hyperactive movements. Thus, the attention deficit hyperactivity disorder (ADHD)-like behavioral abnormality accompanied with a partial learning deficit were observed in juvenile rats following maternal exposure to PCB153.



**Fig. 2.** IntelliCage, an automated system designed for continuous long-term recording of the home cage behavior.



**Fig. 3.** Maternal exposure of mice to TCDD perturbs cognitive flexibility in the reversal task in 'IntelliCage' test. Data represents the mean  $\pm$  S.E. of % of correct choice in each group in the first 100 choice of each training day. #,  $p < 0.05$  vs. chance level. \*,  $p < 0.05$  vs. control group.

**'IntelliCage' test:** In a spatial discrimination task (Day 1 to 7), control and TCDD-exposed animals made fewer errors over training day and reached over 90% correct choices on day 7 (Fig. 3). On the first day of 1<sup>st</sup> reversal task (Day 8), both control and TCDD-exposed animals made lower correct choice ( $p < 0.05$ , vs. chance level), indicating that mice succeeded to learn the spatial discrimination task of 'IntelliCage' test within 7 days, and they could not adopt reversal task at the beginning of reversal task. From the second day of the 1<sup>st</sup> reversal task, the %correct choice in both groups rapidly increased and reached over 90 % correct choices within 7 days. There was no significant difference in performance between each group in spatial discrimination task and the 1<sup>st</sup> reversal task. On the first day of the 2<sup>nd</sup> reversal task (Day 15), TCDD-exposed animals showed significantly fewer correct choices than control ( $p < 0.05$ , Fig. 3), especially in the latter half of the day ( $p < 0.0$ ). These results indicate that maternal exposure of mice to TCDD perturbs cognitive flexibility in the reversal task of 'IntelliCage' test.

### Conclusion

'Flavor Map' test enables to examine a paired-associative learning ability, that thought to reflect a more advanced function among the high-order brain function in rats. 'KODOMO' test enables to examine learning and emotional behavioral performance in juvenile rats. 'IntelliCage' system accompanied with our test protocol makes it possible to examine learning performance automatically, and is useful as a facile test method. the use of these new tests, will be useful to evaluate possible effects not only dioxin and PCBs but also other chemicals on the high-order brain function.

### Acknowledgements

This study was supported in part by Environmental Risk Office Fund (Animal studies on health effects of dioxins) of the Ministry of the Environment, Japan, a grant grants from the Environmental Technology Development Fund to CT, Grants-in-Aid from Food Safety Commission, Japan (0601) and the Grant-in-aid from Ministry of Education, Science and Culture in Japan to CT (15201014) and MK (18681008), and Health and Labour Sciences Research Grants from Ministry of Health , Labour and Welfare, Japan to MK. The views expressed in this presentation do not necessarily reflect the positions or policies of these governmental organizations.

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