Spatial and Non-Spatial Learning in Rats Exposed to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) During Gestation and Lactation

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Abstract

Previously we reported faster acquisition of a radial arm maze (RAM) working memory task in rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) during gestation and lactation. This effect was more striking in males than females. In this study we further investigated the effects of TCDD on learning and memory, by testing male and female TCDD-exposed rats on three spatial learning and memory tasks, the RAM, the Morris water maze, and spatial discrimination-reversal learning (RL), as well as on a non-spatial learning task: visual RL. Time-mated Sprague-Dawley rat dams were gavaged with 0.1 μg/kg TCDD or corn oil vehicle on days 10-16 of gestation. Litters were culled to eight on day two and weaned on day 21. Beginning on day 80, one male and one female from each litter were tested on an eight-arm RAM with all arms baited. The animals were tested for 35 sessions. Following RAM testing, the animals were rested for two weeks and then tested on the Morris water maze for 24 sessions, eight per day. Another male and female from each litter were tested on spatial RL on a T-maze. One of the two goal arms was consistently baited on every trial. After the animal reached a criterion of 10 of 12 trial correct, the opposite arm was baited until the criterion was achieved. Four of these position reversals were given. Following the spatial RL, the same animals were tested on a visual RL task in the same maze. Low-wattage cue lights were attached to the end of each goal arm and either the lit or the unlit arm was baited on every trial. Original learning was followed by four reversals. As expected, the TCDD-exposed rats made significantly fewer errors on the RAM, and the facilitation was more striking in males than in females. However, TCDD-exposed male rats were not facilitated in their learning of either the spatial RL task or the Morris water maze task. These results demonstrate a reliable, but task-specific, facilitation of spatial learning and memory in male rats exposed to TCDD during gestation and lactation. In contrast, the TCDD-exposed rats showed an impairment in learning on the visual RL task. Specifically, they took significantly more trials to learn the first of the four reversals. There was a similar, but non-significant increase in trials to criterion on the second reversal. These findings are consistent with those of earlier monkey studies in which TCDD-exposed monkeys were facilitated in their ability to learn certain spatial learning tasks, but were impaired on visual RL tasks. The monkeys also showed an impairment on the first two reversals, but not on the later reversals.

Introduction

Previously, we reported faster acquisition of a radial arm maze (RAM) working memory task in rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) during gestation and lactation[1]. The effect was more striking in males than females. However, we did not see a similar facilitation on another spatial learning and memory task, delayed spatial alternation. In this study, we further investigated the effects of TCDD on spatial learning and memory by testing male and female TCDD-exposed rats on three different spatial learning and memory tasks: the RAM, the Morris water maze, and spatial discrimination-reversal learning (RL). In an earlier study, monkeys exposed to TCDD during gestation and lactation appeared to be facilitated on spatial learning tasks, but were impaired on a visual RL task[2]. Therefore, we also tested TCDD-exposed rats on a visual RL task to investigate whether they would exhibit a similar impairment.

Methods

Animals and Exposure Time mated Sprague-Dawley rat dams were obtained from Harlan Sprague-Dawley (Dublin, VA) on day one of gestation (date of sperm plug=gestation day 0). On gestation days 10-16, each dam was dosed via gavage with either 0.10 μg/kg/day TCDD or corn oil vehicle. Litters were culled to 8 on day 2 and weaned on day 21. Pups were housed in same-treatment, single-sex pairs after weaning, and were maintained on a 12 hour reverse light-dark cycle. Food and water were available ad libitum except during RAM and T-maze testing. Rats were tested on the RAM or T-maze beginning at day 80. Testing was conducted Mon-Fri during the dark phase of the cycle.

Radial Arm Maze (RAM) One male and one female from each litter were tested on the RAM. The maze consisted of a circular platform with eight arms extending from it radially. All eight arms were baited with a food reinforcer (¼ of a Kellogg's Froot Loop). The rat was then placed inside an opaque ring on the center platform. After 10 sec, the ring was lifted and the rat was free to roam the maze. The session was terminated when the rat had retrieved all eight baits or after 5 min had elapsed. Rats were tested for 35 sessions. Number of errors (reentries in arms already visited) was recorded using a computerized scoring system. The data were averaged into blocks of five sessions for analysis.

Morris Water Maze After the animals completed the RAM, they were placed on free feed for 2 weeks, and then tested on the Morris water maze. The maze consisted of a circular pool of water, which was 174 cm in diameter, with a 10 cm x 10 cm platform located 2 cm under the water. To obscure the rat's ability to see the platform, the water was made opaque by adding non-toxic white paint. The rat was placed in the water at one of four locations and allowed to swim about the maze. The session was terminated when the rat found the platform or after 60 sec elapsed. Rats were tested for 24 sessions, eight per day, and the latency to find the platform was recorded. The data were averaged into blocks of eight trials for analysis.

Spatial Discrimination Reversal Learning (RL) Another male and female from each litter were tested on an RL task on a T-maze. One of two goal arms was consistently rewarded on

every trial. The rewarded spatial location was counterbalanced across sex and treatment. For each trial, the rat was placed in the start box for 10 sec. Then the start box door was raised, and the rat was given 30 sec to chose an arm. The rat was kept in the choice arm for 20 sec and then returned to the start box for the next trial. Rats were tested for 12 trials per day until a criterion of 10 of 12 trials correct was achieved (original learning, OL). OL was followed by a series of four spatial reversals.

Visual Discrimination Reversal Learning After completion of the spatial RL task, the same animals were tested on a visual RL task in the same maze. Low-wattage lights were attached to the end of each goal arm and one of the two lights was lit on each trial. For each rat, the reward was consistently associated with either the lit or the unlit arm, and which condition (lit or unlit) was rewarded was counterbalanced across treatment group and sex. As with the spatial RL, the rats were trained to a criterion of 10 of 12 correct on OL and then four reversals were given.

Statistical Analysis The data were analyzed via repeated measures analysis of variance (ANOVA). The litter was used as the unit of variance, and sex was treated as a within litter variable (repeated measure). For the RAM, working memory errors were analyzed via a three-way ANOVA with exposure group (TCDD or control) as a between subject variable, and sex (male or female) and session blocks (1-7) as repeated measures. For the Morris water maze, latency to find the platform was analyzed via a similar ANOVA with sex and session blocks (1-3) as repeated measures. For RL, trials to criterion was analyzed with sex and reversals (1-4) as repeated measures. Statistical significance was ascribed at $p < 0.05$.

Results and Discussion

There were no statistically significant differences in dam liver weight, gestational weight gain, litter size, percent live births, pup birth weight, or pup weaning weight. However, liver weights were significantly increased and thymus weights were significantly decreased in the TCDD-exposed pups. Both of these changes are typical signs of TCDD exposure. Furthermore, the magnitude of the changes was consistent with what we have observed previously in rats exposed to the same dose of TCDD[3].

Analysis of the RAM data revealed a significant treatment effect ($F_{(1,13)}=4.74$, $p < 0.05$) and a nearly significant sex effect ($F_{(1,13)}=4.62$, $p=0.051$). Further analysis of each sex separately showed that the TCDD exposed males made significantly fewer errors than the control males ($F_{(1,14)}=10.17$, $p < 0.01$; Fig. 1), whereas the TCDD exposed females did not differ significantly from the control females ($F_{(1,14)}=0.86$, $p=0.37$). This finding replicates our previous finding of improved RAM performance in perinatally TCDD-exposed male rats[1]. In contrast, there were no statistically significant differences between the TCDD exposed animals and the controls on latency to find the submerged platform in the Morris water maze task or on trials to criterion on OL or any of the four reversals on the spatial RL task. Previously we also reported that TCDD-exposed rats did not differ from controls on a delayed spatial alternation task[1]. Together, the present results and those of our earlier study demonstrate a reliable, but highly task specific, improvement in spatial learning and memory in perinatally TCDD-exposed rats.

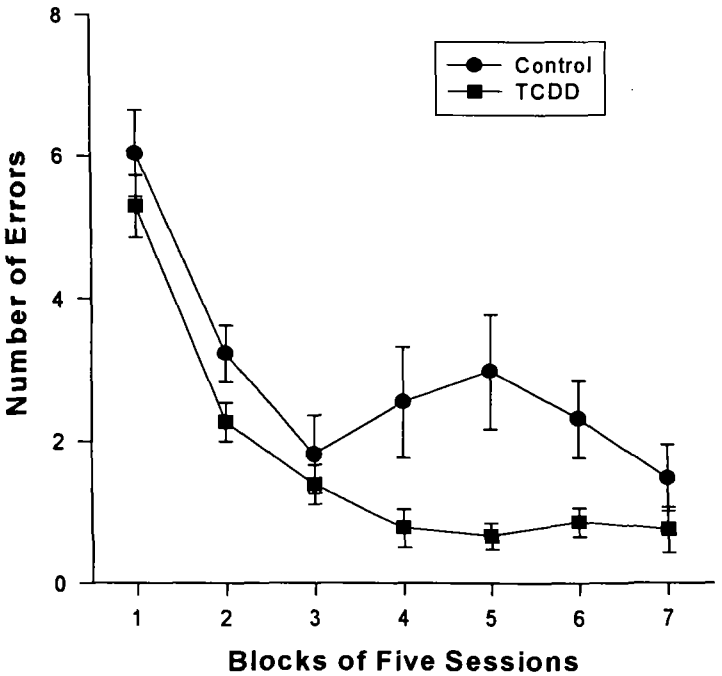

Figure 1. Mean ± SE number of RAM errors on each of seven blocks of sessions for male rats born to dams exposed to TCDD (0.1 μg/kg/day) or corn oil vehicle on gestation days 10-16. N=6-9/exposure group. There was a significant ($p < 0.01$) treatment effect.

Unlike the negative effects for spatial RL, analysis of the visual RL data revealed a significant treatment x reversal interaction ($F_{(4,72)}=2.44$, $p=0.05$). Post hoc analyses of trials to criterion on each reversal indicated a significant effect on the first reversal ($F_{(1,18)}=4.38$, $p=0.05$). TCDD-exposed animals took more trials to reach criterion (Fig. 2). A similar, but non-significant increase in trials to criterion was observed on the second reversal. In an earlier study, we reported that monkeys exposed to TCDD during gestation and lactation showed an impairment on visual RL^2. The monkeys also showed an impairment on the first two reversals, but not on subsequent reversals.

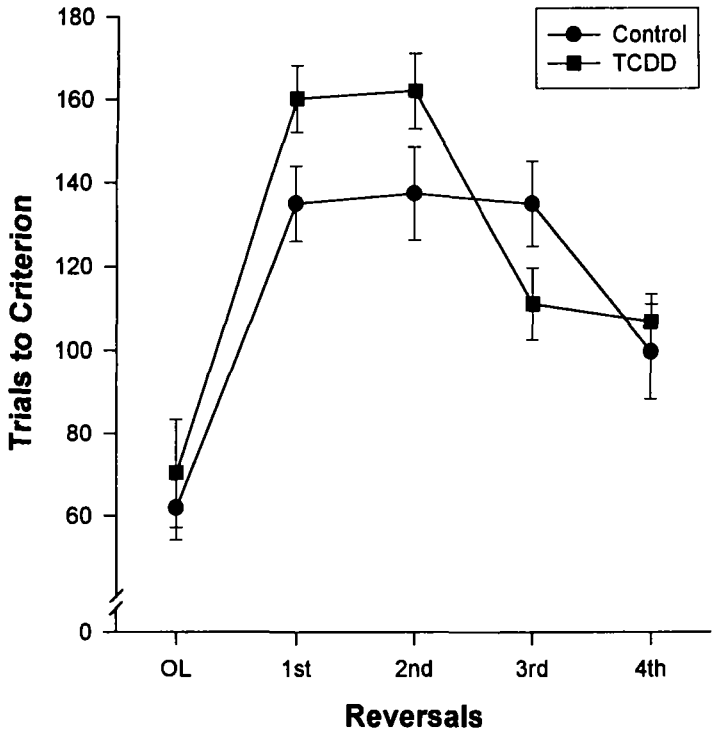

Figure 2. Mean ± SE trials to criterion on OL and four reversals of the visual RL task for rats born to dams exposed to TCDD (0.1 μg/kg/day) or corn oil vehicle on gestation days 10-16. N=10/exposure group. There was a significant treatment x reversal interaction ($p = 0.05$). Post hoc comparisons of the TCDD group to the control group at each reversal indicated a significant difference for reversal one ($p = 0.05$).

Conclusions

In conclusion, these findings demonstrate that rats exposed to TCDD during gestation and lactation show a pattern of behavioral effects very similar to that reported previously in monkeys[2]. That is, both TCDD-exposed rats and monkeys show facilitated learning of spatial tasks, and impaired ability to solve cue-based visual RL tasks[1,2]. The findings reported here demonstrate that the facilitation of spatial learning in rats is highly task specific. The underlying mechanism for this unusual pattern of effects is unknown. However, it is well-documented that various types of brain damage can lead to improvement on some types of learning tasks and impairment on others[4].

Literature Cited

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