Continuous follow-up of feeding and drinking patterns in rats after TCDD exposure

Sanna Lensu[1], Jere Linden[2], Jouko Tuomisto[1], Raimo Pohjanvirta[3]

[1]National Public Health Institute
[2]University of Helsinki
[3]University of Helsinki & National Veterinary and Food Research Institute, Kuopio Department

Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin, TCDD, causes a dose-dependent suppression of feed intake in rats culminating in a wasting syndrome at lethal doses [1]. Despite the drastic nature of TCDD-induced anorexia, it has not been characterized in detail. In the present study, we studied incessantly for 5 days changes in feeding and drinking behaviour of TCDD-sensitive rats treated with a lethal dose of TCDD. This was accomplished with an automated monitoring system.

Material and Methods

13-15-week-old TCDD-sensitive Long-Evans rats (*Turku*/AB; LD50 10 – 20 µg/kg) [2] were habituated to daily handling and eating 45-mg dust-free feed pellets (Bio-Serv, US) for about one week before they were moved into a specific cage allowing continuous recording of eating and drinking (Habitest; Coulbourn Instruments, US). The animal room was controlled for light (12/12 h light/dark cycle, lights on at 7 a.m.), temperature and humidity. The experiment was reviewed and approved of by the Committee for the Welfare of Laboratory Animals of the University of Kuopio and by the Provincial Government. The procedures were conducted in accordance with the Guidelines of the European Community Council directives 86/609/EEC.

The test cage (25 x 30 cm) for a single rat has a wire mesh bottom and plexiglass walls. Autoclaved aspen chips and wooden toys were given for each rat to enrich the environment. A part of the front wall of the cage was covered with black plastic providing visual shelter for the animal. Rats were familiarized to the Habitest cages for approximately one week and experiments were started after they were drinking and eating normally.

Feeding and drinking data were collected continuously with CoulbournHabitest® system which was controlled by Graphic State 3.01 software. In this system, the feeder delivers a single pellet automatically after the rat has removed one from the pellet tray and a computer records the event. Water was available *ablibitum* from a water bottle equipped with an infrared light emitter and sensor to measure the number of tongue licks.

After baseline measurement for one day, rats were treated either with TCDD (UFA-Oil institute, Ufa, Russia; 100 µg/kg, intragastric administration, [*i.g.*], n = 5) or the vehicle, corn oil (4 ml/kg, *i.g.*, n = 5). Feeding and drinking were monitored for five consecutive days following the exposure. The data were analysed with Graphic Sate 3.01, MS-Excel 2000 and SPSS 12.0.1. Group means were compared using independent samples' t-test.

Results and Discussion

On the exposure day, body weights of control and TCDD rats were 305 ± 24 g and 304 ± 40 g, respectively (mean ± SD). After the five-day follow-up period, TCDD-treated rats weighed significantly less ($p < 0.05$) than controls (258 ± 38 g vs. 309 ± 27 g). TCDD-treated rats ate significantly fewer pellets than controls already on day 2 post-exposure, and that difference remained throughout the rest of the five-day experiment.

The clearest effect of TCDD on feeding patterns was detectable in the morning hours. Control rats ate approximately 20 – 30 % of their total daily food intake during morning hours (approximately 5:30 a.m. to 7:30 a.m., figure 1). On the second morning after the exposure (appr. 42 hours from the exposure) there was a significant drop in the TCDD group in the number of pellets eaten at the light/dark transition (6:30-7:30). At three days postexposure, TCDD-treated rats almost totally gave up eating during morning hours (5:30 – 8:30; in figure 1 panels B and D show the

eating on day 5 postexposure). Moreover, there was also a significant decrease in the number of pellets eaten during 17:30 – 21:30, an effect that was detectable from day 3 on. However, feed consumption during these evening hours amounted to 30 % of total daily energy intake in TCDD-treated rats and did not differ from the controls: mean of the relative daily energy intake between 17:30 – 21:30 during the last 3 evenings was 30.0 ± 7 % and that of the controls was 30.7 ± 2 %. It seemed that TCDD exposure shifted feeding to the evening or to night (figure1, panel D). On day 5 (B), the total number of pellets eaten was substantially decreased by TCDD ($p<0.01$): expressed as feed mass, control animals ate 19.5 ± 2 g whereas TCDD-treated rats ate only 1.6 ± 2 g.

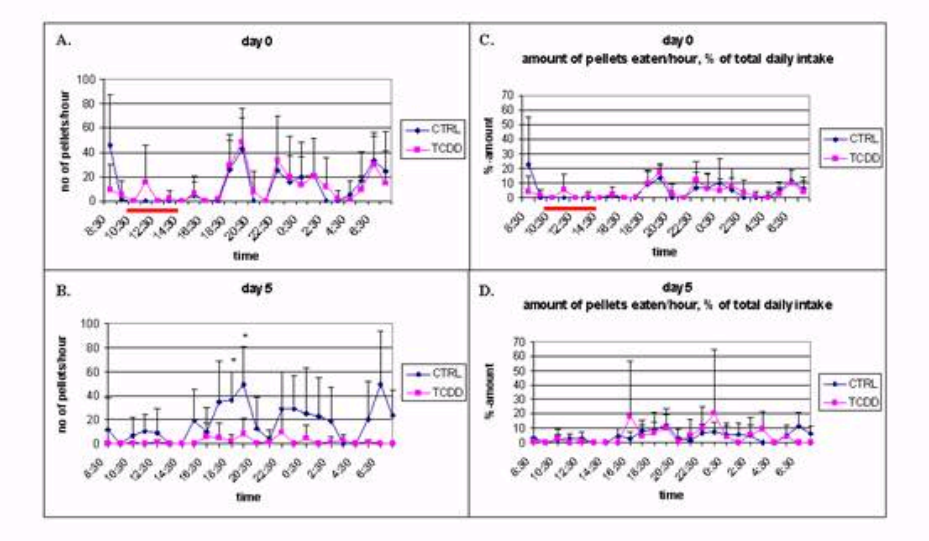

Figure 1. The number of pellets eaten per hour (mean ± SD, n = 5 for both groups) on exposure day (A) and five days after the exposure (B). The time of TCDD exposure is shown with a red bar (A, C). In panels C and D, food intake is calculated as the hourly consumption relative (%) to the total daily consumption.

In drinking behaviour there were no such clear phase shifts as in feeding (figure 2). Interestingly, on the exposure day TCDD exposed rats drank more during daytime ($p<0.05$). Rats were exposed at noon (± 2 hours) and hourly drinking was increased in the afternoon (at 16:30 and 18:30, fig. 2A). Daily drinking was significantly decreased only five days after the exposure.

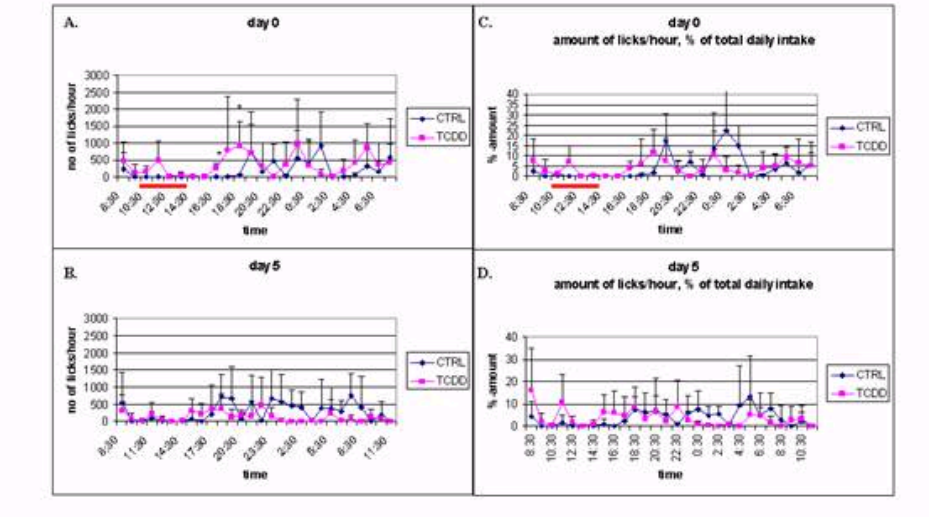

Figure 2. The number of licks per hour (mean ± SD) on exposure day (A, n = 4 for both groups) and five days after the exposure (B, n = 5 for both groups). TCDD exposure is shown with a red bar (A, C). In panels C and D drinking is calculated as the hourly consumption relative (%) to the total daily drinking.

Although the dose of TCDD used (100 µg/kg) is 100% lethal to the L-E rat strain, daily total food intake was not significantly diminished until day 3 postexposure. However, the changes recorded in circadian feeding patterns suggest a role for the central nervous system. Feeding is controlled by the circadian rhythms (e.g.[3]), and as our study shows that TCDD affects the physiological rhythm it supports the notion of a specific disturbance in some regulatory pathway in the central nervous system. TCDD causes a wasting syndrome which finally leads to death, but even if the animals survive, treated animals retain their body weight level below that of the controls [1,4]. Previously our group has reported decreased melatonin levels [5] and increased serotonin turnover [6] in the brains of TCDD-treated rats, but these findings do not completely explain the wasting and the neuronal mechanisms behind the syndrome.

Some immediate responses to TCDD have been reported, including increased dopamine turnover [7] and changes in food selection, especially when the animals were not habituated to food they were offered [8]. In the feeding behaviour we did not find any significant immediate changes, but drinking was significantly, though transiently, increased a few hours after TCDD exposure. Since rats usually drink water postprandially, the behavioural dissociation detected here implies that TCDD may distinctly affect feeding and drinking regulation warranting more in-depth studies.

Acknowledgements

Financial support [S.L.] from the Graduate School in Environmental Health (Ministry of Education) is acknowledged. The work was supported by grants from the Centre of Excellence Program of the Academy of Finland to the Centre of Environmental Health Risk Analysis (Grant no. 53307) and from the Academy of Finland (Grants no. 200980 and no. 211120 [R.P.]). Additional grants from Research and Science Foundation of Farmos and SocietasBiologicaFennicaVanamo[S.L.] are warmly acknowledged.

References

1. Pohjanvirta R., and Tuomisto J. (1990) *Toxicology* 63: 285-299.

2. Unkila M., Pohjanvirta R., MacDonald E., Tuomisto J. T., and Tuomisto J. (1994) *ToxicolApplPharmacol.* 128: 280-292.

3. Kalra S. P., Dube M. G., Pu S., Xu B., Horvath T. L., and Kalra P. S. (1999) *Endocr Rev.* 20: 68-100.

4. Seefeld M. D., Keesey R. E., and Peterson R. E. (1984) *ToxicolApplPharmacol.* 76: 526-536.

5. Pohjanvirta R., Tuomisto J., Linden J., and Laitinen J. (1989) *PharmacolToxicol.* 65: 239-240.

6. Unkila M., Pohjanvirta R., and Tuomisto J. (1998) *ArchToxicol.* 72: 769-776.

7. Russell D. H., Buckley A. R., Shah G. N., Sipes I. G., Blask D. E., and Benson B. (1988) *ToxicolApplPharmacol.* 94: 496-502.

8. Tuomisto J. T., Viluksela M., Pohjanvirta R., and Tuomisto J. (2000) *PharmacolBiochemBehav.* 65: 381-387.