pan (Med Associates, St. Albans, VT, USA). These chambers were accommodated in a sound-attenuating box (550 (w) x 500 (h) x 500 (d) mm) equipped with a speaker in the side wall. To minimize the possible effects of contextual factor for auditory test, two types of chamber configuration, described as context A and B below, were used. For context A, the front, top and back walls of the chamber were made of transparent acrylic resin, and the two side walls were made of modular aluminum. For context B, a white, polyvinyl chloride floor covered the shock-grid bars and a white, polyvinyl chloride round-arched insert was placed inside the conditioning chamber. A tone (80 dB, 1,800 Hz, 30 s) was presented as a CS via a speaker electrically connected to a computer. To control and present the stimulus, a computer software FreezeFrame (MED Associates) was used. An electric foot shock (0.75 mA, 2 s) as a US was delivered via a shock generator (Med Associates). Background noise (65 dB) was provided by internal fans. The behavior of each mouse was monitored and recorded using a digital video camera that was connected to a computer and placed in front of the chamber during the conditioning and test sessions, and was analyzed using FreezeFrame Video-Based Conditioned Fear System software (Actimetrics, Willmette, IL, USA),

2.4. Behavioral test procedures

Behavioral testing was conducted between 13:00 and 17:00. To minimize the possible effects of plasma corticosterone concentrations on animal behavior (Butte et al., 1976; Hui et al., 2004; Thompson et al., 2004) during the testing period, we counterbalanced the task by controlling the order of animals among control, TCDD and TBDD groups.

One day before the conditioning session, the mice were habituated to both context A and B for 10 min. On the day of the conditioning session, the mice were placed in context A of the chamber for 2 min for acclimation, followed by three co-terminating CS-US pairings. That is, at 120, 210 and 300 s after placing the mouse in the chamber, CS was provided for 30 s each time, followed by US in the last 2 s in a 30 s period of the CS. The mice remained in the chamber for 60 s after the third CS-US presentation. Retention tests for contextual and auditory fear memory were carried out 1 and 24 hr after the conditioning session. In the contextual test, the mice were returned to context A for 3 min

in the absence of the tone and foot shock. One hour after the contextual test, the mice were subjected to the auditory test. In this test, the mice were placed in context B without any stimuli for 2 min, and then subjected to CS for 2 min without a foot shock.

The percentage of time freezing after shock deliveries (1 min) in the conditioning session, and during the contextual (3 min) and auditory (the first and last 2 min) test sessions was calculated for each animal using the FreezeFrame software. Freezing was defined as the absence of movement except for breathing.

2.5. Statistics

Data were analyzed by two-way analysis of variance (ANOVA) for repeated measures followed by Tukey's HSD test (Fig. 1-3), and by Student's *t*-test (Fig. 4). All data in the text and figures are expressed as mean and standard error of the mean (SEM).

3. Results

The mean percent freezing (± SEM) after foot shock deliveries during the conditioning session is shown in Fig. 1. In this session, all the groups of animals showed increased percent freezing along with the repetition of the electric foot shocks, and the percent freezing of both TCDD and TBDD groups was slightly lower than that of the control group. A two-way ANOVA showed a significant main effect of shock delivery ($F_{(3, 72)} = 54.6, p < 0.01$). Neither a main effect of group nor an interaction of group × shock delivery was statistically significant.

The mean percent freezing (± SEM) in the contextual test is shown in Fig. 2. Both TCDD and TBDD groups showed a smaller number of freezing responses than the control group at 1 and 24 hr retention intervals, and a two-way ANOVA showed a significant main effect of group ($F_{(2, 23)} = 6.90, p < 0.01$). According to a post hoc analysis by Tukey's HSD test, the percent freezing of both TCDD and TBDD groups was significantly lower than that of the control group ($p < 0.01$), but there was no significant difference between the TCDD and TBDD groups.

The mean percent freezing (± SEM) in the auditory test is shown in Fig. 3. TCDD- and TBDD-exposed groups showed a decrease in freezing response in the 1 and 24 hr

retention intervals. A two-way ANOVA showed a significant main effect of group ($F_{(2, 23)} = 7.05$, $p < 0.01$), and its post hoc analysis by Tukey's HSD test indicated that the percent freezing of both TCDD- and TBDD-exposed groups was significantly lower than that of control group ($p < 0.05$), but there was no significant difference between TCDD and TBDD group.

The mean percent freezing (± SEM) before and during the CS presentation at each retention interval in the auditory test is shown in Fig. 4. All the groups of animals at both retention intervals showed higher freezing responses during the CS presentation than beforethe CS presentation, and differences between before and during the CS presentation in all the groups were statistically significant, as determined by Student's paired *t*-test. The statistical results are shown in Table 1.

4. Discussion

In utero and lactational exposures to dioxins have been reported to induce abnormal reproductive, learning/memory brain functions and immune functions in laboratory animals, such as rodents and primates (Schecter and Gasiewicz, 2003; Kakeyama and Tohyama, 2003), and these endpoints were used in the reevaluation of possible health risks of dioxin and related compounds by the World Health Organization (WHO, 2000). In particular, among these endpoints, alteration of brain functions such as learning and memory has gained much attention owing to epidemiological (Wigle et al., 2008) as well as laboratory animal studies (Wormley et al., 2004). Additionally, in recent years, the risk assessment of not only chlorinated dioxin congeners but also brominated congeners of dioxins has been required because brominated dioxins are produced unintentionally during the combustion of brominated flame retardants that include brominated biphenyls and other related compounds (Birnbaum et al., 2003; Van den Berg et al., 2006). In the present study, we obtained two novel findings on the health risks associated with dioxins. The first is that a very low dose of TCDD induced deficits in the formation and/or retention of fear memory in both the contextual and auditory fear conditioning. The second is a comparison between the risks associated with TCDD and TBDD.

Regarding the TCDD-induced developmental neurotoxicity, the dose used in *in utero*

and lactational exposure to TCDD in the present study induced deficits in fear memory in both the contextual and auditory conditioning in mice under the situations wherein the overall health conditions of the dams and their pups were minimally affected: that is, no effects were observed in the body weight gain of dams during pregnancy, maternal death, number of live pups, and birth weight of pups. To the best of our knowledge, the dose used in the present study is the lowest dose ever reported at which the developmental neurotoxicity of TCDD in mice was demonstrated. That is, the dose (a single oral dose of 3.0 μg/kg b.w. to their mother on gestation day 12.5) was approximately 1/60 of the LD_{50} of TCDD in C57/BL6 mice (Chapman and Schiller, 1985). An earlier report suggested that only a slight degree of immunotoxicity can be induced by a maternal dose of 5.0 μg/kg b.w. in C57/BL6 mice (Ito et al., 2008).

The observation of a deficit in contextual fear memory in mouse pups born to dams *in utero* and lactationally exposed to TCDD is consistent with a previous finding in rats (Mitsui et al., 2006). Although the molecular mechanism of such toxicities is largely unknown, *in utero* and lactational exposure to TCDD has been reported to suppress the expression of two molecules, cyclic AMP response element-binding protein (CREB) (Mitsui et al., 2006) and NMDA receptor subunit NR2B (Kakeyama et al., 2001; Nayyar et al., 2003; Hood et al., 2006), both of which are considered to participate in memory function in the hippocampus (Morris et al., 1990; Collingridge and Bliss, 1995; Cain, 1997; Josselyn et al., 2004; Alberini, 2009). In accordance with these findings, the hippocampus is reported to play a critical role in contextual fear memory, because a lesion or inactivation of the hippocampus impairs fear memory in mice (LeDoux, 2000; Maren, 2001). Taken together, the hippocampus is vulnerable to the neurotoxic actions of dioxins and is considered to be the brain region that mediates TCDD-induced memory impairment, at least, in the contextual fear conditioning.

Besides the toxic TCDD effects determined in the contextual test, we also found that the offspring born to dams *in utero* and lactationally exposed to TCDD show impairments in the auditory test. Since TCDD-exposed mice had significantly higher freezing responses during CS presentation than before CS presentation in the auditory test, such deficit is not considered to be induced by the deficit in auditory perception. Although *in utero* and

lactational exposure to a dioxin-like PCB congener was reported to cause low-frequency hearing deficits (Crofton and Rice, 1999), exposure to TCDD under the present condition might not induce hearing deficits, at least at the level that could affect the performance of the auditory fear test. Thus, the impairments in the auditory test using TCDD-exposed mice in the present study are considered to be caused by the dysfunction of auditory fear memory.

Taken together, *in utero* and lactational exposure to TCDD induces deficits in common function of fear memory in both the contextual and auditory paradigms. Fear memory is required for the associative function between environmental cognition (contextual and/or auditory environment) and fear feeling, and thus consists of memory and emotional functions. *In utero* and lactational exposure to TCDD might have affected not only the hippocampus but also other brain regions, since the hippocampus is suggested to be less important for the auditory fear conditioning than the contextual fear conditioning (Phillips and LeDoux, 1992), although whether the hippocampus is involved in the formation of the auditory fear memory is still controversial (Maren, 2008). The amygdala is considered to be such a putative region which plays critical roles in the acquisition and expression of the fear conditioning (LeDoux, 1995; Fendt and Fanselow, 1999). This notion is supported by the experimental evidence that the lesion of the amygdala and blockade of NMDA receptors in this region hamper the fear memory in mice (Fendt and Fanselow, 1999; LeDoux, 2000; Rodrigues et al., 2001). It has been considered that the acquired information is converted from short-term memory to persistent long-term memory through a consolidation process (McGaugh, 2000), and that NMDA receptors in the amygdala are involved in short-term memory and consolidation process of fear memory. The present result of the TCDD group (Figs. 2 and 3) parallels with that of the blockade of NMDA receptors in the amygdala (Rodrigues et al., 2004). Thus, it is plausible to speculate that *in utero* and lactational TCDD exposure also disrupts NMDA receptor-dependent neural transmission in the amygdala, and thus leads to impairments in shor-term and long-term memory in the fear conditioning. Further investigation is needed to elucidate the possible toxic effects of dioxins on brain regions, such as the amygdala.

In this study, we compared the relative neurotoxicities of TCDD and TBDD, and found

that *in utero* and lactational exposure to TBDD disrupts fear conditioning at the same dose level and degree of effects as *in utero* and lactational exposure to TCDD. Regarding the toxic effects of TBDD, we found that the TBDD-exposed group showed deficits in the contextual and auditory fear conditioning, similarly to the TCDD-exposed group, indicating that toxic effect of TBDD on the developmental brain is a similar to that of TCDD. The presence of brominated dioxins in human tissue was reported in Japan (Choi et al., 2003), China (Li et al., 2007) and Sweden (Ericson et al., 2010), and the amounts of brominated dioxins were estimated to be up to 15 % of the total dioxin TEQ, given that brominated dioxins have toxic equivalency factors similar to those of chlorinated dioxins (Ericson et al., 2010). Because the TBDD dose used in the present study is lower than that used in previous reports in mice, it is considered that the degree of toxicities should be investigated and that continuing efforts to clarify the developmental neurotoxic effects of not only TCDD but also TBDD are needed in the future.

As described above, in the present study, we observed an impairment in the formation of fear memory in adult male mice that were exposed to TCDD and TBDD *in utero* and via lactation. However, since TCDD and TBDD are planar chemicals, they are considered to bind to the arylhydrocarbon receptor (AhR) and elicit AhR-dependent toxicities. Because the structure of human AhR is more similar to that of the DBA/2 strain than to that of the C57BL/6 strain, and because a humanized AhR knock-in mouse study has revealed that humans are less sensitive than the C57BL/6 to at least the teratogenicity of TCDD, it would be reasonable to assume that humans are less sensitive to TBDD toxicities (Moriguchi et al., 2003, Ramadoss and Perdew, 2004). Further studies are needed to address this issue in terms of pharmacokinetics, tissue distribution and dose dependence. In addition, the mice in this study (6 months old) were older than those in other previous studies. Although the performance of memory tasks and brain functions can be considered to similar at least until 9 months of age (Ben Abdallah et al., 2010), further analyses are necessary to clarify the age difference in terms of when the test was performed and when the exposure was carried out. It would also be ideal to confirm the possible effects of these compounds on dams' nursing behavior possibly by cross-fostering experiments.

5. Conclusions

In conclusion, *in utero* and lactational exposure to TCDD in mice at a very low dose caused deficits in the contextual and auditory fear conditioning, which is required for memory and emotional function in offspring. *In utero* and lactational exposure to TBDD showed that TBDD has a similar degree of potency as TCDD and it can induce impairments of contextual and auditory fear memory.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This study was supported in part by Health and Labour Sciences Research Grant from Ministry of Health, Labour and Welfare, Japan (to MK), grants from the Environmental Technology Development Fund and Food Safety Commission (to CT), and Grant-in-aid for Young Scientists (A) and (S) from the Japan Society for the Promotion of Science (to MK). We thank Akiko Shimazaki and Yuki Hirasawa for their technical assistance.

References

Alberini CM. Transcription factors in long-term memory and synaptic plasticity. Physiol Rev 2009;89:121-145.

Ao K, Suzuki T, Murai H, Matsumoto M, Nagai H, Miyamoto Y, Tohyama C, Nohara K. Comparison of immunotoxicity among tetrachloro-, pentachloro-, tetrabromo- and pentabromo-dibenzo-p-dioxins in mice. Toxicology 2009;256:25-31.

Ben Abdallah NM, Slomianka L, Vyssotski AL, Lipp HP. Early age-related changes in adult hippocampal neurogenesis in C57 mice. Neurobiol Aging 2010;31:151-161.

Birnbaum LS, Staskal DF, Diliberto JJ. Health effects of polybrominated dibenzo-*p*-dioxins (PBDDs) and dibenzofurans (PBDFs). Environ Int 2003;29:855-860.

Birnbaum LS, Tuomisto J. Non-carcinogenic effects of TCDD in animals. Food Addit Contam 2000;17:275-288.

Butte JC, Kakihana R, Noble EP. Circadian rhythm of corticosterone levels in rat brain. J Endocrinology 1976;68:235-239.

Cain DP. LTP, NMDA, genes and learning. Curr Opin Neurobiol 1997;7:235-242.

Chapman DE, Schiller CM. Dose-related effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in C57BL/6J and DBA/2J mice. Toxicol Appl Pharmacol 1985;78:147-157.

Choi JW, Fujimaki TS, Kitamura K, Hashimoto S, Ito H, Suzuki N, Sakai S, Morita M. Polybrominated dibenzo-*p*-dioxins, dibenzofurans, and diphenyl ethers in Japanese human adipose tissue. Environ Sci Technol 2003;37:817-821.

Collingridge GL, Bliss TV. Memories of NMDA receptors and LTP. Trends Neurosci 1995;18:54-56.

Crofton KM, Rice DC. Low-frequency hearing loss following perinatal exposure to 3,3',4,4',5-pentachlorobiphenyl (PCB 126) in rats. Neurotoxicol Teratol 1999:21:299-301.

Darras VM. Endocrine disrupting polyhalogenated organic pollutants interfere with thyroid hormone signalling in the developing brain. Cerebellum 2008;7:26-37.

Davis M. The role of the amygdala in fear and anxiety. Annu Rev Neurosci 1992;15:353-375.

Dehay C, Kennedy H. Cell-cycle control and cortical development. Nat Rev Neurosci 2007;8:438-450.

Diliberto JJ, Kedderis LB, Jackson JA, Birnbaum LS. Effects of dose and routes of exposure on the disposition of 2,3,7,8-[3H]tetrabromodibenzo-p-dioxin (TBDD) in the rat. Toxicol Appl Pharmacol 1993;120:315-326.

Ericson Jogsten I, Hagberg J, Lindström G, Bavel B. Analysis of POPs in human samples reveal a contribution of brominated dioxin of up to 15% of the total dioxin TEQ. Chemosphere 2010;78:113-120.

Fendt M, Fanselow MS. The neuroanatomical and neurochemical basis of conditioned fear. Neurosci Biobehav Rev 1999;23:743-760.

Fritz WA, Lin TM, Moore RW, Cooke PS, Peterson RE. *In utero* and lactational 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure: effects on the prostate and its response to castration in senescent C57BL/6J mice. Toxicol Sci 2005;86:387-395.

Guruge KS, Yamanaka N, Hasegawa J, Miyazaki S. Differential induction of cytochrome P450 1A1 and 1B1 mRNA in primary cultured bovine hepatocytes treated with TCDD, PBDD/Fs and feed ingredients. Toxicol Lett 2009;185:193-196.

Hojo R, Kakeyama M, Kurokawa Y, Aoki Y, Yonemoto J, Tohayama C. Learning behavior in rat offspring after *in utero* and lactational exposure to either TCDD or PCB126. Env Health Prev Med 2008;13:162-168.

Hojo R, Stern S, Zareba G, Markowski VP, Cox C, Kost JT, Weiss B. Sexually dimorphic behavioral responses to prenatal dioxin exposure. Environ Health Perspect 2002;110:247-254.

Hood DB, Woods L, Brown L, Johnson S, Ebner FF. Gestational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure effects on sensory cortex function. NeuroToxicology 2006;27:1032-1042.

Hui GK, Figueroa IR, Poytress BS, Roozendaal B, McGaugh JL, Weinberger NM. Memory enhancement of classical fear conditioning by post-training injections of corticosterone in rats. Neurobiol Learn Mem 2004;81:67-74.

Ito T, Inouye K, Nohara K, Tohyama C, Fujimaki H. TCDD exposure exacerbates atopic dermatitis-related inflammation in NC/Nga mice. Toxicol Lett 2008:177:31-37.

Ivens IA, Löser E, Rinke M, Schmidt U, Neupert M. Toxicity of 2,3,7,8-tetrabromodibenzo-*p*-dioxin in rats after single oral administration. Toxicology 1992;73:53-69.

Josselyn SA, Kida S, Silva AJ. Inducible repression of CREB function disrupts amygdala-dependent memory. Neurobiol Learn Mem 2004;82:159-163.

Kakeyama M, Sone H, Miyabara Y, Tohyama C. Perinatal exposure to 2,3,7,8-Tetrachlorodibenzo-p-dioxin alters activity dependent expression of BDNF mRNA in the Neocortex and male rat sexual behavior in adulthood. NeuroToxicology 2003;24:207-221.

Kakeyama M, Sone H, Tohyama C. Changes in expression of NMDA receptor subunit mRNA by perinatal exposure to dioxin. Neuroreport 2001;12:4009-4012.

Kakeyama M, Tohyama C. Developmental neurotoxicity of dioxin and its related compounds. Ind Health 2003;41:215-230.

LeDoux JE. Emotion circuits in the brain. Annu Rev Neurosci 2000;23:155-184.

LeDoux JE. Emotion: clues from the brain. Annu Rev Psychol 1995;46:209-235.

Li H, Yu L, Sheng G, Fu J, Peng P. Severe PCDD/F and PBDD/F pollution in air around an electronic waste dismantling area in China. Environ Sci Technol 2007;41:5641-5646.

Maren S. Neurobiology of Pavlovian fear conditioning. Annu Rev Neurosci 2001;24:897-931.

Maren S. Pavlovian fear conditioning as a behavioral assay for hippocampus and amygdala function: cautions and caveats. Eur J Neurosci 2008;28:1661-1666.

Markowski VP, Cox C, Preston R, Weiss B. Impaired cued delayed alternation behavior in adult rat offspring following exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on gestation day 15. Neurotoxicol Teratol 2002;24:209-218.

Markowski VP, Zareba G, Stern S, Cox C, Weiss B. Altered operant responding for motor reinforcement and the determination of benchmark doses following perinatal exposure to low-level 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Environ Health Perspect 2001;109:621-627.

McGaugh JL. Memory--a century of consolidation. Science 2000;287:248-251.

Mitsui T, Sugiyama N, Maeda S, Tohyama C, Arita J. Perinatal exposure to

2,3,7,8-tetrachlorodibenzo-*p*-dioxin suppresses contextual fear conditioning-accompanied activation of cyclic AMP response element-binding protein in the hippocampal CA1 region of male rats. Neurosci Lett 2006;398:206-210.

Moriguchi T, Motohashi H, Hosoya T, Nakajima O, Takahashi S, Ohsako S, Aoki Y, Nishimura N, Tohyama C, Fujii-Kuriyama Y, Yamamoto M. Distinct response to dioxin in an arylhydrocarbon receptor (AHR)-humanized mouse. Proc Natl Acad Sci U S A 2003;100:5652-5657.

Morris RG, Davis S, Butcher SP. Hippocampal synaptic plasticity and NMDA receptors: a role in information storage? Philos Trans R Soc Lond B Biol Sci 1990;329:187-204.

Mukerjee D. Health impact of polychlorinated dibenzo-*p*-dioxins: a critical review. J Air Waste Manag Assoc 1998;48:157–165.

Nayyar T, Wu J, Hood DB. Downregulation of hippocampal NMDA receptor expression by prenatal exposure to dioxin. Cell Mol Biol (Noisy-le-grand) 2003;49:1357-1362.

Nishijo M, Kuriwaki J, Hori E, Tawara K, Nakagawa H, Nishijo H. Effects of maternal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on fetal brain growth and motor and behavioral development in offspring rats. Toxicol Lett 2007;173:41-47.

Ohbayashi H, Sasaki T, Matsumoto M, Noguchi T, Yamazaki K, Aiso S, Nagano K, Arito H, Yamamoto S. Dose- and time-dependent effects of 2,3,7,8-tetrabromodibenzo-*p*-dioxin on rat liver. J Toxicol Sci 2007;32:47-56.

Petroff BK, Roby KF, Gao X, Son D, Williams S, Johnson D, Rozman KK, Terranova PF. A review of mechanisms controlling ovulation with implications for the anovulatory effects of polychlorinated dibenzo-*p*-dioxins in rodents. Toxicology 2001;158:91-107.

Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 1992;106:274-285.

Ramadoss P, Perdew GH. Use of 2-Azido-3-[125I]iodo-7,8-dibromodibenzo-p-dioxin as a Probe to Determine the Relative Ligand Affinity of Human versus Mouse Aryl Hydrocarbon Receptor in Cultured Cells. Mol Pharmacol 2004;66:129-136.

Rodrigues SM, Schafe GE, LeDoux JE. Intra-amygdala blockade of the NR2B subunit of

the NMDA receptor disrupts the acquisition but not the expression of fear conditioning. J Neurosci 2001;21:6889-6896.

Rodrigues SM, Schafe GE, LeDoux JE. Molecular mechanisms underlying emotional learning and memory in the lateral amygdala. Neuron. 2004;44:75-91.

Samara F, Gullett BK, Harrison RO, Chu A, Clark GC. Determination of relative assay response factors for toxic chlorinated and brominated dioxins/furans using an enzyme immunoassay (EIA) and a chemically-activated luciferase gene expression cell bioassay (CALUX). Environ Int 2009;35:588-593.

Schantz SL, Seo BW, Moshtaghian J, Peterson RE, Moore RW. Effects of gestational and lactational exposure to TCDD or coplanar PCBs on spatial learning. Neurotoxicol Teratol 1996;18:305-313.

Schecter A, Gasiewicz TA. Eds. Dioxins and Health, 2nd ed., John Wiley & Sons, Hoboken, 2003.

Seo BW, Powers BE, Widholm JJ, Schantz SL. Radial arm maze performance in rats following gestational and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Neurotoxicol Teratol 2000;22:511-519.

Seo BW, Sparks AJ, Medora K, Amin S, Schantz SL. Learning and memory in rats gestationally and lactationally exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Neurotoxicol Teratol 1999;21:231-239.

Thompson BL, Erickson K, Schulkin J, Rosen JB. Corticosterone facilitates retention of contextually conditioned fear and increases CRH mRNA expression in the amygdala. Behav Brain Res 2004;149:209-215.

Van den Berg M, Birnbaum L, Denison M, De Vito M,Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N, Peterson RE. The 2005 World Health Organization Reevaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-Like Compounds. Toxicol Sci 2006;93:223-241.

Weber LWD and Greim H. The toxicity of brominated and mixed-halogenated dibenzo-*p*-dioxins and dibenzofurans: an overview. J Toxicol Environ Health 1997;50:195-215.

Widholm JJ, Seo BW, Strupp BJ, Seegal RF, Schantz SL. Effects of perinatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on spatial and visual reversal learning in rats. Neurotoxicol Teratol 2003;25:459-471.

Wigle DT, Arbuckle TE, Turner MC, Bérubé A, Yang Q, Liu S, Krewski D. Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. J Toxicol Environ Health B Crit Rev 2008;11:373-517.

World Health Organization. Consultation on assessment of the health risk of dioxins; re-evaluation of the tolerable daily intake (TDI): executive summary. Food Addit Contam 2000;17:223-240.

Wormley DD, Ramesh A, Hood DB. Environmental contaminant-mixture effects on CNS development, plasticity, and behavior. Toxicol Appl Pharmacol 2004;197:49-65.

Figure legend

Figure 1.

Freezing responses after three shock deliveries (1 min) during the conditioning session. Control, offspring born to dams exposed to corn oil; TB3.0, offspring born to dams exposed to 3.0 μg/kg TBDD; TC3.0, offspring born to dams exposed to 3.0 μg/kg TCDD. Values represent mean ± SEM.

Figure 2.

Freezing responses during the contextual test (3 min). Control, offspring born to dams exposed to corn oil; TB3.0, offspring born to dams exposed to 3.0 μg/kg TBDD; TC3.0, offspring born to dams exposed to 3.0 μg/kg TCDD. Values represent mean ± SEM. Asterisks indicate significant differences from the control group (**$p < 0.01$).

Figure 3.

Freezing responses in the auditory test (the last 2 min). Control, offspring born to dams exposed to corn oil; TB3.0, offspring born to dams exposed to 3.0 μg/kg TBDD; TC3.0, offspring born to dams exposed to 3.0 μg/kg TCDD. Values represent mean ± SEM. Asterisks indicate significant differences from the control group (**$p < 0.01$).

Figure 4.

Freezing responses before and during CS presentations in the auditory test. Control, offspring born to dams exposed to corn oil; TB3.0, offspring born to dams exposed to 3.0 μg/kg TBDD; TC3.0, offspring born to dams exposed to 3.0 μg/kg TCDD. Values represent mean ± SEM. Asterisks indicate significant difference between Before CS and During CS (*$p < 0.05$; **$p < 0.01$).

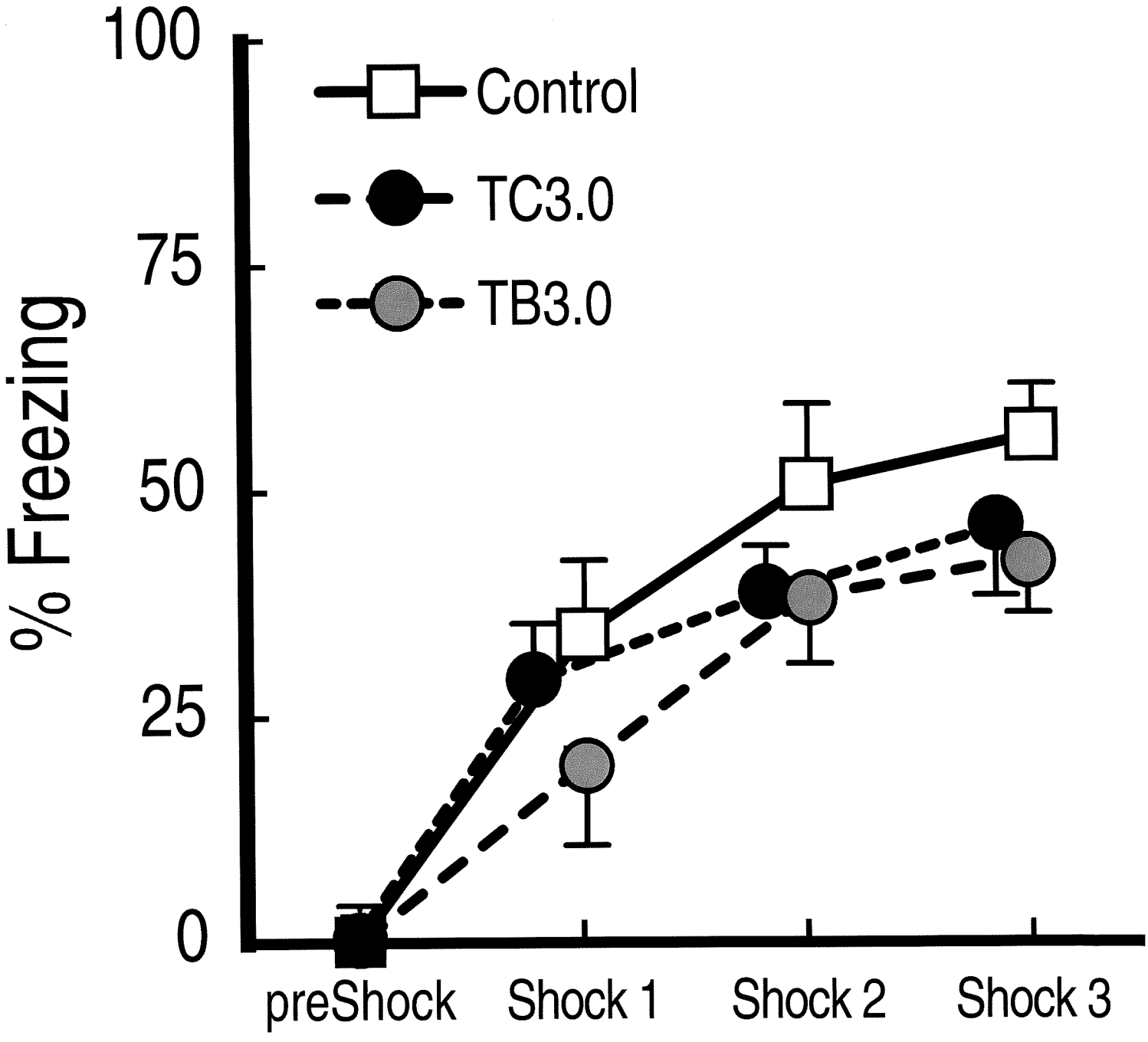
Table 1

The results of the statistical analysis in the auditory test as determined by Student's paired *t*-test

Group	1hr	24hr
Control	$t_{(8)}=3.13$*	$t_{(8)}=6.34$**
TC3.0	$t_{(8)}=3.76$**	$t_{(8)}=2.94$*
TB3.0	$t_{(7)}=2.88$*	$t_{(7)}=4.56$**

Control, offspring born to dams exposed to corn oil; TC3.0, offspring born to dams exposed to 3.0 μg/kg TCDD; TB3.0, offspring born to dams exposed to 3.0 μg/kg TBDD. Values represent mean ± SEM. Asterisks indicate significant difference between Before CS and During CS at each retention interval (*$p < 0.05$; **$p < 0.01$).

Figure 1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preShock
Shock 1
Shock 2
Shock 3

Figure 2

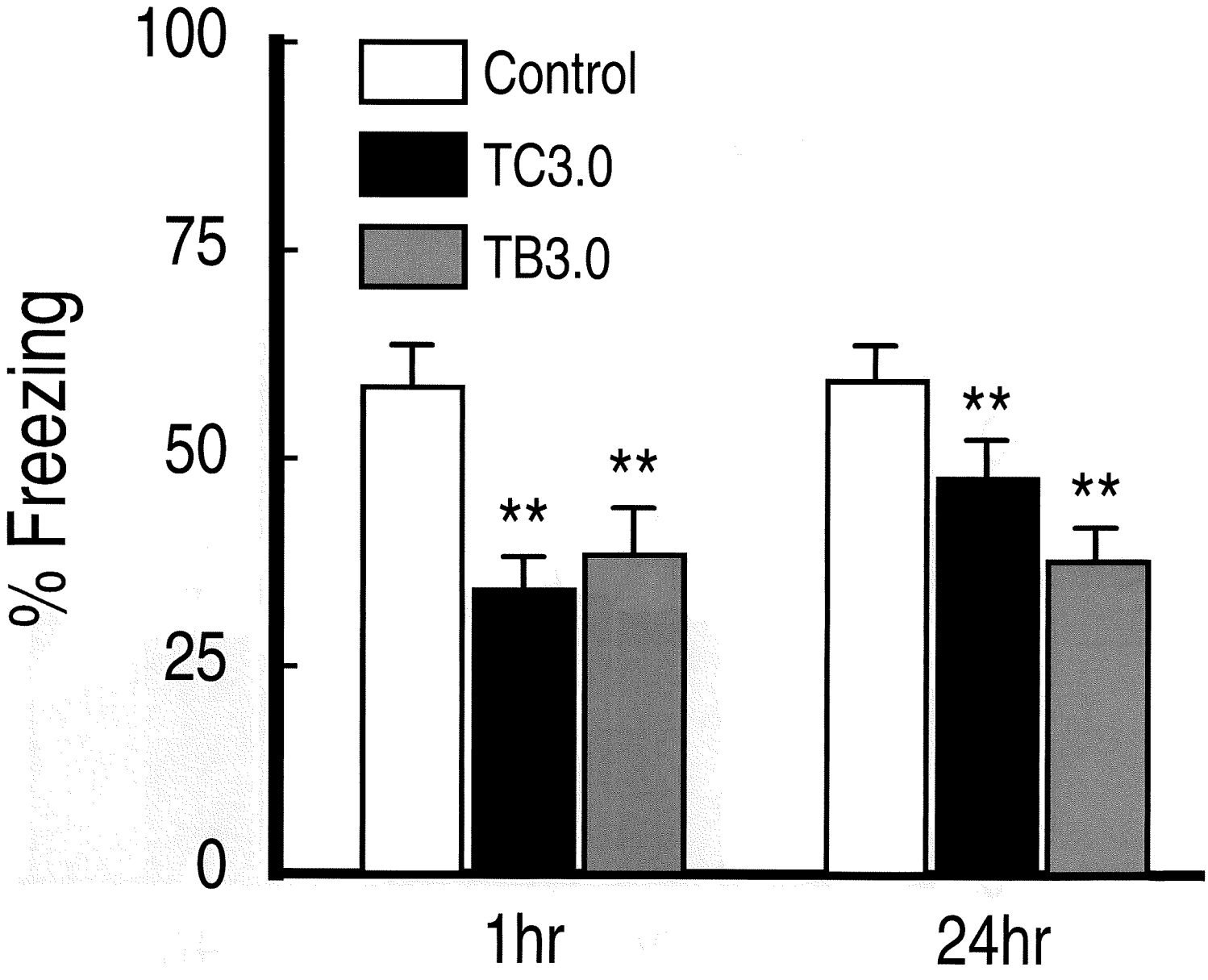


Figure 3

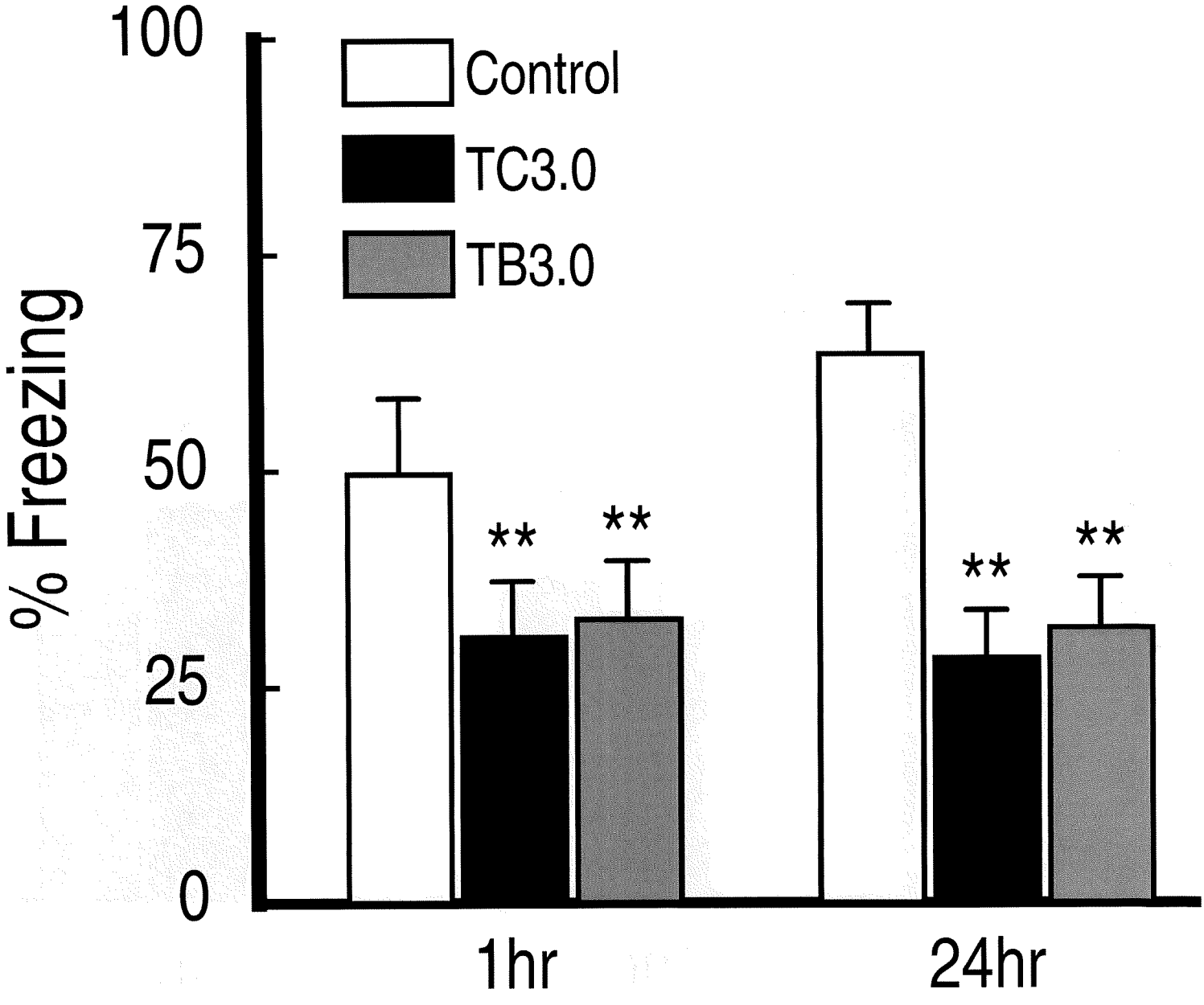
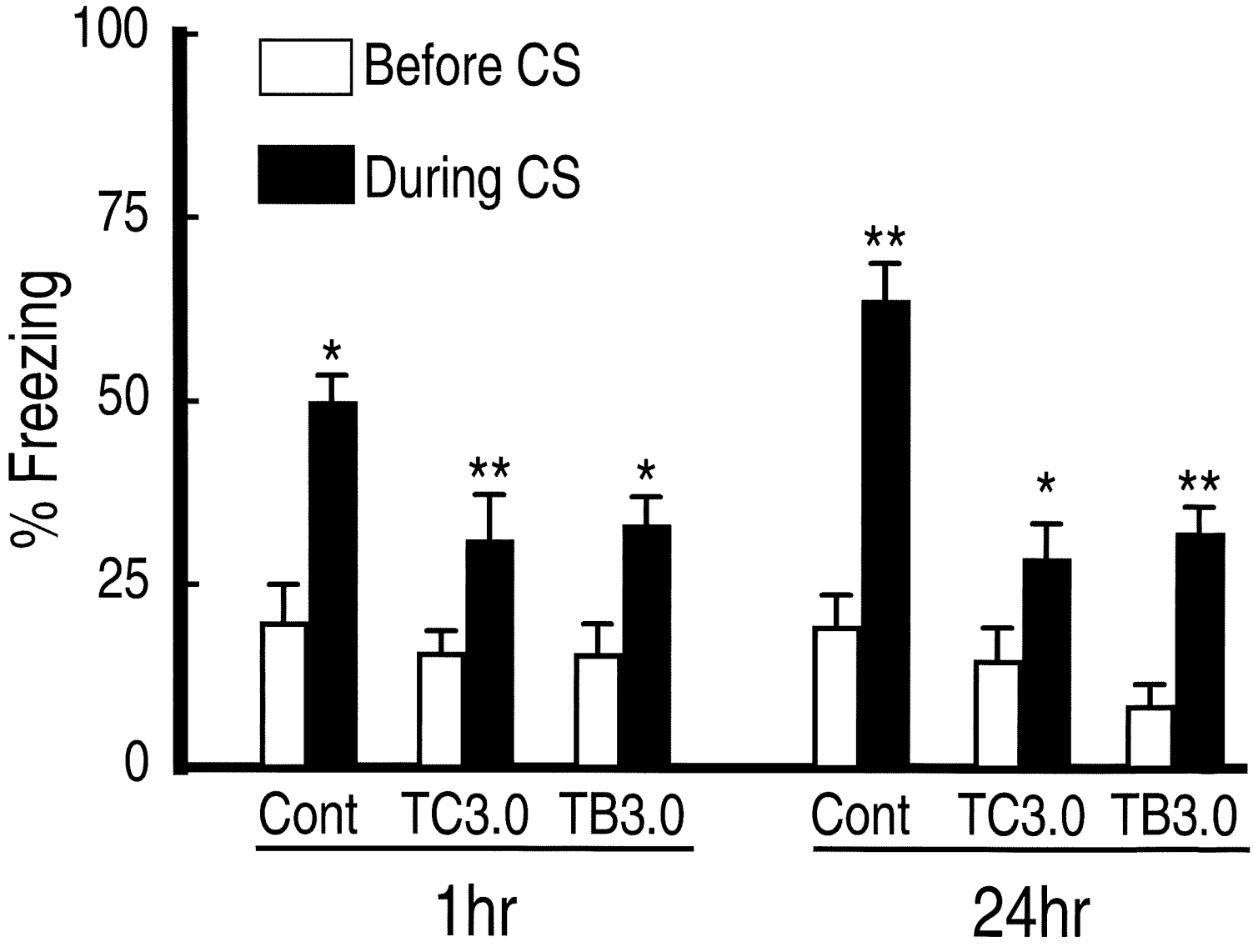


Figure 4


100
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% Freezing
Before CS
During CS
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Cont
TC3.0
TB3.0
Cont
TC3.0
TB3.0
1hr
24hr