of mercury were significantly higher in females than males in both strains. The amount of mercury in the control organs comes from the levels of mercury contained in mouse chow.

Histological examination did not reveal any abnormality in the nerve tissues of the exposed mice regardless of strain or sex (data not shown).

4. Discussion

The present results showed that exposure in utero to a moderate level of Hg^0 resulted in a small but significant aberration of behavioral performance in MT-null mice but not in the wild-type mice. Namely, the exposure depressed the open field activity of males and worsened the learning performance of females in both the avoidance test and spatial learning. Although the extent of each behavioral effect was not so remarkable, all of them were consistently observed in the MT-null mice. Thus, like the neurotoxicity resulting from a prolonged adult exposure to Hg^0 (Yoshida et al., 2004), the developmental toxicity of in utero exposure is more exaggerated in MT-null mice. This is to our knowledge the first demonstration of genetic susceptibility of MT-null mice in terms of the developmental toxicity of metals.

There exist two studies examining the neurobehavioral effects of prenatal exposure to Hg^0, both of which used SD rats. Danielsson et al. (1993) exposed pregnant rats to a level of Hg^0 (1.8 mg/m^3 × 1 or 3 h/day during the latter half of the gestation) comparable to the present study and reported that exposed male pups showed decreased spontaneous motor activity (i.e., locomotion, rearing, rearing time, and total activity) when tested at 12 weeks of age. Fredriksson et al. (1992) exposed newborn male rats to a much lower concentration of Hg^0 (0.05 mg/m^3 × 1 or 4 h/day) for 7 days (day 11–17 postnatal) and reported enhanced locomotion in the open field test and poorer performance in the radial maze at 2 months or 4 months of age. Together with the present results, these studies clearly demonstrated that prenatal exposure to Hg^0 could exert developmental toxicity, which was long lasting. It should be noted that in rats, the open field activity was depressed after exposure in utero (Danielsson et al., 1993), while it was enhanced by the postnatal exposure (Fredriksson et al., 1992). Also, in utero exposure depressed the open field activity (the present study) while adult exposure enhanced it (Yoshida et al., 2004). The observation might merely be a coincidence, but it appears likely that exposure to Hg^0 exerts totally different effects depending on the timing of exposure.

Since the above-mentioned rat studies examined the effects only in males, the present study might have been the first opportunity to reveal the possible sex difference in terms of the developmental toxicity of Hg^0. Although the results were not straightforward, it should be noted that the females appeared to be more susceptible in both learning tests. Goulet et al. (2003) reported that female C57BL/6 mice exposed to methylmercury during fetal and early postnatal development exhibited an altered working memory performance in the modified T maze, but males did not. Yasutake and Hirayama (1988) described that C57BL/6 females are less resistant than males to methylmercury-mediated toxicity. These observations suggest that the nature of the tests rather than nature of the toxin caused females to appear more susceptible in learning tests.

In terms of pathological findings, Steffek et al. (1987) reported that the exposure of pregnant rats at 0.5 mg/m^3 resulted in two fetuses (out of 84 examined) with cranial defects. Gross examination of offspring from pregnant MT-null and wild-type mice exposed to 0.5 mg/m^3 of Hg^0 revealed no spontaneous abortion, stillbirth or gross congenital malformation. The embryotoxic and teratogenic effects on pregnant mice of both strains under the conditions of exposure used in the study could not be observed. In addition, there were no histopathological changes in the nerve tissue of MT-null and wild-type mice at 12 weeks of age (data not shown).

The placenta plays an important role in protection against the transfer of heavy metals from the mother to the fetus. It was shown that placental MT prevents the transfer of mercury from mother to fetus (Yoshida et al., 2002). After in utero exposure to Hg^0, MT-null fetal mice accumulate significantly more mercury than wild-type fetuses, and the elimination of mercury from the organs, except the brain, is remarkably faster in wild-type mice than in MT-null mice (Yoshida et al., 1999b). Mercury concentrations in the brain and kidney of both strains were slightly higher in the exposed group than in the control group, indicating the retention of residual mercury even 12 weeks after the cessation of the exposure. Although no strain difference in brain mercury

levels in male mice were observed, the mean levels were slightly higher in wild-type tissues. Furthermore, female mice had significantly higher levels of mercury in the brain than had male mice. In methyl mercury-treated mice, brain concentrations of mercury were significantly higher in females than in males (Nielsen and Anderson, 1991) and Thomas et al. (1986) reported that a sexual difference in the accumulation of mercury in the brain may be related to a sexual difference in sensitivity to the toxic effects of methyl mercury. MT-null mice lacking MT-I,-II show increased susceptibility to Hg^0-induced neurobehavioral toxicity (Yoshida et al., 2004). Based on these results, we suggest that the increased susceptibility of MT-null females to behavioral changes caused by prenatal Hg^0 exposure is due to the greater retention of mercury and lack of MT-I,-II in the brain. In order to make the relationship between the brain mercury level and the susceptibility clear, we need to perform further experiments in which the kinetics of the brain mercury will be followed after exposure is evaluated.

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