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Transplacental effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on brain dopaminergic neurons in the mouse

An immunohistochemical study*

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Summary. Immunohistochemical studies of monoamine neurons were performed to evaluate toxic effects of 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine (MPTP) on young adult mice and compare them with those of their offspring. Mice, 9–11 weeks old (C57BL/6J), injected subcutaneously with a large dose of MPTP (17 mg/kg per day) during pregnancy on Day 9 and 12 of gestation (G9 and G12) miscarried and were examined at 13 weeks of age. Conversely, mice treated during pregnancy with sequential low dose of MPTP (2.8 mg/kg per day at G9–G17 for 8 days) successfully delivered their babies and were examined at the age of 15 weeks. Baby mice were examined at 1 and 6 weeks of age. The tyrosine hydroxylase-, aromatic L-amino acid decarboxylase- and dopamine (DA)-immunoreactive density of caudoputamen was reduced in 13-week-old mice treated with high dose of MPTP but not in the 15-week-old mothers exposed to a low dose of MPTP as compared to their respective controls. The DA-immunoreactive density of the caudoputamen was the only staining that was reduced in both 1- and 6-week-old baby mice. In conclusion, these results demonstrate that MPTP injected to pregnant mice causes a DA depletion in the striatum of their offspring indicating a transplacental effect of MPTP. The findings also indicate that fetal brain is more susceptible to MPTP toxicity than the brain of young pregnant mice.

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Since 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was accidentally found to cause parkinsonism in humans [4, 11], the neurotoxicity of MPTP was investigated in various animal species to develop a model for the investigation of parkinsonism. At the present time, it is known that the neurotoxicity of MPTP is species dependent. Administration of MPTP to monkeys causes a similar state of toxicity as that seen in patients with parkinsonism [1, 12], but rodents are less sensitive than primates to the toxic effects of MPTP [2, 9]. The difference in sensitivity to MPTP may, at least partially, be explained by species differences in concentration and distribution of monoamine oxidase (MAO)-B in the brain [20] and the existence of neuromelanin [3]. On the other hand, some authors stress that the age of animal is a critical determinant of MPTP toxicity as observed in comparative studies of young adult and old mice [21, 23]. However, until now, the investigation did not include a comparison of MPTP neurotoxicity between adult and immature, baby mice.

In this paper, the toxic effect of MPTP on mother mice (young adults) is compared to that on their offspring after systemic administration of MPTP to preg-

nant mice using tyrosine hydroxylase (TH), aromatic L-amino acid decarboxylase (AADC) and dopamine (DA) immunohistochemistry. A preliminary note of this work was reported previously [16].

Materials and methods

Twenty-two C57BL/6J pregnant mice (9–11 weeks old) were used for this study. Crystalline N MPTP (Aldrich, 97%) was dissolved in ethanol (17 mg/ml) and diluted with saline. MPTP was administered subcutaneously to pregnant mice with two different dosage schedules.

Mice with a large dose of MPTP (17 mg/kg per day) injected on the 9th and 12th day of gestation (G9 and G12, respectively) aborted their fetuses. Mice with a low dose of MPTP (2.8 mg/kg per day) administered from G9–G17 for 8 days successfully delivered their babies at term. Saline solution was subcutaneously injected to control mice.

The neurotoxicity of MPTP was evaluated in the following groups of animals: (A) mother mice, 13 weeks old (high MPTP dose), (B) mother mice, 15 weeks old (low MPTP dose), (C) their offspring at the age of 1 and 6 weeks. The animals were perfused under ether anesthesia with 5% glutaraldehyde in cacodylate buffer (pH 7.2) 11 to 42 days after the last MPTP injection. The brain was postfixed with the same fixative containing 0.2% picric acid for 1 day. After washing with 10% to 30% sucrose containing phosphate buffer (pH 7.4) for 2 days, coronal 40-μm-thick sections were cut with a cryostat. The serial sections were treated using a modified method of Nagatsu et al. [15] as follows: (1) preincubated with 0.1 M phosphate-buffered saline (PBS) containing 0.3% Triton X-100, 0.5% normal goat serum (NGS) and 0.1% sodium azide for a few days; (2) washed with PBS, (3) immersed in freshly prepared 1% sodium borohydride [10] and stirred for 30 min at room temperature; (4) washed with PBS and stirred for 1 h at 4°C; and (5) immersed in 5% NGS for 10 min.

Pretreated floating sections were sequentially incubated with the following sera and substances: (1) anti-TH [15] diluted 1/2000, anti-AADC [15] diluted 1/2000 and anti-DA [15] diluted 1/1000 at 4°C for 5 days; (2) goat anti-rabbit IgG (Miles-Yeda) diluted 1/200 at room temperature for 2 h; (3) a soluble complex of horseradish and rabbit anti-horseradish peroxidase (DAKO) diluted 1/200 at room temperature for 90 min. All sera were diluted with 0.1 M PBS containing 0.3% Triton X-100, 0.5% NGS and 0.1% sodium azide. After washing with PBS, the tissue sections were reacted with 0.04% 3,3′-diaminobenzidine tetrahydrochloride containing 0.01% H_2O_2. The tissue sections, without counterstaining, were dehydrated in a graded series of alcohol and mounted onto slides.

For comparison of smaller brain regions, semiquantitative changes of densitometric intensities derived from TH, AADC and DA levels in the caudoputamen, accumbens, olfactory tubercle, substantia nigra pars compacta and ventral tegmental area were examined by newly developed microphotometry system (Nikon LUZEX 2D) following the method of Nagatsu et al. [17]. It is difficult to measure changes of TH, AADC and DA levels among these areas separately by conventional biochemical assays. Analysis of the image seen on the monitor obtained by microscopy (Nikon microphoto-FX) is shown in Fig. 1. The total density of each pixel in selected area was divided by numbers of pixel in its area by computer. The differences between the value of caudoputamen, accumbens, olfactory tubercle and cortex were compared.

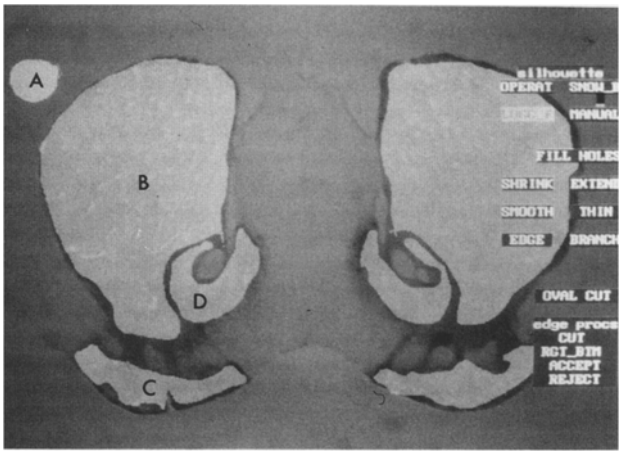

Fig. 1. The image on the monitor obtained by microphotometry system (Nikon LUZEX 2D). The total density of each pixel in selected area was divided by numbers of pixel in its area by computer. The differences between the value of caudoputamen (*B*), accumbens (*D*), olfactory tubercle (*C*) and cortex (*A*) were compared

Results

Caudoputamen

Mother mice, 13 weeks old (high MPTP dose). The TH- or AADC-like immunoreactive (LI) density of caudoputamen showed a markedly reduced staining in MPTP-treated mice as compared to the normal controls. DA terminal staining was also decreased showing a more patchy appearance than that in the normal controls. In the accumbens and olfactory tubercle, a target of mesolimbic system, TH-, AADC- and DA-positive terminals showed either a slight reduction or normal reactivity (Fig. 2). The data analyzed by semiquantitative densitometric assessment of peroxidase-antiperoxidase immunohistochemistry are shown in Fig. 3.

Mother mice, 15 weeks old (low MPTP dose). A definite reduction of TH-, AADC- and DA-LI density was not observed in the staining of caudoputamen, olfactory tubercle and accumbens of MPTP-treated mice (Fig. 4). Semiquantitative densitometric assessment of caudoputamen showed no significant difference between controls and MPTP-treated mice (see Fig. 7).

Baby mice (1 and 6 weeks old). A normal TH- and AADC-LI density was found in the caudoputamen, olfactory tubercle and accumbens of baby mice exposed to MPTP in utero. On the other hand, moderate to severe reduction of DA immunoreactivity was observed in the case of 1- and 6-week-old mice (Figs. 5,

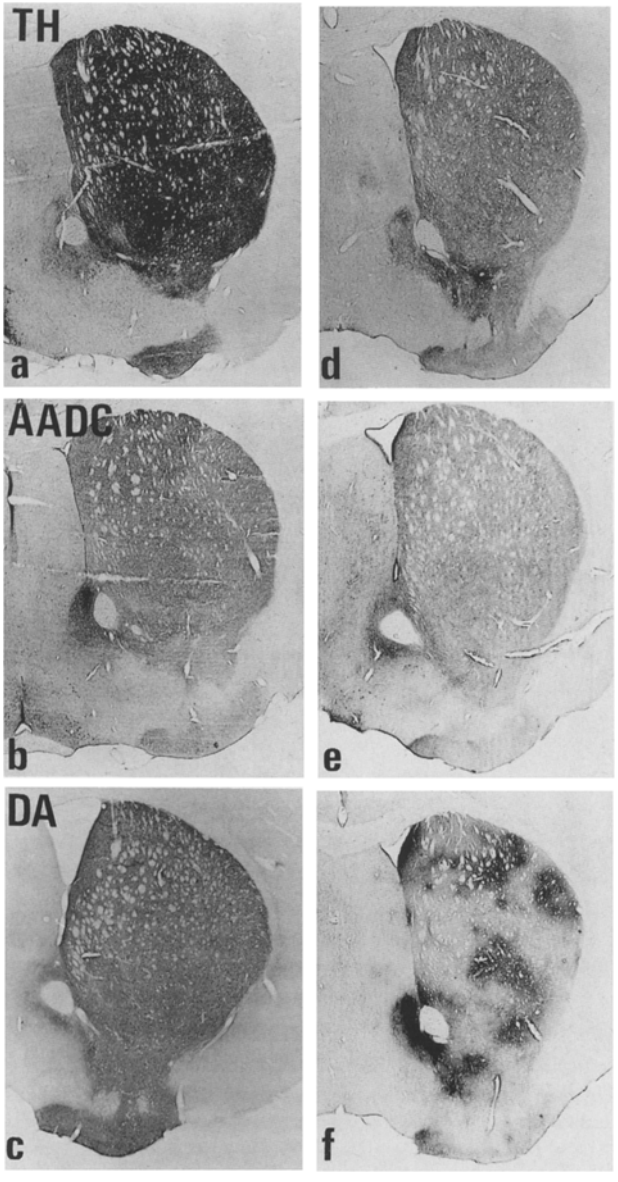

Fig. 2a–f. Immunohistochemical distribution of tyrosine hydroxylase (*TH*), aromatic L-amino acid decarboxylase (*AADC*) and dopamine (*DA*) in the striatum of a 13-week-old mother mouse [high 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) dose]. TH (**a**), AADC (**b**) and DA (**c**) of the control mouse, and TH (**d**), AADC (**e**) and DA (**f**) of the MPTP-treated mouse. **a–f** ×11.25

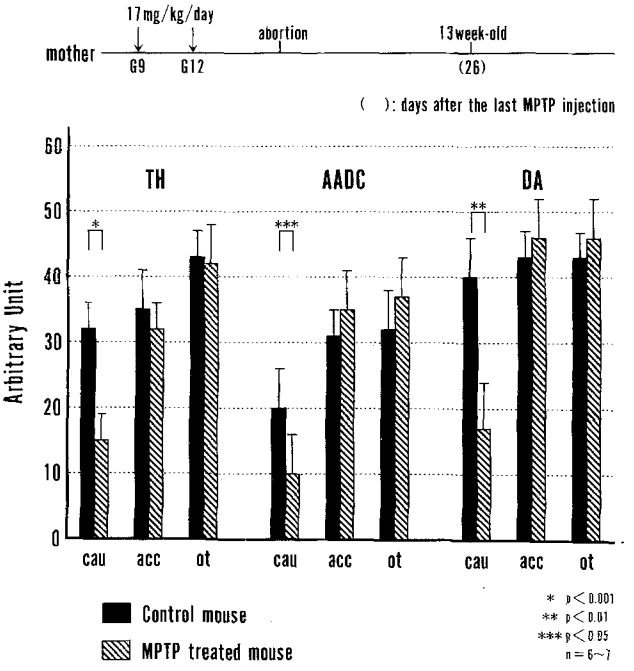

Fig. 3. Histogram of the immunohistochemical densitometric intensities derived from TH, AADC and DA reactivities in the striatum of 13-week-old mother mice (mean ± SD). *cau*: Caudoputamen, *acc*: accumbens, *ot*: olfactory tubercle

6). Semiquantitative densitometric assessment of caudoputamen of 1- and 6-week-old mice showed a significantly different DA-LI intensity of caudoputamen than that of controls. The mean intensity of MPTP-treated mice was 50% in the case of 1-week-old and 62% in the case of 6-week-old mice (Fig. 7).

Substantia nigra (SN) and ventral tegmental area (VTA)

Changes of TH-, AADC- and DA-LI density were not observed in the cell bodies and fibers of the SN (A9 cell groups), VTA (A10 cell group) (Fig. 8) and retrorubral area (A8 cell group) at the light microscopic level.

Discussion

A depletion of DA [7, 8, 25], TH activity and TH protein [13] in the striatum of MPTP-treated mice was observed in biochemical studies. Recently, Naoi et al. [18] found that *N*-methyl-4-phenylpyridinium ion (MPP^+) inhibited AADC activity in rat clonal pheochromocytoma PC12h cells. They suggested that MPP^+ might accelerate the degradation of AADC in neurons and might cause not only direct inhibition of the enzyme activity, but also reduction of AADC protein itself.

In our study, large dose of MPTP (17 mg/kg per day on G9 and G12) reduced the immunostaining of TH, AADC and DA in the caudoputamen of the 13-week-old mother mice observed 26 days after last MPTP injection. These observations agree well with the previous biochemical findings. Nagatsu et al. [17] reported that large TH- and AADC- positive varicose

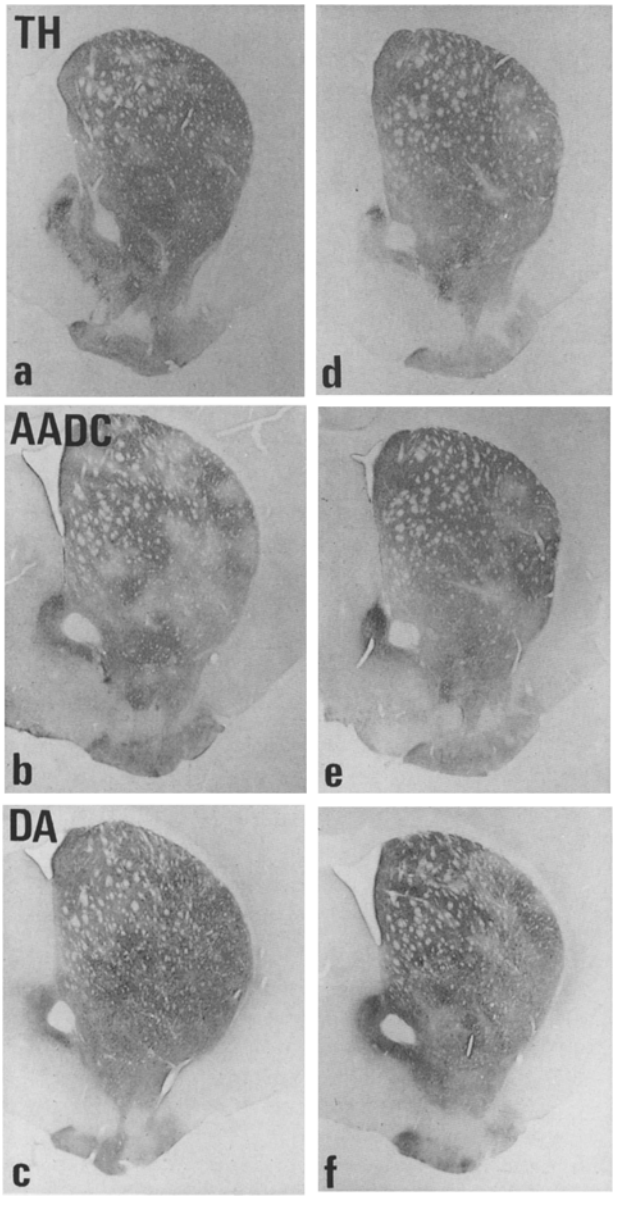

Fig. 4a–f. Immunohistochemical distribution of TH, AADC and DA in the striatum of a 15-week-old mother mouse (low MPTP dose). TH (**a**), AADC (**b**) and DA (**c**) of the control mouse, and TH (**d**), AADC (**e**) and DA (**f**) of the MPTP-treated mouse. **a–f** ×11.25

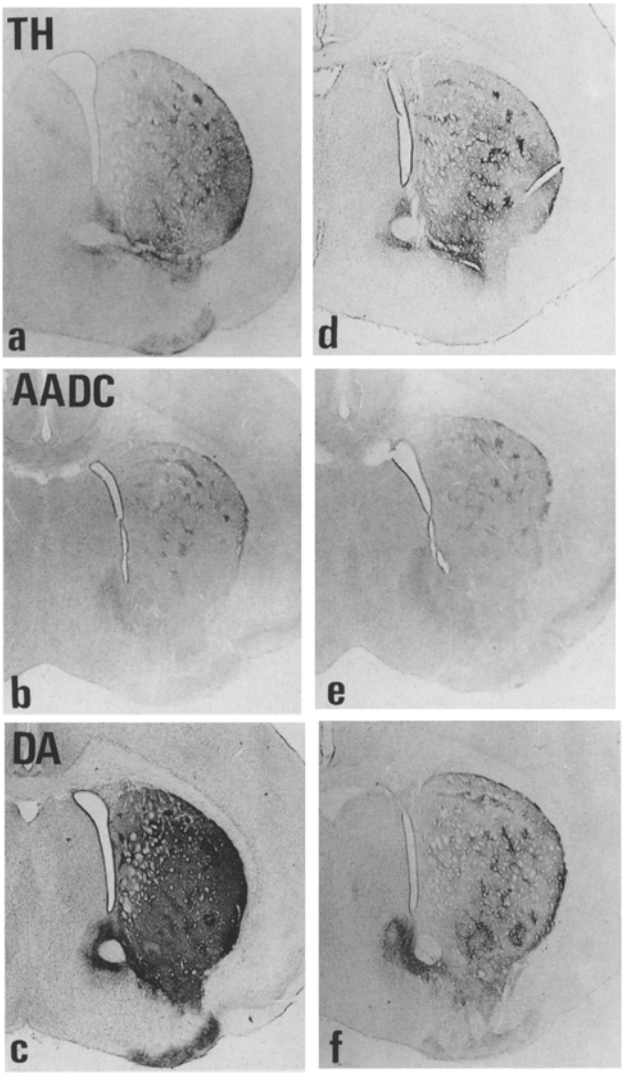

Fig. 5a–f. Immunohistochemical distribution of TH, AADC and DA in the striatum of a 1-week-old mouse. TH (**a**), AADC (**b**) and DA (**c**) of the control mouse and TH (**d**), AADC (**e**) and DA (**f**) of the MPTP-treated mouse. **a–f** ×11.25

fibers were observed in the caudoputamen of MPTP-treated DDY mice. Schneider et al. [24] also found similar changes in cat using TH immunohistochemistry. However, in the present study, large TH- and AADC-positive varicose findings were not seen in the caudoputamen. This discrepancy may be related to the use of different animal species, dose and frequency of MPTP injection. In contrast, 11 days after the last MPTP injection, 15-week-old mother mice exposed to a low dose of MPTP (2.8 mg/kg per day for 8 days) showed almost normal immunostaining of TH, AADC and DA in caudoputamen.

The reports regarding degeneration and depletion of DA neurons in the SN of MPTP-treated rodents are contradictory. A cell loss in SN after MPTP treatment was reported, using TH and DA immunohistochemistry or Falk-Hillarp histochemistry [5, 6, 26]. On the other hand, in our and other studies [22, 27], SN was not affected by MPTP treatment. Our findings indicate that dopaminergic cell bodies containing large amount of TH and AADC do not show significant reduction of immunoreactivity and only nerve terminals containing a small amount of TH and AADC

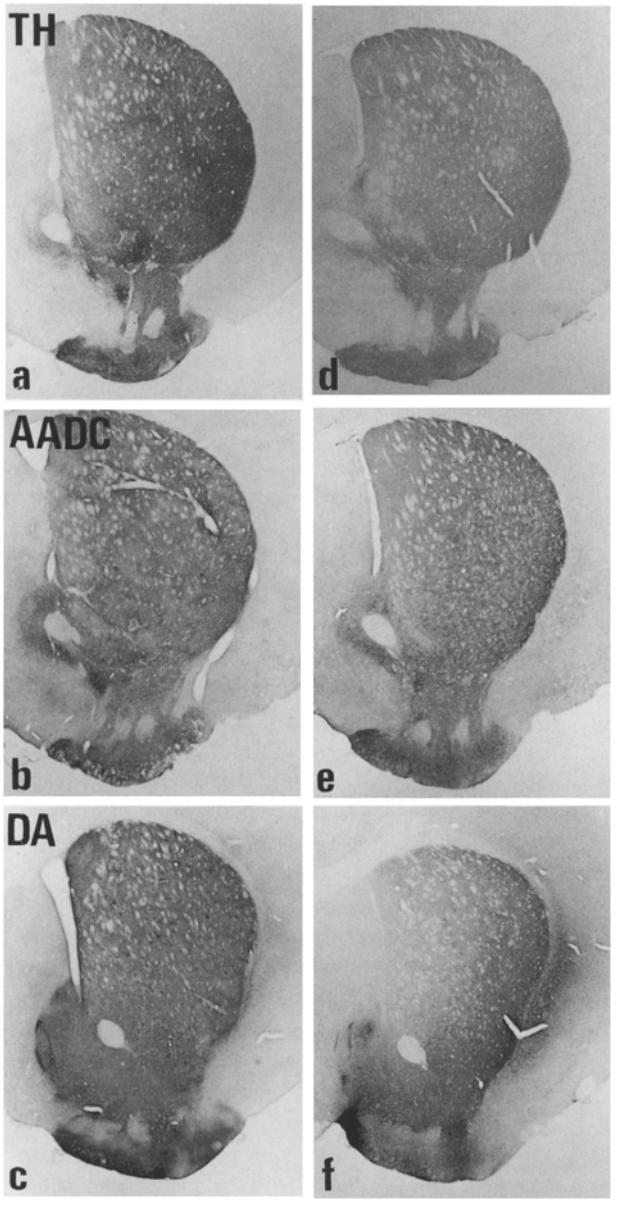

Fig. 6a–f. Immunohistochemical distribution of TH, AADC and DA in the striatum of a 6-week-old mouse. TH (**a**), AADC (**b**) and DA (**c**) of the control mouse and TH (**d**), AADC (**e**) and DA (**f**) of the MPTP-treated mouse. **a–f** ×11.25

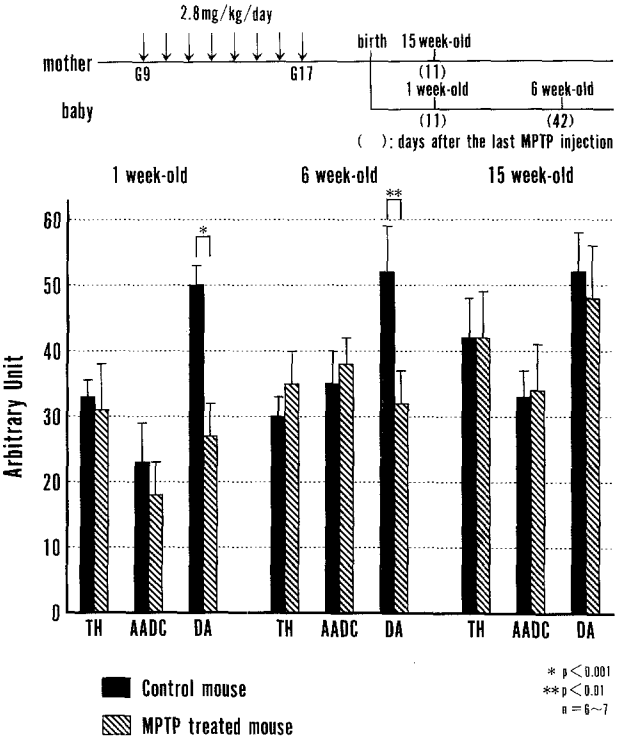

Fig. 7. Histogram of the immunohistochemical densitometric intensities derived from TH, AADC and DA reactivities in the striatum of 1-, 6-, and 15-week-old mice (mean ± SD)

show depletion of immunostaining. Although normal findings of SN were seen in 13-week-old mice, the caudoputamen, a terminal of the nigrostriatal system, showed reduction of immunohistochemical intensity of TH, AADC and DA, but accumbens and olfactory tubercle, a target of mesolimbic system, remained normal or showed slight reduction. These results agree with other reports using immunohistochemical technique [17, 26] and indicate that MPTP has specific neurotoxic effects on nigrostriatal system. Recently, Ohya et al. [19] injected pregnant mice with MPTP and found MPP^+ in fetal brains. Since reduced immunoreactive staining of DA in the caudoputamen was observed in 1- and 6-week-old baby mice exposed to MPTP in utero, this is strongly suggestive that MPTP and/or MPP^+ reached the embryonic brain via the placenta and exhibited neurotoxicity.

The normal immunoreactive density of TH and AADC and the decreased DA staining observed in the caudoputamen, olfactory tubercle and accumbens of 1- and 6-week-old babies strongly suggest that the TH and AADC, but not DA, content recovered 11 and 42 days after last MPTP treatment, respectively. These results agree with those of Mori et al. [14], who reported a remarkable discrepancy in the recovery rate between DA and TH reactivities of the nigral neurons after MPTP treatment, since TH content improved more promptly than that of DA.

Ricaurte et al. [21] reported that MPTP produced extensive damage to the SN of aged mice, but young adult mice were less affected and showed a trend toward recovery of dopamine neurons. Saitoh et al. [23] also reported that the capacity of partial recovery of the striatal DA contents after MPTP treatment was more impaired in aged mice than in young mice. In our study, comparing 15-week-old mother mice (11

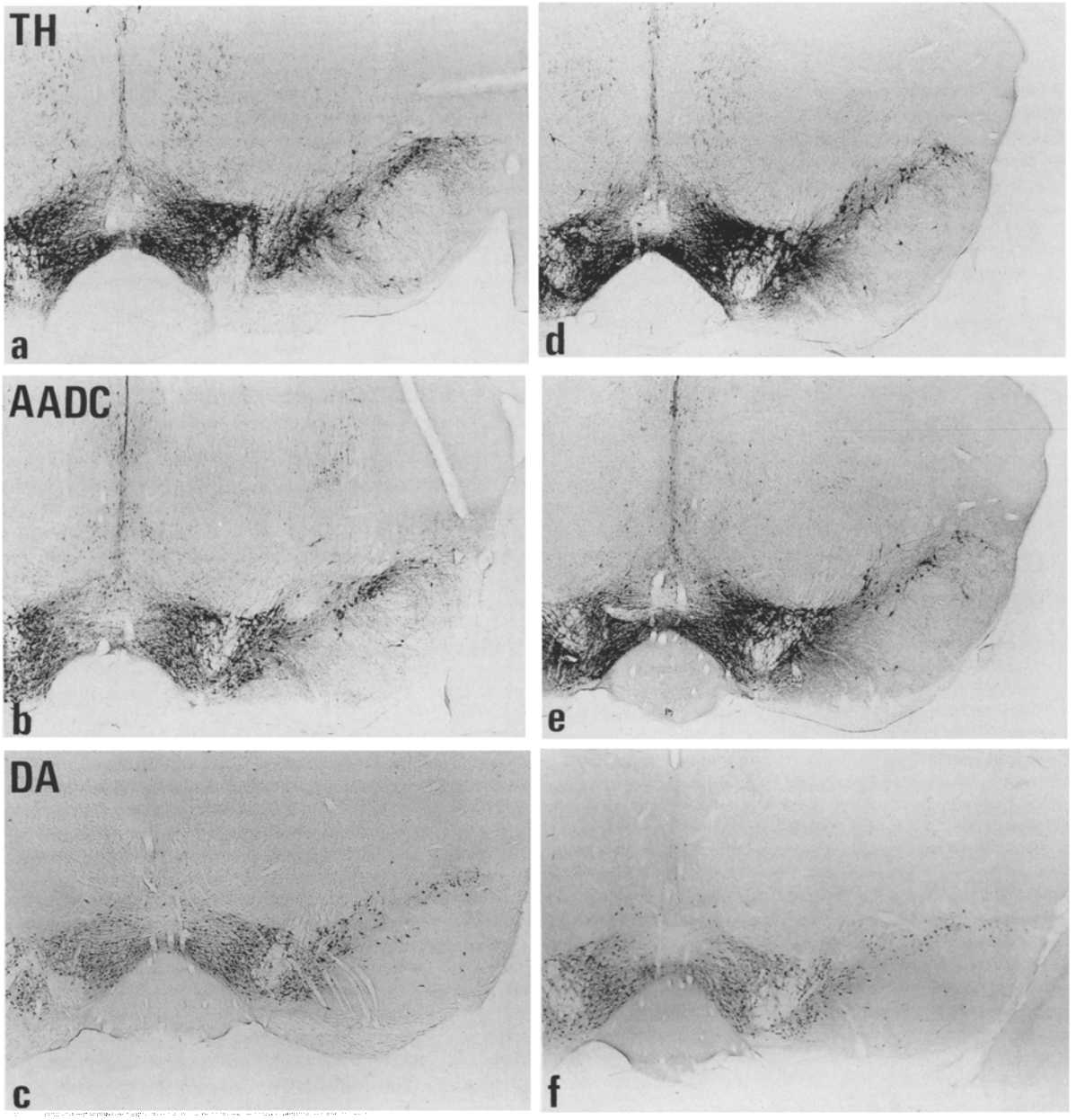

Fig. 8a–f. Immunohistochemical distribution of TH, AADC and DA in the substantia nigra and ventral tegmental area of a 13-week-old mouse (high MPTP dose). TH (**a**), AADC (**b**) and DA (**c**) of the control mouse and TH (**d**), AADC (**e**) and DA (**f**) of the MPTP-treated mouse. **a–f** × 25

days after last MPTP injection) with 1-week-old baby mice (11 days after last MPTP injection), it is evident that reduction of DA immunoreactivity of caudoputamen is more prominent in 1-week-old baby mice than in adult mice. These findings suggest that not only aged mice, but also immature baby mice are more sensitive to MPTP than young adult mice.

In conclusion, injection of MPTP to pregnant mice causes DA depletion in the nigrostriatal terminal field of baby mice and aborted mother mice. The results indicate that fetal brain is more sensitive to MPTP than the young brain of pregnant mice.

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