Proc. Indian natn. Sci. Acad. **B56** No. 1 pp. 95-100 (1990)

MPTP Model of Parkinson's Disease in Monkey: Implications for Neural Transplantation

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Total and hemiparkinson primate models were produced by giving intramuscular and unilateral intracarotid injections of 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine (MPTP). The signs such as tremor, rigidity and movement disorders varied despite the uniform dose of the drug given to the experimental animals. Younger monkeys seem to be more susceptible to the drug. Dopaminergic large multipolar neurons showed marked degenerative changes while the non-dopaminergic spindle-shaped neurons were mostly spared. Some of the earlier affected larger neurons appeared to recover later. These observations may be kept in mind while interpreting the results of nigral transplants to the striatum of MPTP-treated primate models.

Key Words: Rhesus monkey, Parkinson model, MPTP, Substantia nigra

Introduction

Parkinson-like syndrome has been produced in primates by various investigators with the view to studying the restorative effect of fetal substantia nigra transplanted into the striatum (Burns et al. 1983, Kit et al. 1986, Bakay et al. 1987, Bankiewicz et al. 1987, and Sladek et al. 1987). Reports on experiments lasting upto 6 months, suggest that the recovery is wholly or partly due to the dopaminergic neurons of the nigral transplant. Because of the varying degrees of recovery in the primate models, Morihisa et al. (1984) are of the view that the recovery is not graft-induced, but spontaneous. The present study was undertaken to correlate the status of the nigral neurons with the behavioural deficits in primate model produced with 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine (MPTP).

Materials and Methods

Six adult rhesus monkeys, weight ranging from 2.5 to 9 kg, were used for producing Parkinson model with the chemical 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine (MPTP). These monkeys had been quarantined after purchase and kept in captivity on standard diet atleast for a period of one year before the experiments.

Three monkeys used for total Parkinson model were given intramuscular injection of MPTP in sterile normal saline at a dose of 0.5 mg/kg body daily for 5 days.

Three monkeys were used for producing hemiparkinsonism because of the difficulty in maintaining the total Parkinson models. Hemiparkinson model was produced by injecting MPTP into the right internal carotid artery. Common carotid artery was exposed in the neck

after anesthetizing the animal with Ketlar (10mg/kg body wt). A sterile plastic arterial cannula with a stillet was introduced into the common carotid and guided into the internal carotid artery. The injection of MPTP was done slowly over 5 minutes. The cannula was left *in situ* for 5 minutes more. The dose of MPTP for arterial injection was between 0.5 and 1.6 mg/kg body weight (Bankiewicz et al. 1986).

MPTP after weighing carefully was dissolved in 5 ml sterile normal saline for intracarotid injection and 2 ml normal saline for intramuscular injection. The treatment of individual monkey with MPTP was as follows:

Monkey No. 1776: Young male monkey (1·2 years) weighing 2.1 kg was given daily intramuscular injection of MPTP for 5 days. The total dose was 5.25 mg. Eleven days after the injection the animal was sacrificed.

Monkey No. 1777: Young male monkey (1·2 years) weighing 2.2 kg was administered a total dose of 5.5 mg of MPTP over a period of 5 days. Ventral mesencephalon from a 58 day-old fetus was grafted into the head of the left caudate nucleus on the 9th day after the course of MPTP was completed. On the 16th day after the last MPTP injection and 7 days after transplantation the monkey was sacrificed.

Monkey No. 1805: Young female monkey weighing 3·7 kg (2·3 years) was given 8.6 mg of MPTP intramuscularly over 5 days. On the 10th day after the last MPTP injection, substantia nigra from a 58-day-old fetus was transplanted into the head of the right caudate nucleus. This monkey was sacrificed 108 days after the last MPTP injection.

Monkey No. 1806: Adult female weighing 4 kg (5·6 years) was given intramuscular injection of MPTP for 5 days. Total dose was 8.6 mg. Since this animal had not shown any signs of Parkinsonism for 2 months, it was used for producing hemiparkinson model by injecting 1.6 mg of MPTP into the right internal carotid artery. The animal was sacrificed 60 days after the intracarotid injection.

Monkey No. 1579: Adult male weighing 9.2 kg (over 8 years old) was given a single dose of 3.2 mg. MPTP into the right internal carotid artery. The procedure was repeated with 3.7 mg of MPTP after a month. After 4 days, injection was repeated with 14 mg. On the 18th day of the last injection, substantia nigra from a 55-day-old fetus was grafted into the head of the right caudate nucleus at three different sites. The monkey was sacrificed 4 months after transplantation.

Monkey No. 1812: Adult monkey weighing 8.5 kg (over 8 years) was given 3.4 mg of MPTP into the right internal carotid artery as a single dose. Injection was repeated after 4 days with the same dose but the quantity injected could not be determined due to leakage while injecting. One month after the last injection the animal was sacrificed.

All monkeys were kept under close clinical observations during the period of experimentation.

Transplantation Procedure

Donor tissue: Donor tissue used was substantia nigra obtained from fetuses of dated pregnancy. For dated pregnancy cyclicity of the female monkeys was first determined by daily examination of the vaginal smear. Regularly cycling females were mated with males and sperm positivity in the vaginal swab was determined the next morning. Sperm positive day was kept as zero day of pregnancy. Pregnancy was later confirmed by rectal palpation of the uterus and the uterine artery on the 30th and 40th day postcoitum. Caesarean section of the pregnant monkey was done on the desired date under aseptic conditions after Ketlar anesthesia. After delivering the fetus the wound was closed in layers and the animal was returned to the cage. Adequate antibiotics cover and postpartum care were given to these animals.

The brain of the fetus was rapidly dissected out and immersed in chilled ringer lactate solution. The meninges and blood vessels were carefully removed from the brain. The midbrain was sliced by neat horizontal cuts cranial and caudal to the tectum. There after tectum alongwith a part of the dorsal tegmentum was removed from the ventral midbrain. 8-10 microliters of the ventral midbrain tissue were taken into a glass capillary needle attached to a synringe (Das et al. 1979) for injection into the host striatum. According to the quantity of tissue available 2-3 such injections were given into the host striatum using stereotaxic coor-

dinates. Steretaxic coordinates for larger monkeys were calculated on the heads of autopsy specimens already available in the laboratory.

Host: Two of the total Parkinson models and one of the hemiparkinson models were used for transplantation of fetal substantia nigra into the striatum. Host was anesthetized and the head was shaven and cleaned. Head was fixed on a stereotaxic apparatus after giving adequate support to the chest. The scalp was cut midsagittally and reflected towards either side. The skull was cleaned off the connective tissue and with an electric drill a burr whole was made on the right side using the stereotaxic coordinates for the head of the caudate. The glass capillary needle attached to the syringe was slowly lowered and brought to position in the head of the caudate according to the measurements. The donor tissue was injected by lowering the plunger and the needle was retained in position for two more minutes and then withdrawn.

The monkeys were sacrificed at different time intervals after heavy anesthesia. Monkeys transplanted with substantia nigra were anesthetised and the brain were removed either after fixation with intracardiac perfusion of buffered 4% paraformaldehyde for histology and tyrosine hydroxylase (TH) immunofluorescence or before fixation for monoamine histofluorescenece (Torre 1980). For immufluorescence and monoamine histofluorescene cryostat sections were used. For routine histology paraffin sections were stained with cresyl violet. Numerical density of the neurons in the pars compacta of 3 total Parkinson model and 3 adult control monkeys were determined by semiautomatic method using IBAS Image Analyser.

Results

Monkey No. 1776: Following the third injection, rapid blinking of the eyelids, slowing down of the limb movements and short periods of immobility were observed in the monkey. On the last day of injection monkey assumed a crouching position and remained immobile for long periods of time. Sluggish movements were observed only on prodding. Handling of the food became clumpsy and it could manage self feeding only for 4 more days. Rigidity of the limbs was also noticed. Inspite of oral feeding and intravenous fluid, general condition of the monkey deteriorated. It was sacrificed on the 11th day of the experiment.

Majority of the neurons of the pars compacta of the substantia nigra, when compared to the control, appeared shrunken (figures 1 & 2). Mean diameter in control (3 monkeys) was 43.853 $\pm$ 0.546 while in MPTP treated monkeys (3) the measurement was 38.736 $\pm$ 2.940. The difference was statistically significant. Nuclei of the neurons appeared cloudy and the margin was indistinct. Many of the cells were hyperchromatic. A few neurons appeared normal, but were smaller in diameter.

Monkey No. 1777: This monkey started showing signs of Parkinsonism only on the last day of injection. Movements became slow and sluggish and after 2 days it assumed flexed posture and moved only when disturbed. Postural tremors were observed at times. This animal was fed orally with soft fruits and intravenous fluids for 6 days following which it was grafted with fetal substantia nigra. The condition of the monkey continued to deteriorate and it had to be sacrificed on the 21st day.

Neurons of the pars compacta had more or less the same morphology as in the previous monkey. Only very few fluorescing neurons were seen in the compacta region when compared to the large number seen in the control animals (figures 3 & 4). Transplant site in the striatum showed fluorescene for monoamines. However, it was difficult to differentiate individual neurons (figure 5).

Monkey No. 1805: Only on the last day of the injection any kind of disorder in movements was noticed. Rapid blinking of the eyelids, slowing and clumpsiness of the movements were seen. After 2 days the monkey remained stationary in flexed posture. Clumpsy movements were elicited only on prodding. Postural tremors also became apparent. At this stage the monkey was fed on soft fruits orally and fluids intravenously.

On the 10th day after the last injection, substantia nigra from a 58-day-old fetus was grafted into the striatum. On the 6th day after grafting, movements showed improvement and in 2 days monkey was able

to transfer food to the mouth. Within the month, movements had improved considerably and the monkey was able to handle food independently. But postural tremors were still present. Suddenly it started deteriorating again and had to be fed orally. In this condition the monkey was maintained for two more months.

Neurons in pars compacta were few in number. Many of them showed chromatolytic changes with eccentrically placed nuclei and clumping of Nissl substance. Some of the spindle-shaped neurons appeared apparently normal (figure 6).

On microscopic examination of the transplanted area of the striatum, a small localised area showed diffuse fluorescence for monoamines. Surrounding area showed red auto fluorescence suggesting tissue necrosis.

Monkey No. 1806: No abnormal signs were noticed in this monkey either during or after the intramuscular injection of MPTP. Following the intracarotid injection of 1.6 mg of MPTP, anticlockwise circling and noticeable rigidity of the left forelimb were noticed. It preferred the right limb for support in the cage. Within the next few days the signs extended to the left limb also. All these signs continued for a month and thereafter there was a gradual recovery. By the end of the second month after intracarotid injection, the monkey appeared normal except for the reduced power in the left forelimb while holding a stick.

Histofluorescence for monoamines in the compacta region of substantia nigra showed a number of fluorescing neurons on the right side, though the density was less compared to the left untreated side. A few chromatolysed and hyperchromatic neurons were seen on routine histology examination. Same features were seen in relation to a few neurons, but much less in number, on the left side.

Monkey No. 1579: Only after the third intracarotid injection signs of motor abnormalities were noticed in this monkey. Flexed frozen position and rapid blinking of the eyelids were noticed after 2 days. Monkey was apathetic and disinterested in food and the surroundings for 2-3 days requiring assisted feeding on soft fruits. After 12 days the monkey started responding to repeated prodding by lunging and open mouth threats Though right limbs appeared normal it preferred to remain in the crouched position most of the time.

At this time substantia nigra from a 55-day-old fetus was transplanted into the striatum. Four days after transplantation food intake increased. Attemps were made to handle food with the left hand without success. But the monkey improved steadily and gradually and became active and started feeding on regular diet within a month. Only very little movement deficit was observed on the left side after two months.

Immunoreactivity for TH on the right side pars compacta of substantia nigra was seen in relation to a few neurons while on the left side many cells were stained positive (figure 7).

The area of the transplant processed for tyrosine hydroxylase had not shown any positive immunoreactivity.

Monkey No. 1812: On the second day after the intracarotid injection the monkey became quiet and listless but no movement disorders were noticed. During this period food intake was also reduced. After 4 days, behaviour of the monkey reverted back to the preinjection status. After the second injection a mild flexion deformity on the left limbs and an occasional anticlockwise circling were noticed for a short while.

Microscopic observation of the pars compacta in this monkey was comparable to the observations in the control monkey.

Discussion

A few significant observations emerge from this study, despite the small number of animals used to produce the Parkinson-like model.

Onset and severity of signs varied from animal to animal belonging to the younger age group treated with identical dose and injection regime of MPTP. Moreover, the younger monkeys appeared to be more sensitive to MPTP when compared to the older age group. Thus, one monkey showed signs after the third injection while the other two started signs only on completion of five injections. This may depend on the fate of conversion of MPTP to MPP (Langston et al. 1984) by monoamine oxidase B. Another interesting observation was the structural details of the neurons in the pars compacta

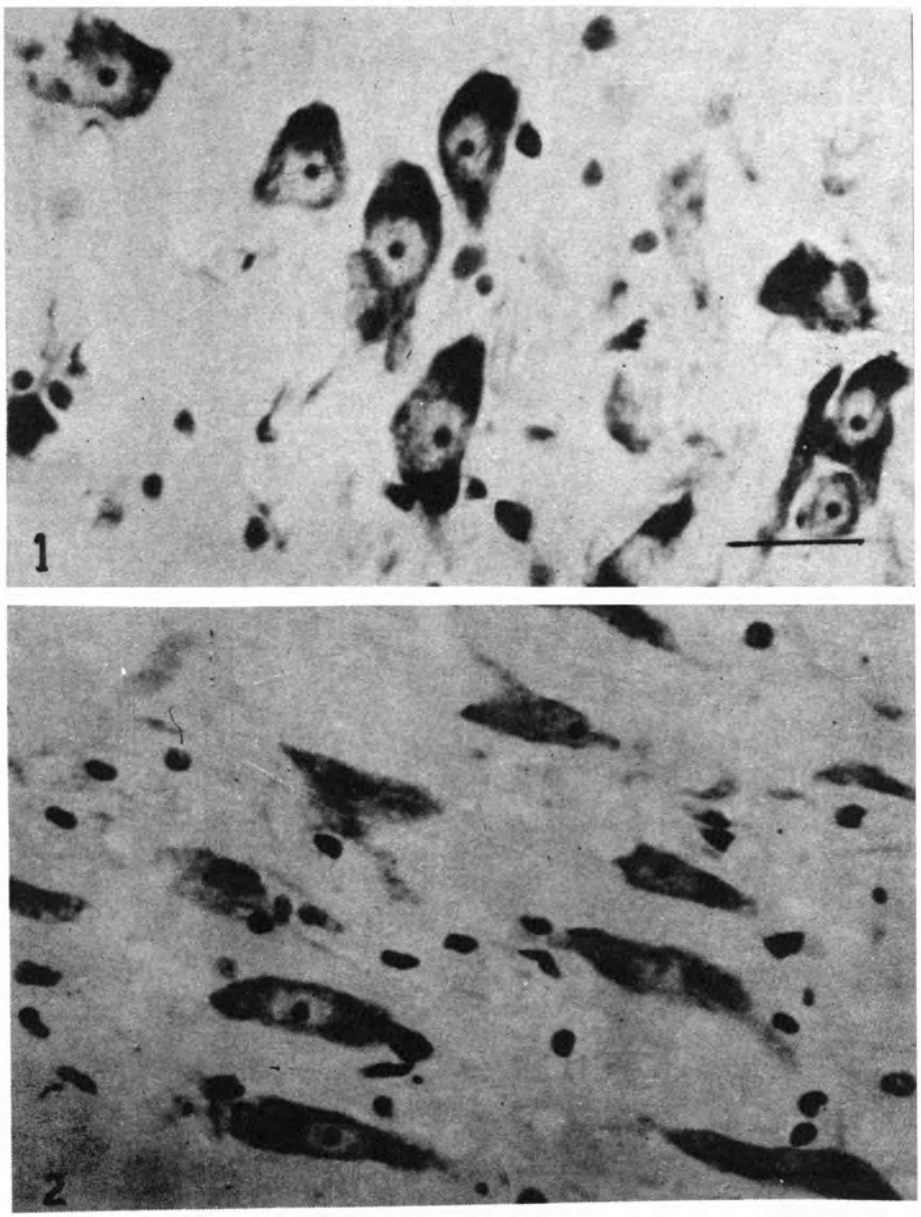

Figure 1-2 Photomicrograph of pars compacta in adult control monkey (Bar = 40μ) Cresyl violet stain; **2:** Pars compacta neurons of monkey, after 3 weeks of MPTP injections. (Bar = 45 M) cresyl violet stain.

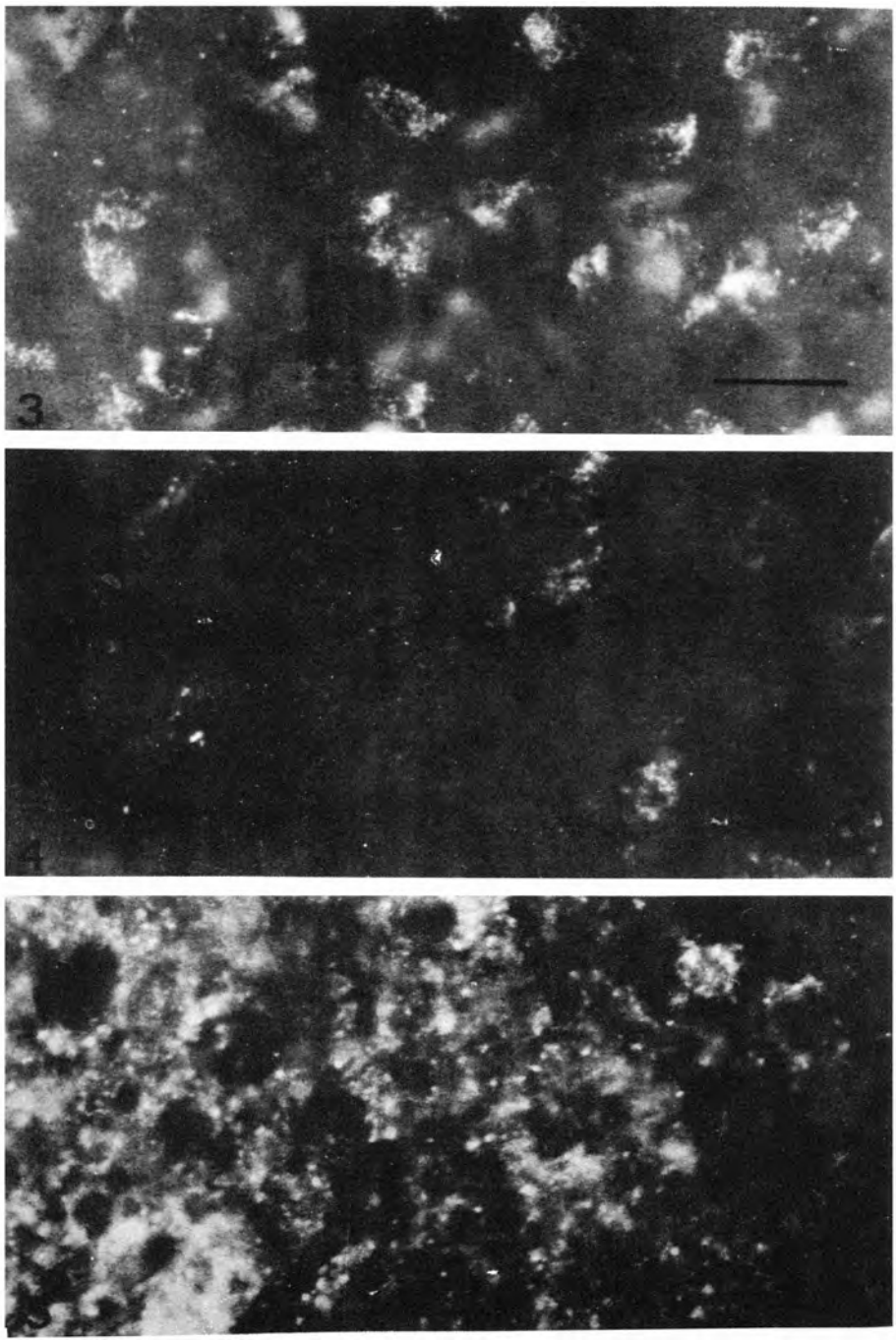

Figure 3-5 Monoaminergic fluorescing cell bodies in pars compacta of normal adult monkey (Bar = 45 M) Glyoxylic acid fluoresence; **4**: Monoaminergic fluorescence in the pars compacta of MPTP treated monkey (Bar = 45 M) Glyoxylic acid fluorescence; **5**: Monoaminergic fluorescence of the nigral transplant in the striatum of the MPTP-treated monkey. (Bar = 45 M) Glyoxylic acid fluorescence.

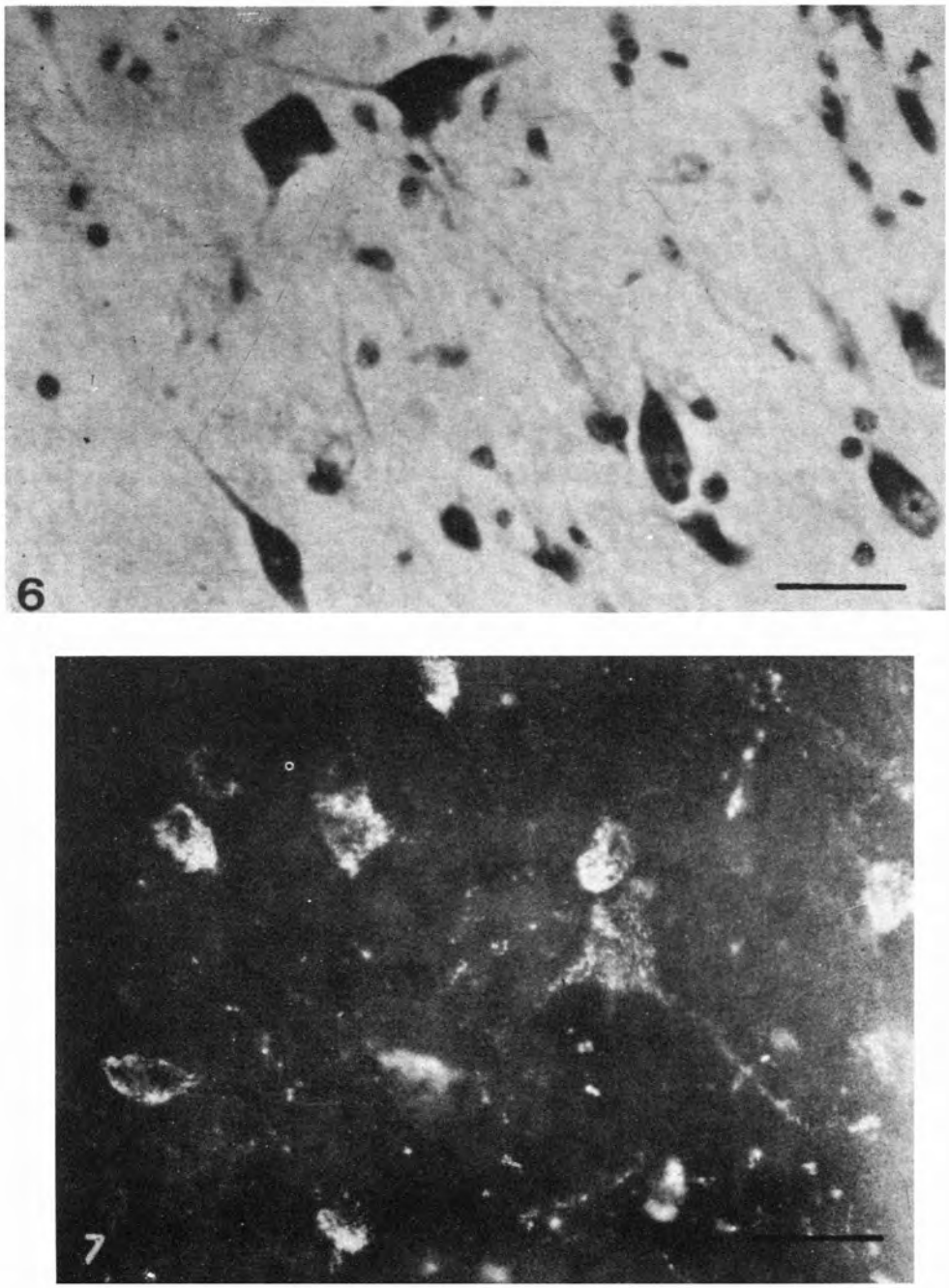

Figure 6-7 Pars compacta showing reduced neuronal density. 108 days after MPTP treatment. Some of the affected large neurons are still retained. Smaller spindle shaped neurons appear normal (Bar = 45 M) Cresyl violet stain; **7**: Tyrosine hydroxylase positive neurons in pars compacta of the contralateral side on the injection showing normal immunoreactivity (40 M)

of the substantia nigra in the three young Parkinson models. Morphological characteristics of the cells more or less are the same in all the three monkeys except for the reduced density of neurons in monkey no. 1805. A few apparently normal neurons are seen in all the monkeys. These observations indicate that neurons are damaged as early as first week after the intramuscular drug administration and all the neurons are not uniformly affected. Mostly the large neurons which are reported to be dopaminergic showed chromatolytic changes. The unaffected neurons are the smaller, spindle-shaped variety, which are non-dopaminergic (Fallon & Loughlin 1985). Though most of the large affected neurons are lost subsequently, it is apparent from monkey no 1805 that some of the affected neurons are retained, which may recover later. This may account for the recovery in monkey no. 1579, which had no surviving dopaminergic neurons in the transplant. Recovery in the immediate post-transplantation period in this monkey could be due to the diffusion of dopamine from the freshly transplanted substantia nigra. In monkey 1805, the recovery during the first month after transplantation appears to be due to the release of dopamine from the degenerating graft, evident from the diffuse fluorescence restricted to a very small area in the transplanted site in this animal.

In our study the older rhesus monkeys appeared more resistant to MPTP toxictity as compared to the younger animals. This is in contrast to the earlier report by Bankiewicz *et al* (1987) in *Macaca fascicularis*. According to Bakay et al. (1987), older monkeys already depleted of nigral neurons due to progressive accumulation of pigment, are more sensitive to the insult by MPTP.

In conclusion, younger rhesus monkeys seem to be more sensitive to MPTP toxicity when compared to older animals. The onset and severity of the Parkinson-like signs depend on the effect of MPTP on the substantia nigral neurons which seem to vary from monkey to monkey. Large multipolar neurons which are dopaminerigc appear to be more sentitive to toxicity compared to the nondopaminergic neurons. Some of the dopaminergic neurons seem to recover over a period of time after administration of MPTP. While interpreting the results of substantia nigral transplantation in primate Parkinson model these points should be taken into consideration.

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