The effect of fetal mesencephalon implants on primate MPTP-induced parkinsonism

Histochemical and behavioral studies

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Parkinsonism or hemiparkinsonism was induced by administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in four rhesus monkeys, which then received homologous fetal mesencephalon implants into the caudate nuclei. Cavities were prepared in the medial caudate nucleus 2 to 5 weeks before the fetal grafts were implanted. Control studies were conducted in unoperated MPTP-treated animals. Significant behavioral improvement, which occurred within weeks of implantation of fetal mesencephalon, was sustained for up to 7 months. No recovery was seen in the unoperated control animals. Histological examination revealed numerous surviving tyrosine hydroxylase (TH)-immunoreactive cell bodies. In addition to the graft, abundant TH-immunoreactive fibers were observed in the host caudate nucleus ventral to the region of the implanted and the nonimplanted cavities. Since TH-immunoreactive cell bodies of the substantia nigra compacta (A-9 cells) were destroyed by MPTP treatment and the ventral tegmental area (A-10) remained intact, it is concluded that sprouting of remaining host dopaminergic fibers occurs. These newly formed fibers appeared to emanate from the mesolimbic projection to the striatum. It is likely that the newly sprouted dopaminergic fibers account for the motor improvement elicited by precavitation and fetal mesencephalon implantation.

These results suggest that the mechanism of recovery of parkinsonian primates after implantation of fetal dopaminergic tissue into the caudate nucleus is by stimulation of sprouting from host neurons. They also suggest that, with identification of the factors responsible for the formation of the new dopaminergic neuronal processes and with further development, tissue implantation may be an effective therapy for Parkinson’s disease in humans.

KEY WORDS • Parkinson’s disease • MPTP • mesencephalon • fiber sprouting • fetal mesencephalon transplant • primate

DOPAMINERGIC tissue implantation into the denervation striatum reverses, at least in part, the motor abnormalities in rodent models of parkinsonism. Attempts to replace deficient neurons represent a new direction in the therapy of neurodegenerative disorders and differ markedly from the current treatment of Parkinson’s disease, in which systemic administration of dopaminergic precursors restores striatal levels of dopamine. Neurotransmitter replacement therapy for Parkinson’s disease, however, fails to halt progression of the disease, resistance to treatment develops, and unacceptably severe side effects are common. Tissue implants might potentially eliminate these problems. Cell replacement may limit the progression of disease and, because increases in dopamine are limited to the sites of dopamine deficiency, systemic side effects may be avoided.

The efficacy of implants in reversing abnormal motor responses in rodent models of Parkinson’s disease does not ensure that tissue implants will be of value in treating humans. Adrenal medullary implants into the striatum of rats appear to ameliorate the motor deficits attending dopaminergic neuron destruction. However, the first human trial of adrenal medullary implants in parkinsonian patients was not successful. Recent reports of the successful use of tissue transplants in
humans with Parkinson’s disease\textsuperscript{21,22} are encouraging, but results are still preliminary, the basis of the recovery reported is not fully understood, and the results have not been consistently confirmed. It is evident that much needs to be learned about the effects of transplanting tissue to brain; the mechanisms involved in the survival and function of the transplanted tissue and the responses of the host to the transplant, its secretions, and the trauma of its insertion are poorly understood.

Treatment of monkeys with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) destroys nigrostriatal dopaminergic neurons and produces a motor deficit that closely resembles Parkinson’s disease.\textsuperscript{10,11,20} This primate model of Parkinson’s disease has been used to test the efficacy of implanted tissues in reversing the motor abnormalities attending toxic destruction of the nigrostriatal neurons.\textsuperscript{3,4,7,26,28} Administration of MPTP into the internal carotid artery of monkeys produces ipsilateral destruction of dopaminergic neurons in the substantia nigra and a hemiparkinsonian syndrome.\textsuperscript{6} Experimental hemiparkinsonism provides a useful model because the intact side may be used as a control for examination of volitional movement, “turning behavior” in response to dopamine agonists is an index of receptor sensitivity asymmetry,\textsuperscript{19} and interactions of transplanted tissue with the denervated and the intact host caudate nucleus may be compared in the same animal. We have previously shown that adrenal medullary allografts only transiently improve apomorphine-induced turning behavior in hemiparkinsonian monkeys and fail to reverse the deficit in volitional motor responses.\textsuperscript{7} In those animals, the grafted adrenal medullary tissue did not survive.

In the present study, the effects of fetal mesencephalic tissue implants were examined in monkeys rendered parkinsonian or hemiparkinsonian by the administration of MPTP either systemically or into the internal carotid artery.\textsuperscript{6,10,20} Monkey fetal mesencephalon was implanted into preformed cavities in the caudate nucleus, and behavioral responses were studied for up to 7 months. Immunohistochemical and autoradiographic studies were performed when the animals were sacrificed. The motor improvements after implanting fetal dopaminergic cells in the caudate nucleus of these animals, as well as those occurring in parkinsonian monkeys in other studies of transplants,\textsuperscript{3,4,12,26,28} suggest that tissue implant therapy may be applicable to patients with Parkinson’s disease. The extensive sprouting of dopaminergic fibers into the damaged striatum observed in the present communication indicates that stimulation of new growth from surviving host dopaminergic neurons may be responsible for the behavioral recovery.

**Materials and Methods**

**Subjects**

Ten adult rhesus monkeys (Macaca mulatta) of either sex and two adult female cynomolgus monkeys (Macaca fascicularis) were used for this study. The monkeys were housed in quarters with a 12-hour light/dark cycle and were fed Purina Monkey Chow twice daily with free access to water. The experimental protocol was approved by the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke, and all animals were treated in accordance with the National Institutes of Health guidelines for animal care and use.

**MPTP Administration**

Four rhesus monkeys were made parkinsonian by five daily intravenous injections of MPTP-HCl (0.3 mg/kg)\textsuperscript{10} followed 1 month later by two additional injections of MPTP-HCl (0.2 mg/kg). Six rhesus monkeys were made hemiparkinsonian by intracarotid infusion of MPTP as previously described.\textsuperscript{8,7} These animals were anesthetized with intramuscular ketamine (Vetlar, 7 mg/kg) followed by intravenous pentobarbital (Somnifer, 7.5 mg/kg). One common carotid artery was exposed at the level of its bifurcation and the superior thyroid and external carotid arteries were temporarily clamped. A No. 27 needle was inserted into the common carotid artery and 60 ml saline containing 3 mg MPTP-HCl was infused in a retrograde fashion at a rate of 4 ml/min. The two cynomolgus monkeys were made hemiparkinsonian by infusion (4 ml/min) of 60 ml saline containing MPTP-HCl (0.4 mg/kg) into one internal carotid artery via a transfemoral catheter.

**Assessment of Motor Function**

The four monkeys with bilateral parkinsonism were followed for 3 months after MPTP treatment. Motor function was assessed by examination and videotaping of spontaneous activity. Changes in motor activity in response to administration of L-dopa/carbidopa (200/20 mg by mouth), apomorphine (0.2 mg/kg intramuscularly), or amphetamine (3 mg/kg intramuscularly) were recorded on videotape. Spontaneous and drug-induced motor activities were examined at various intervals before and after tissue implantation (see below).

Five weeks after unilateral intracarotid artery administration of MPTP, when the hemiparkinsonian syndrome was fully developed and stable, spontaneous activity, volitional motor responses, and apomorphine-induced locomotor activity were examined. Volitional responses to presentation of pieces of food were recorded on videotape. Two pieces of food were offered sequentially; the second piece was presented when the first piece had been taken by one hand and brought to the animal’s mouth. In normal monkeys, the second piece of food was always taken with the other hand. In hemiparkinsonian monkeys, however, the first piece was transferred and held in the mouth while the same hand that was used initially was again used to obtain the second piece of food. The limb contralateral to MPTP infusion, which was rigid and showed tremor, remained unused. The percentage use of the parkinson-
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ian arm to obtain the second piece of food provided an index of improvement in volitional motor function.

Monkeys with hemiparkinsonism, when spontaneously active, turn predominantly toward the affected side. Treatment of hemiparkinsonian animals with apomorphine (0.2 mg/kg intramuscularly) enhances the general level of locomotor activity and, because of supersensitive receptors on the MPTP-treated side, reverses the direction of spontaneous turning. The rate of turning away from MPTP-treated side after apomorphine administration was taken as an index of dopamine receptor supersensitivity. To facilitate quantification of the locomotor response, the animals were dressed in primate jackets which held an infrared phototransmitter. The monkeys were housed in cages equipped with infrared radiation detectors which signaled a computer when activated. The number of clockwise or counterclockwise turns were recorded during sequential 5-minute intervals for 1 hour after apomorphine injection and were stored in the computer. Responses to three to five of the weekly apomorphine injections established the basal turning pattern for each animal.

To enhance graft survival, cavities were prepared in the head of the left and right caudate nuclei 2 to 5 weeks before tissue was implanted. The animals were anesthetized with pentobarbital (7.5 mg/kg) and surgery was performed with sterile technique. A diamond saw was used to cut and remove a right frontal bone flap (5 x 3 cm) extending just across the midline. The dura was incised and retracted medially to expose the interhemispheric fissure and the hemispheres were gently separated. With an operating microscope and microsurgical technique, a small incision was made through the body of the corpus callosum; the most dorsal aspect of the septum was resected, thus exposing both lateral ventricles. Two small cavities were made with pituitary rongeurs on the mediodorsal aspect of the head of each caudate nucleus anterior to the foramen of Monro. Each cavity was filled with a trypan blue-stained gelatin sponge (Gelfoam). The dura was closed, the bone flap was replaced, and the scalp incision was closed.

Implantation Groups

Five hemiparkinsonian monkeys and three fully parkinsonian monkeys were left unoperated and served as controls. Two to five weeks after precavitation of the caudate nuclei, four monkeys were implanted with fetal mesencephalon; one fully parkinsonian monkey received a unilateral implant and three hemiparkinsonian animals received bilateral implants (see below).

Procurement of Fetal Tissue for Implants

Fetal dopaminergic brain tissue was obtained from 35- to 42-day gestational monkey fetuses. In preliminary studies (see below) it has been established that, at this stage of development, tyrosine hydroxylase (TH)-immunoreactive neurons are present in the fetal mesencephalon, but projections to the striatum are not fully developed (Fig. 1). Rhesus monkey fetuses were obtained via Caesarean section carried out under general anesthesia. Under sterile conditions, the ventral part of the mesencephalon was dissected under an operating microscope and cut into 1 x 1 x 2-mm pieces. The tissue was placed in ice cold phosphate-buffered saline (PBS) until implanted 20 to 40 minutes after removal from the donor.

Transplantation Procedure

Two to 5 weeks after precavitation, transplants were inserted into the caudate nucleus cavities. The caudate nuclei were exposed via the transcaldosal approach, the previously created cavities were located, and the Gel-
foam was removed. In the fully parkinsonian monkey an implant was inserted into only one caudate nucleus, leaving the other side with Gelfoam-filled cavities (Fig. 2A). In the hemiparkinsonian animals, cavities on both sides were implanted with one to three 1 x 1 x 2-mm pieces of tissue (Fig. 2B). Gelfoam was used to secure the implants within the cavities; the bone flap was replaced and the wound was closed. Two hemiparkinsonian animals received tissue from a single fetus, whereas the third animal received tissue from two fetuses.

Fig. 2. Schematic representation of the delayed implantation protocol. A: MPTP-parkinsonian monkey with unilateral implant. B: MPTP-hemiparkinsonian monkey implanted bilaterally.
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Examination of Brain Tissue
For histopathological studies, three monkeys that had received fetal mesencephalon, two unoperated hemiparkinsonian animals, and one fully parkinsonian monkey were killed 5 to 7 months after implantation or after MPTP infusion, respectively. Animals were killed by an overdose of pentobarbital (460 mg intravenously) and were perfused through the ascending aorta with 500 ml of ice cold PBS containing 0.5% sodium nitrite, followed by 2 liters of ice cold 10% formalin in PBS (pH 7.0). The brains were removed rapidly, cut into 6-mm coronal slices, and postfixed for 30 minutes in the same fixative. The tissue slices were rinsed for 48 hours in 20% sucrose in PBS, frozen on dry ice, cut into 20-μm coronal sections in a cryostat, mounted on slides coated with chrome-alum, and processed for immunofluorescence microscopy. Brain sections were exposed for 2 to 3 days at 4°C to rabbit serum containing antibody to TH diluted 1:1000 in PBS or to glial fibrillary acidic protein (GFAP) diluted 1:1000 in PBS containing 0.3% Triton X-100 and 1% normal goat serum. The sections were washed three times (10 minutes each) and then incubated for 30 minutes in fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G diluted 1:300 in PBS with 0.3% Triton X-100. The sections were washed as described above, rinsed in PBS, and mounted in glycerin/PBS (3:1). Sections were examined under a fluorescence microscope equipped with a Ploem illuminator. Sections adjacent to those examined for TH immunoreactivity were stained with hematoxylin and eosin (H & E).

Dopamine uptake sites were visualized by using tritiated (3H)-mazindol autoradiography as described by Javitch, et al.18 For autoradiographic studies, 20-μm sections were cut in a cryostat at −20°C, thaw-mounted on gelatin-coated slides, dessicated, stored at −70°C, and then preincubated at 4°C for 5 minutes in 50 mM Tris buffer (pH 7.9 at 4°C) with 300 mM NaCl and 5 mM KCl. Sections were incubated for 40 minutes in 50 mM Tris buffer (300 mM NaCl and 5 mM KCl) with 4 nM 3H-mazindol (24.5 μCi/mmol) and 0.3 μM desmethylimipramine to block noradrenergic uptake sites. Nonspecific binding was determined in alternate sections in the presence of 30 μM benztropine. Sections were then rinsed in buffer twice for 1 minute each followed by a 10-second rinse in distilled water, then dried under a stream of cold dry air. Slides were apposed to LKB Ultrafilm, along with a set of calibrated 3H standards, for 4 weeks.* The autoradiograms were analyzed by computerized densitometry.† Color-coded transformations of binding site densities were made to enhance visualization.

Results
Effects of MPTP Treatment
Systemic MPTP treatment produced severely parkinsonian animals which were unable to sustain themselves. The three unoperated fully parkinsonian monkeys did not improve over time and for humanitarian reasons were sacrificed 3 months after MPTP injections. The brain of the animal that was examined for TH immunoreactivity showed that the cells in the substantia nigra pars compacta were depleted almost totally, whereas the number of dopaminergic cell bodies in the ventral tegmental area of the midbrain (A-10) and the density of fibers in the mesolimbic projection sites (nucleus accumbens septi, olfactory tubercle, and septum pellicudum) did not appear to be affected. This is consistent with previous observations.17 Dopaminergic projections to the caudate nucleus and to the putamen were severely damaged. In the two other unoperated monkeys treated systemically with MPTP, levels of dopamine and dopa-decarboxylase in the neostriatum were reduced dramatically (data not shown).

Monkeys made hemiparkinsonian by intracarotid infusion of MPTP developed rigidity, bradykinesia, and tremor of the limbs on the side contralateral to the injection.6 Although these animals did not use the affected limbs, they were able to take food with the unaffected upper extremity and to drink, and they remained healthy. Spontaneous activity was attended by circling toward the MPTP-treated side; treatment with apomorphine reversed the direction of turning and enhanced spontaneous activity, as described previously.6 Contralateral turning begins 2 to 5 minutes after injection of apomorphine, reaches a peak at about 30 minutes after, and lasts for about 1 hour. The total number of turns during the hour is a measure of the intensity of the response. All hemiparkinsonian monkeys showed a consistent magnitude of drug-induced turning in response to doses of intramuscular apomorphine (0.2 mg/kg), repeated at various times up to 40 months after unilateral MPTP infusion.7 The animals remained unable to obtain food treats with their af-

* Ultrafilm manufactured by LKB Produkter, Bromma, Sweden.
† Densitometer manufactured by MCID Imaging Research, St. Catherine’s, Ontario, Canada.
fected arm, which continued to display rigidity, bradykinesia, and tremor.

Fetal Mesencephalic Implants in Bilateral Parkinsonian Monkeys

The monkey with bilateral parkinsonism showed dramatic improvement during the 1st week after receiving the unilateral fetal mesencephalic tissue implant. This animal had demonstrated no indication of improvement in the 3 months after MPTP administration nor in the week after cavitation. Three weeks after the implant there was bilateral recovery and it was no longer possible to distinguish between movements of this monkey and those of normal animals. The animal reached for food and brought it to its mouth as rapidly as did the normal animals. Feeding, grooming, and general activity all increased, tremor disappeared, and freezing episodes were no longer observed. Five months after implantation, the monkey started to turn spontaneously away from the implanted side. After amphetamine injection (Fig. 3) turning away from the implant was dramatically increased, whereas after apomorphine administration motor activity consisted of turning toward the implanted side (Fig. 4). Food was accepted without evident arm preference. These responses to amphetamine and apomorphine and the general activity level persisted until sacrifice 7 months after implantation.

The solid fetal mesencephalic implant was easily located in sections of the monkey brain stained with H & E. Consistent with its placement into one of the

![Image](https://via.placeholder.com/576x774.png?text=Fig. 3. A and B: Charts depicting amphetamine (AMPH)-induced turning in a monkey with bilateral parkinsonism before (A) and 5 months after (B) receiving a unilateral fetal mesencephalic tissue implant. CW = clockwise turning; CCW = counterclockwise turning. Each column represents 5 minutes. C: Amphetamine (AMPH) stimulated the implant (black dot) to release dopamine which interacted with dopaminergic receptors (DA) only on the implanted side (right side), inducing turning away (counterclockwise on the charts) from that side.)
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Preformed cavities, the implant was evident on the medial aspect of the caudate nucleus on one side; the vacant cavity from cavitation only was seen on the opposite side (Fig. 5A). Many histofluorescent TH-immunoreactive dopamine cell bodies and processes were present in the implant (Fig. 5B). There was also an abundance of dead tissue (giving rise to lipofuscin autofluorescent debris) in the central and dorsal portions of the implant. Many TH-positive fibers were evident in the medial portion of the caudate in close proximity to the transplant. However, there was no evidence of continuity between the TH-immunoreactive fibers in the transplant and those in the caudate at any site along the periphery of the implant.

In the opposite caudate nucleus, which had received cavitation but no implant, many fluorescent fibers traversed the tissue in a dorsoventral direction just medial and ventral to the cavity (Figs. 5C and 6 left). These fibers emanated from the ventral aspect of the striatum adjacent and just lateral to the nucleus accumbens septi. Immunoreactivity to GFAP in the region of the cavity shows reactive astrocytes that are oriented parallel to the sprouted fibers (Fig. 6 right).

In contrast to areas of the caudate-putamen away from the surgical sites, the nucleus accumbens septi and olfactory tubercle contained abundant TH-immunoreactive dopaminergic fibers which occurred on both sides and were comparable in density and distribution to those in normal untreated animals. Examination of the ventral mesencephalic region revealed a marked

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**Fig. 4.** A to C: Charts showing apomorphine (APO)-induced turning in a monkey with MPTP-induced parkinsonism before (A) and 2 (B) and 6 months after (C) receiving a unilateral fetal mesencephalic tissue implant. CW = clockwise turning; CCW = counterclockwise turning. Each column represents 5 minutes. D: The release of dopamine from the implant (black dot) decreased sensitivity of the dopaminergic receptors (DA) on that side. Supersensitive receptors on the nonimplanted side caused the animal to turn toward (clockwise on the chart) the implant (right side) after apomorphine injection.
reduction in the number of A-9 cell bodies (substantia nigra compacta), whereas the A-10 cells in the midline (ventral tegmental area) had a normal density of TH-immunoreactive dopaminergic perikarya and fibers.

**Fetal Mesencephalic Implants in Hemiparkinsonian Monkeys**

During spontaneous activity, circling toward the MPTP-treated side of the hemiparkinsonian monkeys was at about the same level 7 months after the fetal tissue implants as before implantation. However, after implantation the circling response to apomorphine was strikingly reduced (Fig. 7). As indicated above, the number of turns contralateral to the MPTP-treated side during the 1 hour after administration of a standard dose of apomorphine was used as an index of asymmetry of receptor sensitivity in the hemiparkinsonian animals. Decrements in asymmetry of sensitivity to apomorphine after implantation were evidenced by the reduction in drug-induced turning. This was expressed as a percentage decrease of turns elicited by the standard dose of apomorphine compared to the same animal before implantation. In all animals, apomorphine-induced rotation decreased during the first 3 weeks after fetal mesencephalic tissue implantation; in the two hemiparkinsonian animals that received tissue from a single fetus, decreased drug-induced turning persisted longer than 6 months.

The animal with implantation of tissue from two different fetal donors appeared to be different. In this animal, 6 weeks after implantation the number of turns in response to apomorphine began to increase, and by 4 months the turning response was back to the baseline level. Histological examination revealed immunological rejection of the implants (Fig. 8).

Volitional arm use was evaluated by the response of the animals to food offerings during three video-recorded sessions. One month after implantation, there was a marked increase in use of the involved arm by all three implanted hemiparkinsonian animals; this persisted for 7 months after implantation in the two monkeys that had received tissue from a single fetus. The animal that was implanted with tissue from two fetuses had regression of volitional use of the affected arm at about 6 weeks after implantation, simultaneously with the increase in apomorphine-induced rotation.

Histological examination of the brain sections of the hemiparkinsonian monkeys with fetal tissue implants
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FIG. 6. Tyrosine hydroxylase (left) and glial fibrillary acidic protein (right) immunoreactivities in the region of the caudate nucleus cavity. Left: × 125, reduced 32%; Right: × 160, reduced 42%.

provided information about the viability of the implanted tissue and its interaction with the host brain. In one monkey the implant was attached to the medial surface of the caudate nucleus contiguous with the lateral ventricle (Fig. 9A). The implant contained many fluorescent TH-positive (presumably dopaminergic) cell bodies and processes (Fig. 9B). Long thick processes were seen throughout the implant; however, there was no obvious ingrowth of fibers from the implant into the host caudate nucleus. The medial region of the caudate nucleus, ventral to the cavity and lateral to the attached transplant, contained abundant dorsoventrally oriented TH-immunoreactive fibers (Fig. 9C). These processes appeared to arise from ventral structures and were projecting in a dorsal direction toward the cavity.

The implant on the opposite (untreated) side had become dislodged and was attached to the septum. A few fluorescent cell bodies with long processes were noted in the implant. In the medial caudate region, ventral to the cavity, many long vertical fibers were observed, similar to the sprouted fibers seen on the lesioned side. In both cerebral hemispheres the nucleus accumbens septi, the region lateral to the nucleus accumbens septi, and the olfactory tubercle contained abundant TH-immunoreactive fibers. These fibers were similar to those found in normal animals. In the midbrain, however, there was almost complete depletion of dopaminergic cells in the A-9 area of the substantia nigra on the MPTP-treated side.

Autoradiography

The third hemiparkinsonian monkey was processed for specific binding of 3H-mazindol to dopaminergic uptake sites. The area of the transplant (identified by H & E stain, not shown) displayed an active dopamine uptake (Fig. 10). Dopaminergic binding activity was not only confined to the graft, but extended also in the dorsoventral direction of the medial portion of the caudate nucleus. This correlates with the area of the TH-immunoreactive sprouting that was observed in other implanted monkeys; however, we were unable to obtain TH-immunohistochemical staining in this animal.

Discussion

This study shows that monkey fetal mesencephalic tissue implanted into the caudate nucleus of monkeys with MPTP-induced parkinsonism survives for up to 7 months and produces behavioral changes indicative of functional recovery. We have reported previously that 6 months after implantation of adrenal medullary allografts in hemiparkinsonian primates, apomorphine-induced turning is reduced by about 40% to 50%. A similar reduction in apomorphine-induced rotation oc-
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FIG. 7. Apomorphine (APO)-induced turning in a hemiparkinsonian monkey before (A) and 3 (B) and 7 months after (C) bilateral fetal mesencephalic tissue implants. CW = clockwise turning; CCW = counterclockwise turning. Each column represents 5 minutes. D and E: As a result of unilateral dopamine depletion caused by MPTP administration, dopaminergic receptor supersensitivity developed on the injured side. Apomorphine stimulation of these receptors caused the hemiparkinsonian monkey to turn away (counterclockwise on the chart) from the lesioned side. After implantation of the fetal mesencephalic tissue (black dots), dopaminergic activity on the implanted side reduced the level of supersensitivity and decreased apomorphine-induced turning.

curred in animals that received adrenal cortex or fat allografts; however, none of these animals regained permanent use of the MPTP-affected extremities. This contrasts sharply with the recovery of arm use and almost complete cessation of turning reported here for the fetal dopaminergic implant recipients. Within weeks after implantation, the animals regained use of their parkinsonian limbs, strongly suggesting restoration of dopaminergic activity in the host striatum. This functional improvement persisted for as long as the animals were allowed to live. The hemiparkinsonian monkey that received tissue from two fetuses initially improved to the same extent as the two hemiparkinsonian mon-
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FIG. 8. Photomicrographs of the hemiparkinsonian monkey that received mesencephalic implants from two fetuses. At the time of sacrifice, there were no tyrosine hydroxylase (TH)-immunoreactive cells or fibers present in the implants. This animal showed no long-term behavioral recovery. LV = lateral ventricle; CC = corpus callosum; CN = caudate nucleus; SP = septum. A: Overview photomicrograph. At this level, implant on the MPTP-treated side is present in the corpus callosum (CC). × 15, reduced 42%. B and C: Immunological rejection of both grafts. There were no TH-immunoreactive cell bodies within the grafts on the adjacent sections stained against TH (not shown). × 125, reduced 42%. D and E: Closer views of the lymphocytic infiltration. × 250, reduced 42%.

keys that received tissue from only one fetus, but at 3 months regressed to its preimplantation state.

Increased dopamine receptor density in the caudate nucleus and putamen on the MPTP-treated side of hemiparkinsonian monkeys probably accounts for the dopamine agonist-induced turning. Fetal implants effect a decrease in the density of dopaminergic D1 and D2 receptors in the caudate nucleus, as measured by specific agonist binding (unpublished data). This is consistent with downregulation of dopaminergic receptors by substantia nigra grafts in denervated rat striatum. Similarly, reduced apomorphine-induced turning after fetal implants in hemiparkinsonian monkeys can be attributed to diminution in supersensitivity of dopamine receptors in the striatum. It appears likely that both the reduction in supersensitivity and the functional recovery are related to enhanced dopaminergic activity following surgical cavitation and implantation of fetal dopaminergic tissue into the caudate nucleus.

Several sources for increased dopaminergic activity after the creation of cavities and implantation are possible. There are clearly TH-positive cells and fibers within the graft, but we observed no evidence that these fibers cross into the host caudate which makes synaptic connectivity unlikely. While it is possible that dopamine from the graft diffuses into the host striatum, radiolabeled dopamine diffuses a minimal distance from the tip of a cannula in the basal ganglia. Our observation of sprouted TH-positive fibers oriented from the ventral striatum toward the implant site indicates that these fibers may be a source of recovery of dopaminergic activity. Sprouting of dopaminergic neurons has been reported after implantation of adrenal medulla tissue into the MPTP-damaged striatum of mice and monkeys.

As previously shown, MPTP selectively injures the dopaminergic cells in the substantia nigra pars compacta while sparing the mesolimbic system originating from the ventral tegmental area. The surviving dopaminergic neurons in the areas spared from the toxic effects of MPTP appear to be the source of these
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FIG. 9. Photomicrographs from a hemiparkinsonian monkey implanted bilaterally with fetal mesencephalon. × 125. A: Right caudate nucleus and implant. H & E. B and C: Tyrosine hydroxylase (TH) immunohistochemistry of the areas marked on the H & E stain. The TH-immunoreactive cells with their processes were present 7 months after the implantation into the caudate nucleus. However, the fibers did not cross from the implant into the host caudate nucleus. In the host caudate nucleus below the implant, sprouted TH-immunoreactive fibers are oriented from the ventral striatum toward the implant.

dopaminergic fibers. In the animals that received fetal mesencephalic implants, both dopaminergic neurons in the graft and sprouted host dopaminergic fibers may contribute to the enhanced dopaminergic activity and associated behavioral improvement.

The monkey with bilateral parkinsonism that received a unilateral fetal mesencephalic tissue implant developed asymmetrical dopaminergic receptor responsiveness expressed by spontaneous and drug-induced turning. During spontaneous activity the animal turned away from the implanted side, suggesting that dopamine was being released from the side of the implant and activating receptors on that side. Furthermore, by stimulating release of dopamine, amphetamine caused turning away from the grafted side for over 12 hours. Enhanced dopaminergic activity on the implanted side presumably produces downregulation of the supersensitive receptors on that side. Apomorphine, by activating the residual supersensitive postsynaptic dopamine receptors on the nonimplanted side, causes turning toward the implanted hemisphere. Despite this turning asymmetry, this monkey had symmetrical bilateral recovery from rigidity and tremor. The sprouting of dopaminergic fibers, which infiltrate the caudate nucleus...
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FIG. 10. Color-coded images derived from autoradiographs of specific $^3$H-mazindol binding to dopamine uptake sites in the brains of a monkey with hemiparkinsonism after a fetal mesencephalic implant (upper) and from an unoperated monkey with hemiparkinsonism (lower). In the hemiparkinsonian monkey implanted with fetal mesencephalon (upper), in addition to high dopaminergic activity of the graft there is mazindol binding to the areas where dopaminergic sprouting was observed in the other implanted monkeys.

after adrenal medullary or nondopaminergic tissue allografts, after placement of adrenal autografts or cavities only or after fetal nondopaminergic implants suggests that a neurite-promoting factor released from the graft (or from the host tissue near the graft) may serve as a stimulus for this sprouting.

In addition to providing indications of the mechanism of functional recovery after implantation, other useful information was obtained from this study. 1) The relatively young fetus (32 to 42 days of gestation) was found to be a good source for dopaminergic tissue. Procurement of fetal mesencephalon obtained before completion of development of the dopaminergic projection from the substantia nigra to the neostriatum provides tissue in which the grafted dopaminergic cells survived and elicited behavioral recovery. 2) The transient behavioral recovery which occurred in the monkey implanted with tissue from two fetuses combined with the histological evidence of immunological rejection of the grafts in this monkey suggest using a single fetus as the source for tissue implants until the immunological aspects of central nervous system grafting are better understood. 3) The precavitation technique employed in this study offers two advantages for neuronal survival: first, within the first 2 weeks after tissue trauma, ingrowth of vessels creates a nutritive bed for the graft; and second, as shown in rodents, neurotrophic substances which enhance neuronal survival may be formed at the injured site. Histological observations in the current study indicated that the implanted tissue survived best at the periphery of the implant where the vascularity and trophic factors would have the greatest influence. This suggests that multiple small cavities and small grafts may permit optimal implant survival. 4) One possible disadvantage of precavitation and solid-tissue grafting is the development of gliosis at the interface between graft and host. Staining with antibody against GFAP revealed a dense pattern of reactivity along the cavity and surrounding the graft (not shown). The lack of dopaminergic fiber growth from the graft into the host caudate was perhaps in part due to glial reaction. However, it is also possible that the reactive host astrocytes play a beneficial role as they may be responsible for dopaminergic fiber sprouting from the host.

This study demonstrates that fetal dopaminergic im-

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plants into the caudate nuclei of MPTP-parkinsonian monkeys survive and induce significant clinical improvement for at least 6 months after transplantation. Since motor deficits in MPTP-treated monkeys require greater than 80% striatal depletion of dopamine, relatively small increments of dopamine can restore function. However, it is not clear at this point if the dopamine that is responsible for behavioral recovery is derived from the fetal dopaminergic graft, the dopaminergic sprouts derived from the host brain, or both.

References


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