Autologous transplantation of the superior cervical ganglion into the brain of parkinsonian monkeys

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The effect of autologous transplantation of the superior cervical ganglion (SCG) into the brain of parkinsonian monkeys was studied through quantitative measurement of animal behavior. The motor activity of the monkey was measured with a telemetry system during the experiment. After experimental parkinsonism was induced by repeated intravenous injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), three monkeys were treated with autologous transplantation of the SCG into both caudate nuclei. One monkey served as a control without SCG transplantation after MPTP treatment. Three SCG-transplanted monkeys showed biphasic (acute and chronic) behavioral amelioration of parkinsonism after transplantation. In the acute stage, the animals showed transient hyperkinesia with aggressive behavior and loss of circadian rhythm. In the chronic stage following acute hyperkinesia, the animals regained normal behavior and circadian rhythm without aggressiveness. In contrast with the transplanted monkeys, the control monkey failed to show recovery of the bradykinesia and muscle rigidity.

KEY WORDS • Parkinson's disease • transplantation • MPTP • superior cervical ganglion • monkey

It is well known that Parkinson's disease can be treated by systemic administration of L-3,4-dihydroxyphenylalanine (L-dopa) to replace dopamine in the nigrostriatal system. 14,28 This treatment depends on the fact that the nigrostriatal system executes its function through the tonic modulatory mechanism rather than conveying temporally or spatially patterned signals. 7,8 Clinical problems such as the diminution of the drug's effect, the on-off phenomenon, and/or the incidence of dyskinesia have occurred in patients with long-term administration of L-dopa due to desensitization of the dopaminergic receptors in the striatum. 3,4,24,25 These phenomena indicate that treatment for Parkinson's disease requires not only the substitution of the neurotransmitter but also the reconstruction of the neuronal circuit in the nigrostriatal system. 16,27 The substitution of dopamine from transplanted neural tissue may have greater benefit than simple pharmacological replacement because of its capability to be controlled by the patient's own neural system.

It has been reported that transplantation of embryonal nigral tissues into the brain with substantial lesions of the nigrostriatal dopamine pathway produced a new nigral dopamine input to the neostriatum and promoted recovery of functional animal behavior. 8,9,23 Clinical application of neural transplantation using human embryonal brain may be difficult because of ethical and immunological problems. We have reported transplantation of the autologous superior cervical ganglion (SCG) into the brain to circumvent these limitations and have demonstrated the extension of fibers from the graft to the striatum with a morphological method. 17 The present animal experiment was designed to quantify the behavioral change in experimental parkinsonism treated with transplantation of SCG cells in the striatum.

Materials and Methods

Four male monkeys (Macaca fuscata), weighing 3.1 to 7.6 kg, were used for this study.

Measurement of Motor Activity

A telemetric method was introduced to quantify the motor activity of the animals. Each monkey wore a jacket with a custom-made accelerometer device and an electrical transmitter connected to the device. * Electrical signals generated by the animal's movement were transported to a receiver outside the cage, and pulses triggered by the signals were summed for each 10 min-

* Electrical transmitter manufactured by Nihon-Kohden, Tokyo, Japan.
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utes during the experiment (Fig. 1). The mean number of pulses per hour over 24 hours was evaluated to indicate the time course of the motor activity of the animals.

**SCG Transplantation**

In order to simulate Parkinson's disease, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was intravenously injected into the monkeys. For intensive application of the drug, 1.0 mg/kg of MPTP was injected at 2- to 3-day intervals over 2 weeks in Monkeys 1, 2, and 4. For gradual drug application, MPTP (0.15 to 0.3 mg/kg) was injected at intervals of several days to 1 week over 2 months (Monkey 3). After a total dose of 1.0 to 3.0 mg/kg had been injected, the monkeys manifested bradykinesia, muscle rigidity, and occasional tremor of the head and trunk. Three animals (Monkeys 1, 2, and 4) were fed through a gastric tube because of severe bradykinesia and loss of appetite. After the activity of each animal’s movements decreased to 20% or less of that prior to treatment with MPTP, autologous SCG transplantation was performed in three monkeys (Monkeys 1, 2, and 3).

The SCG was microsurgically removed from one side under general anesthesia with a gas mixture of 1% to 2% halothane, 40% O2, and 60% N2O. After removal of its fibrous capsule, the SCG was cut into small pieces (1 cu mm in size) in sterile minimum essential medium, which were kept in the same solution for 20 to 30 minutes until transplantation. The animal’s head was fixed in a stereotactic apparatus under general anesthesia, and a skin incision and two bilateral burr holes were made. The small pieces of SCG were transplanted through a polyethylene cannula (1.6 mm in outer diameter and 1.3 mm in inner diameter) into the bilateral caudate nuclei (A 22 mm, L 2 mm, D 15 mm, according to the atlas of Kusama and Mabuchi). The method of SCG transplantation has been described in detail elsewhere. As a control animal, one monkey (Monkey 4) received no SCG transplantation after induction of parkinsonism by MPTP administration. Table 1 shows the experimental protocol used in this study.

### Results

**Effect of MPTP Injection**

The telemetry system employed to measure the activity of the animal’s behavior showed an apparent biphasic circadian rhythm of the normal monkey before MPTP injection (Fig. 2, graph 1). Of the three monkeys

![Figure 1](image1.png)

**FIG. 1.** Diagram of the telemetry system and the visual monitor employed in this experiment. The electromagnetically induced current signal is transmitted to the receiver, and triggered pulses are calculated by the computer system. VTR = videotape recorder.

![Figure 2](image2.png)

**FIG. 2.** Block graphs showing the change of spontaneous activity in Monkey 1 over 24 hours. **Graph 1:** Normal activity showing the circadian rhythm. **Graphs 2 and 3:** Activity decreased after large-dose rapid intravenous (i.v.) MPTP injections, with the disappearance of the circadian rhythm. **Graphs 4 and 5:** The activity increased to the normal level regaining the circadian rhythm after a transient hyperactive period following transplantation of superior cervical ganglion (SCG).

### Table 1

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Body Weight (kg)</th>
<th>Total Dose of MPTP (mg/kg)</th>
<th>Duration of MPTP Administration (days)</th>
<th>Gastric Feeding Tube</th>
<th>SCG Transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.1</td>
<td>3.0</td>
<td>9</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>7.6</td>
<td>1.0</td>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>6.2</td>
<td>2.9</td>
<td>57</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>3.1</td>
<td>2.5</td>
<td>5</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

*SCG = superior cervical ganglion. + = procedure performed; – = procedure not performed.*
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with rapid induction of parkinsonism (Monkeys 1, 2, and 4), two (Monkeys 1 and 4) manifested hyperactive behavior (the number of movements rose to 200% or more of the preinjection state) and loss of circadian rhythm several days after injection of one or two doses of MPTP. After the injection of successive doses of MPTP, all three monkeys revealed bradykinesia, a flexed posture, and muscle rigidity. The measuring system indicated a remarkably decreased amount of movements without circadian rhythm (Fig. 2, graph 3).

In the monkey with gradual induction of parkinsonism (Monkey 3), hyperkinetic behavior was not observed during the experimental course. The telemetry system indicated mild bradykinesia. As the serial injections of MPTP continued, activity gradually decreased and the monkey progressively lost appetite and showed the peculiar behavior of dropping food as if it had lost the normal ability to concentrate on the taking of food (sensory inattention). After the total dose of injected MPTP reached 2.9 mg/kg, the animal revealed severe bradykinesia, a flexed posture, and muscle rigidity, but it maintained a circadian rhythm of motor activity (Fig. 3, graph 2).

SCG Transplantation

Three monkeys (Monkeys 1, 2, and 3) were treated with SCG transplantation. In Monkey 1, after the animal was given three injections of MPTP and the activity of movement decreased to less than 20% of the preinjection state, the SCG was transplanted bilaterally into the caudate nuclei. This monkey showed a remarkable change in behavior, including rapid increase in activity 6 days after SCG transplantation. The increase in movement activity continued and overshot the normal level with disappearance of the biphasic circadian rhythm (Figs. 2 and 4). This hyperactive movement consisted of frequently alternating hypo-/hyperkinetic states (Fig. 2, graph 4) with the animal manifesting aggressive moods such as rage when facing researchers. Following this period of hyperactive behavior for about 10 days, the monkey gradually became calmer, losing its irritable mood. Four weeks after the SCG transplantation it regained normal behavior. The spontaneous activity of movements was normalized with the biphasic circadian rhythm (Fig. 2, graph 5). The bradykinesia, muscular rigidity, and flexed posture completely disappeared.

Monkey 2 suffered from severe akinesia following the injection of one dose of MPTP. Alimentation was maintained through a gastric tube because of the akinesia, muscular rigidity, and loss of appetite. The monkey showed only an incomplete recovery of behavior.

**FIG. 3.** Block graphs showing the change of spontaneous activity in Monkey 3 over 24 hours. Graph 1: Normal activity showing the circadian rhythm. Graph 2: Activity decreased after low-dose extended intravenous (i.v.) MPTP injections. Graphs 3 and 4: Activity gradually improved after transplantation of superior cervical ganglion (SCG).

**FIG. 4.** Graphs showing the entire course of spontaneous activity for each animal injected with MPTP in the present study. Monkeys 1 (Graph 1) and 2 (Graph 2) received larger doses in a short period, while Monkey 3 (Graph 3) was given smaller doses over an extended period of time. Superior cervical ganglion (SCG) transplantation was made in Monkeys 1, 2, and 3. Monkey 4 received no treatment for parkinsonism.
following SCG transplantation. Spontaneous movements transiently increased to 40%, but this eventually decreased to low levels (Fig. 4, graph 3). The circadian rhythm and the muscular rigidity remained unchanged.

In Monkey 3, the SCG was transplanted following the induction of parkinsonism by gradually administered MPTP. The spontaneous activity of movements slowly started to increase on the 5th day after transplantation, maintaining the biphasic circadian rhythm, and reached almost normal level by 4 weeks (Fig. 4, graph 3). The animal’s flexed posture, muscular rigidity, and sensory inattention had completely disappeared by the same time. Appetite recovered concomitantly with behavioral activity. No abnormal hyperkinetic behavior or hyper-/hypokinetic state was observed in this animal throughout the experiment.

Monkey 4, the control animal treated with MPTP administration without SCG transplantation, continued to show severe akinesia and muscular rigidity during the period of the experiment up to 5 weeks after MPTP injection (Fig. 4, graph 4).

Discussion

Parkinson’s disease may be treated by transplantation of various kinds of neural tissues since the grafted catecholamine-producing neurons are able to restore tonic and regulatory neurotransmission at denervated synaptic sites in the striatum. It has been reported that homologous transplantation of embryonic substantia nigra into the caudate putamen of rats subjected to 6-OHDA (hydroxydopamine) lesions in the nigrostriatal pathway induced recovery, as reflected in many behavioral tests after pharmacological activation as well as in spontaneous behavior. The transplantation of human fetal nigral tissue into the striatum of patients with Parkinson’s disease has also been reported to improve parkinsonism in combination with immunosuppressive therapy. However, clinical application of the human fetal tissue imposes immunological and ethical problems. To avoid these problems, autologous transplantation of the adrenal medulla has been applied in human Parkinson’s disease. However, except for a few reports, the efficacy of the transplantation has been unsatisfactory in most cases. We have transplanted autologous sympathetic ganglia into the monkey brain to ameliorate MPTP-induced parkinsonism, and our results indicate that the SCG is a favorable candidate for neural transplant; not only does SCG have considerable regenerative capacity, but it can also supplement both norepinephrine and dopamine in the brain suffering from Parkinson’s disease.

The telemetric method employed for measuring the motor activity of the monkeys was suitable to quantify the changes in movement in the present study. Thus, any minor changes in motor activity, circadian rhythm, and long-term course of movement were assessed in freely moving animals following MPTP treatment and SCG transplantation. The present study clearly showed by means of telemetry that SCG transplantation accomplished recovery from Parkinson’s syndrome induced by MPTP administration, in contrast to lack of recovery in the monkey without SCG transplantation.

Previous reports have shown that transplantation is effective in influencing the host brain function through several mechanisms: 1) trophic actions on the host brain; 2) diffuse release of hormones or transmitters; 3) reinnervation of elements in the host brain by the graft; 4) establishment of reciprocal graft-host connections; and 5) more complete integration into the host circuitry. There may be two possible mechanisms for the amelioration of MPTP-induced parkinsonism in the monkeys in this study. First, catecholamine released from surviving neurons in the graft affected dopamine receptors in the striatum of MPTP-induced parkinsonian monkeys whether or not the transplanted catecholamine terminals synapsed on target cells in the striatum. Our previous report has demonstrated that many transplanted catecholamine cells survived in the graft and extended their axons into the striatum of MPTP-induced parkinsonian monkeys. Homovanillic acid (a metabolite of dopamine) content was markedly elevated in the cerebrospinal fluid after SCG transplantation. These morphological and biochemical data suggest that a considerable amount of dopamine was released from the transplanted SCG tissue into the monkey brain.

Some monkeys revealed transient hyperactive movement with aggressive behavior after SCG transplantation. This abnormal behavior corresponded to the action of diffuse release of catecholamines from the grafted SCG neurons on the dopamine receptors in the striatum. Penn, et al., reported that most patients who received autotransplantation of the adrenal medulla experienced transient delusion during the first 2 to 4 weeks after surgery. This side effect is probably due to an excessive amount of catecholamine released from the transplanted medullary tissue. In the present study, the telemetry system clearly revealed the disappearance of the circadian rhythm after MPTP administration and its reappearance after SCG transplantation. This phenomenon indicates that an uncontrolled release of transmitters was produced by the destruction of catecholamine cells after MPTP injection and SCG transplantation. The reappearance of the circadian rhythm suggests that transmitters were released under the control of the host brain.

Secondarily, the trophic actions of the SCG graft may be a probable mechanism for improving parkinsonism in the monkey. Trophic factors released from therafted tissue or from the injured host brain may promote regeneration of nigrostriatal dopaminergic fibers in the host brain. Bohn, et al., reported that transplanted adrenal medullary tissues were not found in the striatum 5 weeks after transplantation, and they observed processes sprouting from the remaining dopaminergic fibers in the mouse striatum treated with MPTP. They concluded that the elevation of the dopamine content after transplantation resulted from the
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Sprouting of dopaminergic terminals promoted by transplanted adrenal medulla. Furthermore, Cooper showed that a stereotactic insult of the caudate nucleus resulted in recovery in humans with Parkinson’s disease. In the present study, autologous SCG was transplanted into the caudate nuclei. The transplanted SCG contained many Schwann cells which could produce potent neurotrophic substances. The trophic substances may promote regeneration of remaining nigrostriatal dopaminergic fibers in the MPTP-treated monkeys, which may be a possible mechanism for the amelioration of Parkinson’s syndrome in this experiment.

The present study indicated that the autologous transplantation of the SCG ameliorated induced Parkinson’s disease in experimental animals. It is suggested that this method may be clinically applicable to human Parkinson’s disease.

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References

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