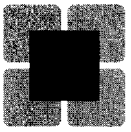

Fetal substantia nigra grafts

Effect on dopamine receptors in the rat corpus striatum

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■ Effects of fetal substantia nigra grafts on the dopamine receptors in the corpus striatum in rats were investigated after the destruction of the nigrostriatal dopaminergic pathways with intraventricular 6-hydroxydopamine injections. The expected dopamine receptor denervation supersensitivity was demonstrated by a 53.7% increase of [^{3}H] spiroperidol binding in rats with sham grafts compared with normal control-group rats. In contrast, rats with grafts showed a significant reduction of supersensitivity, with a 25% decrease in binding to the graft-bearing caudate when compared with the sham-graft group. A nonsignificant 15% decrease in binding on the nongrafted side was also observed. The fetal substantia nigra grafts thus reduced the denervation supersensitivity toward a normal level.

□ INDEX TERMS: CORPUS STRIATUM; RECEPTORS, DOPAMINE; SUBSTANTIA NIGRA; TRANSPLANTATION □ CLEVE CLIN J MED 1989; 56:259–262

MUCH RESEARCH has been devoted to transplanting fetal brain tissue into adult host animals. One of the most commonly studied systems is the dopamine-containing substantia nigra. Unilateral lesions of the rat substantia nigra eliminate the ipsilateral dopaminergic innervation to the corpus striatum, causing supersensitivity of dopaminergic neurons.[1–5] This supersensitivity is believed to be produced by an increase in receptor density. Amphetamine stimulates the release of dopamine from the intact striatum only, causing rotation or asymmetrical locomotion toward the side of the lesion.[2,5] Due to the diminished spontaneous dopamine release, postsynaptic neurons in the striatum become supersensitive to dopamine, as has been shown by electrophysiological studies, receptor binding assays, and measurement of adenylate cyclase activity.[3,6] Because of this supersensitivity, postsynaptic dopamine agonists such as apomorphine have a greater effect on the striatum ipsilateral to the lesion, resulting in agonist-induced rotation away from the side of the lesion.

■ *See the editorial by Sweeney (pp 287–289)*

Rotational behavior has been extensively employed as an animal model of nigrostriatal function. It has been repeatedly demonstrated that fetal substantia nigra grafts decrease apomorphine-induced rotation in animals with unilateral substantia nigra lesions and increase the concentrations of dopamine in parts of the striatum adjacent to the graft.[7–14] These grafts have been shown to be capable of re-establishing dopamine inner-

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vation of a host caudate, forming ultrastructurally normal synapses within a previously denervated corpus striatum.[8,15–17] In addition, graft-derived catecholamine axons appear to release dopamine within the host central nervous system.[7,13,17,18] However, only one previous study has investigated the effects of nigral grafts on dopamine receptors.[19] In this study, fetal substantia nigra grafts were found to restore dopamine receptor densities to normal levels in adjacent areas of the corpus striatum, demonstrated by light-microscopic autoradiography with [^{3}H] spiroperidol.

We used [^{3}H] spiroperidol homogenate binding to evaluate the effects of nigral transplants on whole striatal dopamine receptors.

MATERIALS AND METHODS

Randomly bred Norvegicus albino rats (weight, 175–250 g) were used to evaluate the effects of nigral grafts on dopamine receptors. For destruction of dopaminergic neurons, intraventricular 6-hydroxydopamine (OHDA) (250 μg/25 μL) was injected, with the rats under light ether anesthesia, at coordinates 1 mm lateral to the bregma and 3.5 mm below the dura, as described by Konig and Klippel.[20] The solution was made up just before use and kept on ice in order to retard auto-oxidation. The 6-OHDA, expressed as free base, was injected in 25-μL increments. Control animals were injected with an equal volume of the saline vehicle solution. Two weeks later, tyrosine hydroxylase activity was measured in the right corpus striatum of 10 denervated and six control rats for evaluation of drug effects.[21] The rats were rapidly decapitated, and their brains were quickly removed. The right corpus striatum was dissected away, weighed, and homogenized in 10 volumes of Tris-HCl buffer (50 mM, pH = 6.2). Tyrosine hydroxylase activity was measured in 0.05-mL homogenates by the radioenzymatic method described by Waymire et al.[22]

Four weeks after the 6-OHDA injection, a 2 × 3-mm cavity was made by suction unilaterally on the surface of the right caudate nucleus of 34 rats that were under thiopental anesthesia (45 mg/kg); this technique was explained by Stenevi et al.[23] Four to six weeks later, the cavity was re-opened and fetal nigral grafts were inserted in 20 rats; this became the "grafted group." These monoamine-containing ventral mesencephalon grafts were obtained from rat embryos of 17- to 18-day gestation, as described previously.[23–25] The remaining 14 denervated rats served as the "sham-grafted" group, undergoing all surgical procedures except graft insertion. Six vehicle-injected rats served as normal controls.

TABLE 1
THE EFFECT OF INTRAVENTRICULAR 6-OHDA ON TYROSINE HYDROXYLASE (TOH) ACTIVITY IN THE RIGHT CORPUS STRIATUM TWO WEEKS AFTER INJECTION

Group	n	Mean TOH values (nmol $^{14}CO_2$/mg tissue/h)	% control
Normal control	6	3.57 ± 0.05	100
Denervated (6-OHDA)	10	1.77 ± 0.08	49.4 ($P < .001$)

Two months after grafting, rats were rapidly decapitated, and their brains were quickly removed. The right and left corpora striata were dissected away separately, as described by Waddington et al.[6] Gross graft survival was determined by visual observation of the graft tissue filling the suctioned cavity. [^{3}H] spiroperidol binding was assayed in striatal membranes prepared by homogenizing striatal tissues in 10 volumes of ice-cold sucrose (0.32 M) using a Teflon-glass homogenizer, and the homogenates were centrifuged for 15 min at 18,000 g and 4°C. The pellets were then re-homogenized in a sufficient volume of 50 mM sodium/potassium phosphate buffer (pH = 7.4) to produce a concentration of approximately 10 mg tissue/mL. The homogenates were either used immediately or stored in aliquots at -20°C for up to three days. Saturable binding of [^{3}H] spiroperidol (28 Ci/mmol [103.6×10^{10} Bq/mmol]) was determined by incubating 0.2 mL of the striatal homogenate with increasing concentrations (0.1–7.5 nm) of the radioligand in a final volume of 0.25 mL of sodium/potassium phosphate buffer (pH = 7.4) 37°C. The incubations were terminated after 20 minutes by vacuum filtration through Whatman GF/B filters. The filters were washed three times with ice-cold buffer (3 mL) and placed in scintillation vials containing Aquasol-2 (4 mL). Radioactivity was determined at least three hours later in a liquid scintillation counter. Specific binding was taken as that portion of total binding not inhibited by 1 μM of haloperidol. K_d and B_{max} were determined by Scatchard analysis of bound v bound/free [^{3}H] spiroperidol concentration. B_{max} was expressed as fmol/mg tissue.

Sample data were expressed as mean ± standard deviation. Differences were tested using a two-tailed Student's t test. Significance was accepted at the $P < .001$ level.

RESULTS

Table 1 shows the effect of intraventricular 6-OHDA on tyrosine hydroxylase activity in the corpus striatum

TABLE 2
THE EFFECTS OF UNILATERAL SUBSTANTIA NIGRA GRAFTS ON THE [^{3}H] SPIROPERIDOL BINDING ON THE RIGHT (GRAFTED SIDE) AND LEFT (NONGRAFTED SIDE) CORPUS STRIATUM

Group	[^{3}H] spiroperidol (fentomol/mg tissue)	% of normal
Normal control (right side)	12.60 ± 3.50 (6)	100
Sham grafted (right side)	19.36 ± 3.30 (14)*	153
Grafted (right) side	14.46 ± 4.30 (20)	115
Nongrafted (left) side	16.52 ± 5.20 (20)	131

Results are given as mean ± standard deviation. Number of animals in each group are shown in parentheses.
*Differs from both normal control ($P < .001$) and grafted side ($P < .001$).

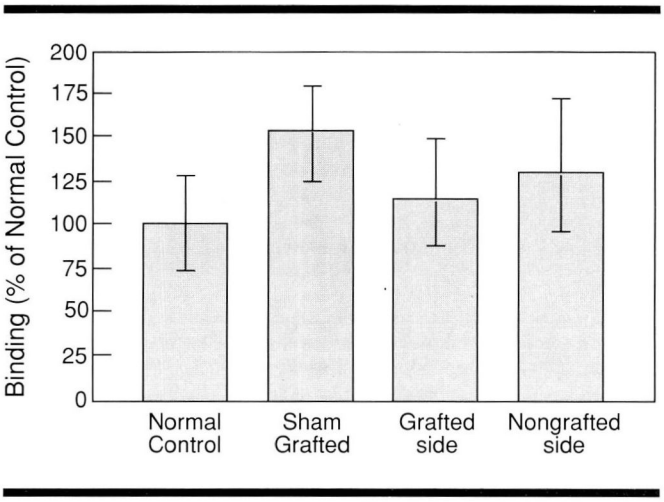

FIGURE 1. The effects of unilateral substantia nigra grafts on [^{3}H] spiroperidol binding in corpus striatum. The sham-grafted group was significantly different from either the normal control or grafted-side groups ($P < .001$).

two weeks after injection. There was 49.4% reduction of tyrosine hydroxylase activity in the right corpus striatum of the denervated rats compared to the normal control group ($P < .001$).

Two months after grafting, 80% of the grafts appeared grossly to have survived. [H^3] spiroperidol binding was measured in all rats of the grafted group, including those without grossly apparent surviving grafts. *Table 2* and *Figure 1* show the results and comparison of the measurement of [^{3}H] spiroperidol binding in the right and left corpora striata. In the normal control group, [^{3}H] spiroperidol binding on the right side was 12.6 ± 3.5 fmol/mg tissue. In the sham-grafted group, [^{3}H] spiroperidol binding to the right caudate was increased to 153% of normal controls ($P < .001$). The grafted group showed a reduction in [^{3}H] spiroperidol binding from 153% of normal to 115% and 131% on the grafted and nongrafted sides, respectively (*Table 2*). The binding on the grafted side was significantly lower ($P < .001$) than in the sham control group. Although the binding on the nongrafted side also showed a tendency to decrease when compared to the sham-grafted group, this difference was not significant.

DISCUSSION

Brain tissue transplantation has been used in several central nervous system areas to correct hormone deficiencies[25,26] or to reverse the effects of lesions produced by various methods.[7–11,13,14,16,27–30]

Although numerous investigators have performed many studies of the effects of substantia nigra grafts on drug-induced rotational behavior, dopamine reinnervation, and re-establishment of dopamine content, only one study has investigated the effects of nigral grafts on dopamine receptors.[19] In that study, intraventricularly placed fetal substantia nigra grafts were found to restore dopamine receptor densities to normal levels in adjacent areas of the corpus striatum, demonstrated by light microscopic autoradiography with [^{3}H] spiroperidol.

We used [^{3}H] spiroperidol homogenate binding to evaluate the effects of nigral grafts on dopamine receptors in the whole striatum. Two months after grafting, 80% of the grafts appeared grossly to have survived. In the denervated sham-grafted group, [^{3}H] spiroperidol binding in the striatum was increased 53% ($P < .001$). Several investigators have found a 15%–45% increase in receptor density in whole striatal homogenates after dopamine denervations.[1,31,32] These findings are consistent with our results. In grafted rats, binding on the grafted side decreased 25% from the supersensitive levels of the denervated sham-grafted group. On the nongrafted side, a smaller (nonsignificant) 15% decrease was also observed. These results demonstrate that the grafts produced a reduction toward normal of [^{3}H] spiroperidol binding in denervated striatum. By themselves, these results cannot support a conclusion about the mechanism by which the grafts reduced binding. However, other studies support our findings and show that reinnervation after substantia nigra grafting only takes place in adjacent areas.[14,19] For example, substantia nigra grafts placed in the ventricle usually reinnervate only the dorsomedial quadrant of striatum, as determined from concentrations of dopamine in punch

samples or from histochemical fluorescent studies.[17] Our results suggest that dopamine receptor binding changes occur over larger areas than the region actually reinnervated. This may be an explanation for the bilateral improvement seen in some patients with Parkinson's disease with unilateral adrenal medulla autografts.[33]

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