

Functional Compensation by Transplantation of Cell Suspensions of Embryonic Mesencephalon into the Striatum of Rats with 6-Hydroxydopamine Lesions

Hiroshi WATANABE, Yukitaka HIYAMA*, Hiroshi SUDA,
Shun-ichi SEKIHARA and Katsutoshi TERASAWA*

Research Institute for Wakan-yaku and *University Hospital, Toyama
Medical and Pharmaceutical University, Toyama 930-01, Japan

Accepted January 16, 1986

Abstract—Neuronal cell suspensions were implanted into the striatum of female rats that showed apomorphine-induced rotation and a reduction in striatal dopamine after intranigral treatment with 6-hydroxydopamine. The apomorphine-induced rotation was significantly suppressed by the transplantation in 12 out of 64 rats. DOPA accumulation and dopamine level (6.3 and 3.4%, respectively, compared with those of uncompensated rats) in the striatum following treatment with an amino acid decarboxylase inhibitor were slightly restored in compensated rats.

Transplantation of neuronal tissue pieces to the brain has been achieved with a high degree of success (1), although it limits the ability to reinnervate many targets located in ventral areas of the brain. To overcome the limitation, a procedure for intracerebral grafting of neuronal cell suspensions was introduced recently (2, 3). We investigated the reliability of the method for the transplantation of cell suspensions of embryonic mesencephalon into the striatum with 6-hydroxydopamine (6-OHDA) lesions.

Female rats of the Wistar strain weighing 150–180 g were stereotaxically injected with 6-OHDA (8 μ g in the base) into the right substantia nigra in a volume of 4 μ l at the rate of 1 μ l/min (4). The stereotaxic coordinates of the injection site were A: 2.4, L: 1.8, H: –2.5, according to the rat brain atlas (5). They were given apomorphine hydrochloride (0.1 mg/kg in the salt, s.c.) to determine rotational behavior once a week after 6-OHDA treatment and transplantation of neuronal cell suspensions. Embryos were removed from the mother at 18–20 days of gestation. Ten to twelve tissue pieces of mesencephalon were incubated at 37°C for 20 min in 200 μ l sterile saline containing 0.6% D-glucose and 0.1% trypsin. The trypsin was washed off,

and the tissue was suspended by repeated pipetting with a Pasteur-pipette as described by Schmidt et al. (3). Five microliters of a suspension were injected with a Hamilton syringe into the striatum of a rat which showed constant rotation after apomorphine. The injection site was selected in the striatum to accomplish the transplantation in a short period. The stereotaxic coordinates were A: 8.0, L: 3.0 and H: +1.0, according to the rat brain atlas (5).

Some of rats were given 100 mg/kg of *m*-hydroxybenzylhydrazine (NSD-1015) and 30 min later sacrificed by decapitation at 12 weeks after the transplantation. Striatal DOPA and catecholamines were extracted with the alumina method and assayed using HPLC with electrochemical detection (6). [³H]-Spiperone binding to striatal membranes was estimated according to the method of Sibley et al. (7). Specific binding was defined as that displaced by 10 μ M of (+)-butaclamol, and 1 μ M of ketanserin was used to remove 5-HT₂ receptor binding. For the fluorescence histochemistry, the striatum was freeze-dried, treated with paraformaldehyde gas, embedded in paraffin and observed using a fluorescence microscope according to the previously report method (8). Apomorphine

hydrochloride (Dainippon Seiyaku), 6-OHDA hydrobromide (Sigma Chemical Co.) and NSD-1015 (Sigma) were dissolved in sterile saline containing 0.03% ascorbic acid. Trypsin (Sigma) was dissolved in sterile saline. [^3H]-Spiperone (26.4 Ci/mmol) was purchased from Amersham Japan. The chemicals used for HPLC assay were of analytical grade obtained from commercially available sources.

In the present study, we determined the total number of rotations induced by apomorphine to estimate functional recovery of lesioned striatum. Twelve rats (compensated group) out of 64 rats implanted with neuronal cell suspensions showed 40–86% decrease in apomorphine-induced rotation, and 52 rats (uncompensated group) showed no significant decrease in rotation on the seventh day of the implantation (not estimated until the day). Five control rats injected with suspension medium showed no significant changes in drug-induced rotation (data not shown). As shown in Fig. 1, the decreasing effect of the implantation on drug-induced rotation continued through at least 16 weeks. On the contrary, rats that showed no decrease in rotation exhibited rather an increase of the behavior through 12 weeks after the implantation.

Levels of DOPA and dopamine for the lesioned striatum of the uncompensated group were 9.6 and 1.4%, respectively, of those observed for the unlesioned striatum.

In the compensated group, those levels for the lesioned striatum were slightly restored; 15.9 and 4.8% of DOPA and dopamine levels, respectively, for the unlesioned striatum (Table 1).

[^3H]-Spiperone B_{max} values for lesioned striatum (776 ± 15 fmol/mg protein) were significantly greater than those observed for unlesioned striatum (542 ± 17 fmol/mg protein, mean \pm S.E. of three separate experiments) in uncompensated rats. The difference

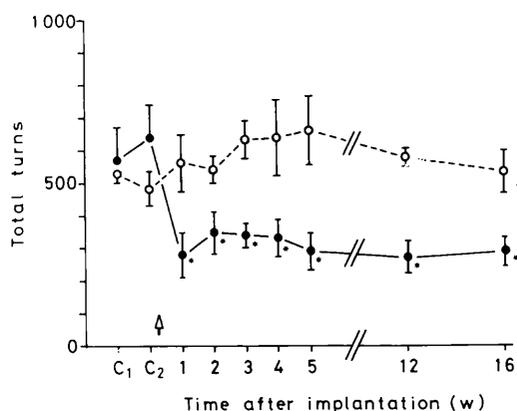


Fig. 1. Effect of intrastriatal transplantation of neuronal cell suspensions on apomorphine-induced rotation in unilateral 6-hydroxydopamine-lesioned rats. C₁ and C₂: Two control measurements prior to the implantation. Group: ○-----○ (uncompensated) and ●-----● (compensated). Each point represents the mean \pm S.E. of four animals. * $P < 0.01$ vs. C₂ value (paired t -test).

Table 1. 3,4-Dihydroxyphenylalanine (DOPA) and dopamine levels in the striatum implanted with neuronal cell suspensions following treatment with an amino acid decarboxylase inhibitor, *m*-hydroxybenzylhydrazine

Group	Side	N	DOPA (ng/mg)	Dopamine (ng/mg)
Naive control		6	1.97 \pm 0.12	14.16 \pm 0.48
Uncompensated	Unlesioned	4	1.77 \pm 0.14	12.62 \pm 0.91
	Lesioned	4	0.17 \pm 0.04* (9.6)	0.18 \pm 0.04* (1.4)
Compensated	Unlesioned	4	1.64 \pm 0.10	14.33 \pm 0.35
	Lesioned	4	0.26 \pm 0.02* (15.9)	0.69 \pm 0.02* (4.8)

Twelve weeks after the transplantation, animals were treated with *m*-hydroxybenzylhydrazine (100 mg/kg, i.p.) and sacrificed 30 min later. Values represent the mean \pm S.E. N: number of animals. Values in parentheses indicate percent of unlesioned striatum. * $P < 0.01$ vs. unlesioned striatum (Student's t -test).

in [³H]-spiperone B_{max} values for lesioned versus unlesioned striatum was not modified by the implantation in the compensated group. Fluorescent DA neurons in surviving implants were found in the compensated rat as a few scattered neurons along the needle track within the host striatum, but not found in the uncompensated rats.

Present study corresponds to the fact that the transplantation of neuronal cell suspensions compensates function of the striatum for 6-OHDA lesions with a slight restoration of dopamine levels and local reinnervation of dopaminergic neurons (9). It has been reported that the rate of rotation produced by apomorphine does not reflect the compensation as compared with amphetamine test (10), while our data have shown that total number of rotations induced by apomorphine can be used for evaluation of functional compensation of the transplantation in the striatum. We found no reduction in increased DA receptors that is responsible for functional compensation estimated by the administration of apomorphine. The reason for this discrepancy is unclear, but it may be related to a little change if any, since the reinnervated portion of the striatum appears to be extremely restricted when compared with a piece of the striatum homogenized for the receptor binding study. Data presented here have also shown that functional compensation appears a week earlier than that reported previously (10). The reliability of the transplantation, however, seems to be relatively low in the present experimental condition.

Acknowledgement: The author is grateful to Prof. Dr. Y. Nomura for his advice on receptor binding. This work was supported in part by a Grant-in-Aid (No. 59570939) for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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