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DOPAMINE-RICH GRAFTS IN THE NEOSTRIATUM AND/OR NUCLEUS ACCUMBENS: EFFECTS ON DRUG-INDUCED BEHAVIOURS AND SKILLED PAW-REACHING

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Abstract—This study compares the behavioural efficiency of dopaminergic mesencephalic neurons implanted into the rat neostriatum and/or the nucleus accumbens. The dopaminergic mesotelencephalic pathway was unilaterally destroyed by injection of 6-hydroxydopamine into the medial forebrain bundle at the level of the lateral hypothalamus. Three weeks later, embryonic dopaminergic mesencephalic neurons were implanted into the denervated neostriatum, or the nucleus accumbens or into both locations (double grafts). All animals were tested over a four month period for amphetamine- and apomorphine-induced rotation, apomorphine-induced locomotor activity, and on a skilled paw reaching task.

The characteristic ipsilateral rotation induced by amphetamine observed in lesioned animals was significantly reduced by neostriatal and double grafts, but persisted in animals with grafts in the nucleus accumbens alone. Four months after grafting, an overcompensation of rotation was observed for the neostriatal and double grafted animals, which now rotated contralaterally, i.e. away from the grafted side. The rotation induced by apomorphine in lesioned rats was decreased by neostriatal and double grafts and to a lesser extent by grafts implanted into the nucleus accumbens. Apomorphine-induced locomotor hyperactivity in lesioned animals was ameliorated by the nucleus accumbens and by double grafts. In the paw-reaching task, lesioned animals showed severe impairment in the use of the contralateral limb, which none of the grafts alleviated. Pretreatment with amphetamine had variable effects on the paw-reaching task which persisted in subsequent drug-free trials, suggesting that a conditioning mechanism may be involved.

These findings suggest that the simultaneous reinnervation of the neostriatum and the nucleus accumbens by dopaminergic transplants is not sufficient to re-establish normal function in more complex behavioural tasks.

The functional effects of dopaminergic-rich (DA) grafts implanted into the CNS have been widely studied. It has become apparent that although DA grafts can have beneficial effects compensating many deficits induced by a DA lesion,[2,4,6,8,16–23,25–28,30,31,39] they do not compensate all components of the post-lesion behavioural syndrome[2,13,18–21,28,35] and may even produce deleterious effects.[2,12,22,26,28,31] It has been hypothesized that the lack of complete functional recovery may be due to the persisting disconnection of the nigrostriatal circuitry even though DA grafts, implanted in an ectopic location, provide extensive terminal innervation in the neostriatum itself.[10] Alternatively, as the reinnervation of the host tissue by DA grafted neurons is limited to the transplanted areas,[1,9] several target structures of the DA systems, deprived of their afferents following the DA lesion, are not reinnervated by the transplants. It has been hypothesized that the different DA systems act in a coordinated manner, their terminal fields being functionally interdependent.[33] According to this hypothesis, the limited functional recovery brought about by DA grafts could be linked to the imbalance between the activity of different DA terminal fields, resulting from the absence of reinnervation by the DA grafted neurons of telencephalic structures denervated by the lesion.[28]

We have addressed this issue using behavioural tests in which DA grafts have already been shown not to have compensated for post-lesion deficits. In particular, it is well known that the unilateral lesion of the DA mesotelencephalic pathway disrupts use of the contralateral forelimb.[37,42,49] This deficit is not compensated by DA grafts implanted in the neostriatum.[23,37] Recently, Mandel and colleagues[36] have suggested that lesions of the nucleus accumbens could also be important in paw-reaching deficits as the neostriatum and nucleus accumbens work in synchrony during the performance of this task. Thus, DA depletion of both structures was found to yield greater deficits than DA depletion of the neostriatum alone. The authors suggested that the failure of earlier studies to restore skilled paw-reaching could be a consequence of the absence of the simultaneous reinnervation in the nucleus accumbens and the neostriatum by DA implants.[36]

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Abbreviations: DA, dopaminergic; 6-OHDA, 6-hydroxydopamine; TH, tyrosine hydroxylase.

The present experiment has been designed to evaluate the functional effects of embryonic grafts implanted into either the neostriatum or the nucleus accumbens, or both, on paw-reaching behaviour. Additionally, other drug-induced behavioural tests, such as rotation and locomotor activity, have been included to evaluate the efficacy of grafts in the striatum and nucleus accumbens respectively.[19,20,26]

EXPERIMENTAL PROCEDURES

Animals

Forty-five young female rats of the Sprague–Dawley strain (C.F.Y., Huntingdon) were used, weighing 160–220 g at the start of the experiment. They were housed in groups of four to six per cage in a colony room, under a natural light–dark cycle with *ad libitum* access to food and water.

Lesion surgery

Lesions were made on the hemisphere contralateral to the preferred limb, as designated by preoperative paw-reaching ability (see below). The DA mesencephalic system of 35 rats was destroyed unilaterally by the stereotaxic injection of 6-hydroxydopamine hydrobromide (6-OHDA, Sigma) into the medial forebrain bundle, under equithesin anaesthesia (3 ml/kg). The neurotoxin was dissolved in isotonic saline containing 0.01% ascorbic acid (pH 4) at a concentration of 4 μg/μl. The solution (1.5 μl) was injected at each of the two sites along the anteroposterior axis, via a stainless steel cannula. Stereotaxic coordinates were anterior AP 0 and −1 mm behind bregma, lateral L ± 1.6 mm from the midline, vertical V −8.6 mm below the level of the skull, with incisor bars set 5 mm above the level of the interaural line. After completion of the injection, the cannula was left in place for an additional 2 min to allow diffusion of the neurotoxin away from the injection site. Ten control sham animals received an injection of vehicle alone under the same conditions as previously described.

Transplantation surgery

Three weeks after lesioning, 25 lesioned animals received DA grafts, implanted as a dissociated cell suspension into the host striatum and/or nucleus accumbens.[9] Briefly, DA-rich tissue was obtained from 62 embryos (crown–rump length 13 mm, approximately 14 days gestational age) from pregnant females of the same inbred strain. The ventral mesencephalic tissues were dissected out in isotonic saline. The tissue pieces were collected in two batches (32 and 30 embryos per batch) in sterile isotonic saline containing 0.6% of D-glucose, then incubated in 0.1% trypsin at 37°C for 20 min, washed and mechanically dispersed to form a cell suspension. The suspensions were prepared in a final volume of one embryonic piece per 6 μl of glucose saline. One embryo per host was used. They were injected with 1.5 μl of cell suspension at each of the two sites for the neostriatal and accumbens grafts, and with 1.5 μl at each of four sites for the double grafts. Stereotaxic coordinates were as follows, with incisor bars set 2.3 mm below the interaural line:

Striatal grafts	($n = 9$)	A + 1.2	L ± 3.0	V − 4.5
		A 0.0	L ± 3.5	V − 5.0
Accumbens grafts	($n = 8$)	A + 2.2	L ± 1.4	V − 7.0
		A + 1.2	L ± 1.4	V − 7.4
Double grafts	($n = 7$)	A + 1.2	L ± 3.0	V − 4.5
		A 0.0	L ± 3.5	V − 5.0
		A + 2.2	L ± 1.4	V − 7.0
		A + 1.2	L ± 1.4	V − 7.4

Using the same procedure and coordinates, 10 sham and 11 lesioned animals received a vehicle injection into the striatum and/or nucleus accumbens.

The following abbreviations are used for the different groups: SH, sham animals; LE, lesioned animals; ST-G, DA grafts in the neostriatum; AC-G, DA grafts in the nucleus accumbens; ST.AC-G, DA grafts in the neostriatum and the nucleus accumbens.

Behavioural tests

Turning behaviour. In order to evaluate the extent of the lesions, turning behaviour induced by D-amphetamine (2.5 mg/kg, i.p.) was studied one week after surgery. Animals were placed for 1 h in automatically recording rotometer bowls[48] immediately following drug injection and the number of full 360° turns/min over 1 h was recorded by an on-line connection to a microcomputer. Rotations in ipsilateral and contralateral directions were recorded separately, but all analyses are based on the net (ipsilateral minus contralateral) scores.

To assess the functional viability of the grafts, amphetamine (2.5 mg/kg, i.p.) and apomorphine (0.1 mg/kg, s.c.) induced rotation activity was measured two months (amphetamine, apomorphine) and four months (amphetamine) after grafting. Rotational activity was measured either over 300 min (amphetamine) or a 60 min period (apomorphine).

Locomotor activity. Two months after grafting, locomotor activity was studied in activity cages which record the number of interruptions of an infrared photocell beam over a set time period. Locomotor activity was recorded for 60 min after an injection of saline, and then for a further 120 min following an injection of apomorphine (0.1 mg/kg, s.c.).

Skilled paw-reaching: test apparatus. Paw-reaching was measured using a modified version of the "staircase" test apparatus described by Montoya and colleagues.[37,38] The apparatus consists of a clear Perspex chamber (203 mm long × 108 mm high × 103 mm wide) with a hinged lid, into which a rat is placed. Leading off from this chamber is a narrower compartment (165 mm long × 108 mm high × 60 mm wide) with a central platform running along its length, creating a 19 mm wide trough on either side. The narrowness of the chamber prevents rats from turning around, so that the left paw can only reach into the left trough, and the right paw only into the right trough. The top surface of the platform, 35 mm wide, overhangs the sides so that rats cannot scrape food pellets up the side of the platform. A removable double staircase is inserted from the end of the box into the troughs to either side of the central platform. Each of the eight steps of the staircase contains a small 3 mm deep well into which two 45 mg chow pellets (Custom Biological Inc.) are placed. The highest step of the staircase is 13 mm and the bottom step is 64 mm below the platform. The animal can retrieve the pellets by reaching down into the trough and the number of steps from which pellets are removed provides an index of reaching ability.

Procedure. Paw-reaching was studied before lesion surgery and between two and three months after grafting. Animals were food-deprived to 90% of their free-feeding weight and were maintained at this weight during the period of testing. Two chow pellets were placed in each well of the double staircase. Rats were placed in the test boxes for 15 min. At the end of each session the staircases were removed, and the number of food pellets remaining on each side of the apparatus was recorded.

Pre-lesion procedure. Training extended over a period of two weeks, consisting of 17 trials in total. The data from the last five trials were used to match animals on the basis of both skill and any natural paw bias. The "preferred" paw was defined for each rat as the paw with which each rat obtained more pellets. Each animal was then assigned to one of the five groups so that average performance (on either paw) did not differ significantly across the groups. The lesions were conducted on the hemisphere contralateral to the preferred paw. As a result of surgery on the contralateral side, in post-operative tests the "preferred" paw will then be described as the "experimental" paw, and

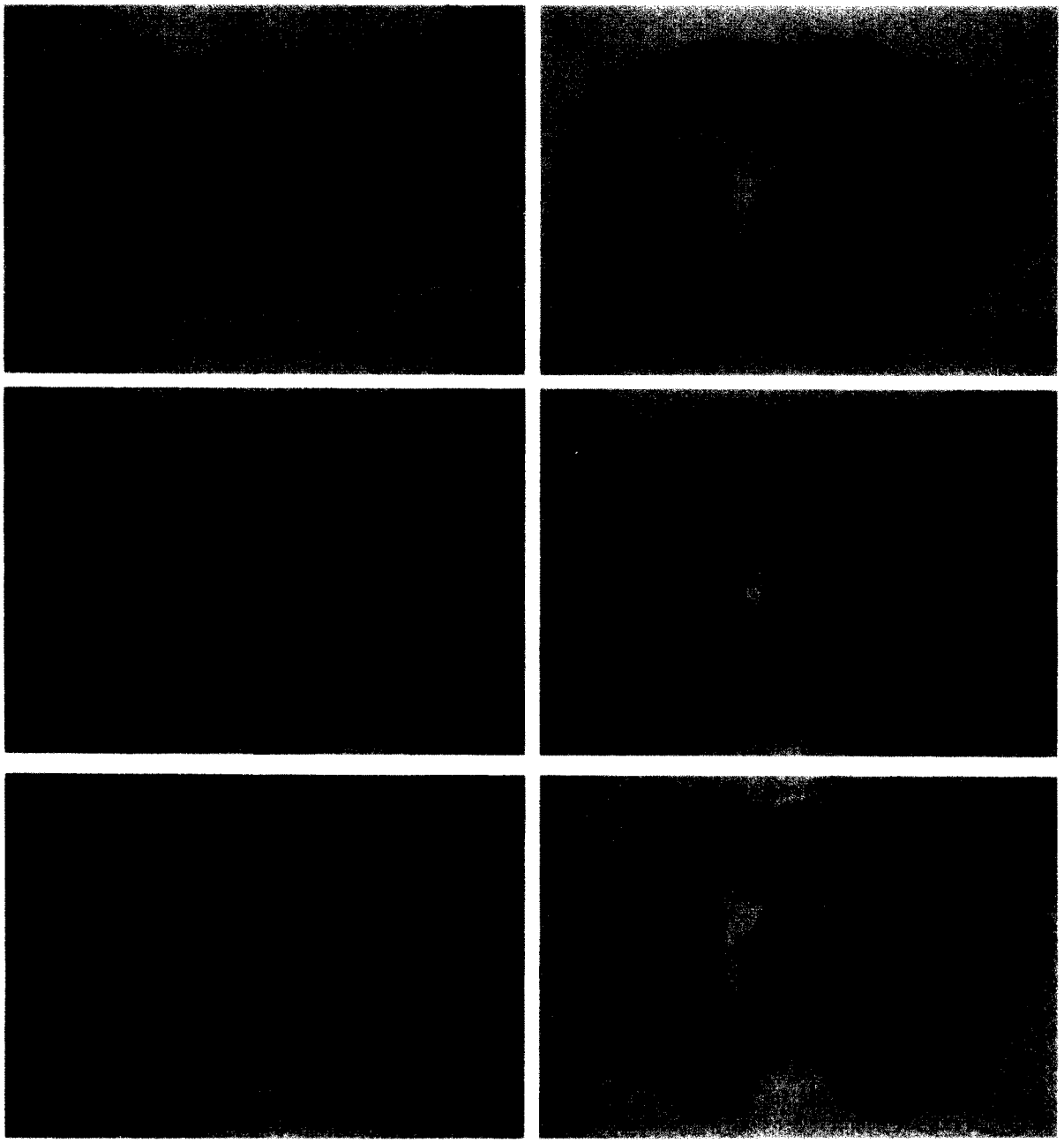

Fig. 1. Low magnification photomicrographs of tyrosine hydroxylase immunohistochemistry of sections from lesioned and grafted animals. (a) Mesencephalon of a lesioned rat. (b) Striatal region of a lesioned rat. (c) Striatal region of a neostriatum grafted animal. (d) Striatal region of an accumbens grafted animal. (e) Striatal region of a double grafted animal: DA neurons grafted in the nucleus accumbens. (f) Striatal region of a double grafted animal: DA neurons grafted in the neostriatum. Scale bars = 800 μm.

the "non-preferred" as the "intact" paw which is under the control of the intact hemisphere.

Post-graft procedure. Testing was recommended two months after grafting, following the drug-induced rotation and locomotor activity tests. Animals received 18 daily tests according to the pre-lesion procedure. The effects of D-amphetamine on performance were then investigated over a further 13 daily trials in which the drug was injected 10 min before paw-reaching was tested. Increasing doses of D-amphetamine were used (0.1, 0.2, 0.4 and 0.8 mg/kg, s.c.), interspersed with days of either isotonic saline injections (0.1 ml/100 g, s.c.) or no injections at all. The 0.8 mg/kg and 0.2 mg/kg doses were then repeated, to test the consistency of any effects.

Histology. Animals were killed for immunohistochemical analysis four months after the grafting procedure, after completion of behavioural testing. Under general barbiturate anaesthesia, they were perfused transcardially with 50 ml phosphate-buffered saline (pH 7.2) containing 5×10^4 IU/ml heparin, followed by 300 ml of 4% paraformaldehyde in 0.1 M phosphate buffer containing 14% saturated picric acid solution buffered to pH 7.4. After a 24 h post-fixation period in the same fixative, 50-μm-thick coronal sections were cut using a Vibratome

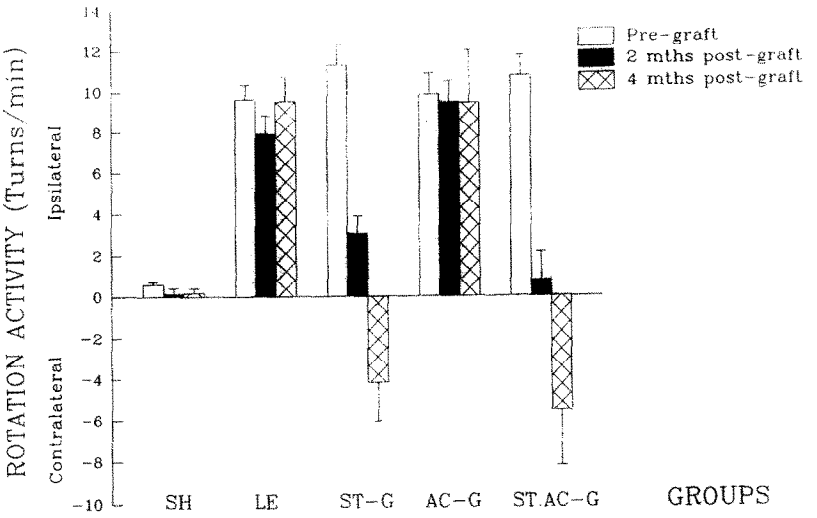

Fig. 2. Rotation activity induced by D-amphetamine (2.5 mg/kg) before and two and four months after grafting. Pre-graft and post-graft results are expressed as mean rotation rate (turns/min, mean ± S.E.M.) calculated over 1 h.

(Oxford Instruments). Free-floating sections were processed through a standard immunohistochemical procedure[5] to demonstrate tyrosine hydroxylase (TH, used at 1:5000 dilution; Boy, Paris, France). TH-positive staining was visualized by the biotin–streptavidin technique (ABC kit, Dako Labs, Glostrup, Denmark) using 3,3′-diaminobenzidine as the chromogen.

Data analysis

All data were analysed using a multifactorial analysis of variance, with groups as the single between-subjects factor, and test and/or time as additional within-subject factors appropriate to the particular behavioural measure. In cases where there was a significant interaction, groups were compared using the Newman–Keuls, Dunnett and Sidak tests to correct for multiple comparisons dependent on the particular comparison to be made.[45,51]

RESULTS

Immunohistochemistry

The extent of the lesion was determined using an antibody against TH, to show the loss of TH-positive (presumed DA) neurons in the mesencephalon. As shown in Fig. 1a, no surviving TH-positive neurons were observed in the substantia nigra on the lesioned side. Within the ventral tegmental area, a few small round TH-positive cells were visible. Correspondingly, there was an almost total loss of TH-positive fibres in the lesioned striatum and nucleus accumbens of lesioned rats (Fig. 1b). Thus, by histochemical criteria, all animals used in the experiment were considered to have received effective lesions.

All grafts contained numerous TH-positive neurons, grouped at the host–graft interface or within the graft itself (Fig. 1c–f). When the suspension was injected in the nucleus accumbens, a few DA neurons were in some cases observed along the needle tract and in the medial part of the striatum lining the ventricle, probably as a result of diffusion along the injection cannula. In three animals in the AC-G group, a small cluster of TH-positive neurons was found in the corner of the neostriatum, under the corpus callosum and lining the ventricle (Fig. 1d). Grafts implanted into the neostriatum or the nucleus accumbens gave rise to a rich TH-positive reinnervation of the surrounding host tissues (Fig. 1c, d). The diffusion of DA cells from accumbens grafts back up into the medial striatum was accompanied by an additional fibre reinnervation of the anteromedial striatum (Fig. 1d). However, the central and lateral parts of the neostriatum in these cases remained denervated, in contrast to rats with striatal grafts. In the double graft animals, TH-positive fibres were present both in the nucleus accumbens and in the neostriatum (Fig. 1e, f).

Turning behaviour

Amphetamine-induced rotation. Amphetamine-induced rotation was measured after the lesion and after grafting. The results of pre- and post-graft

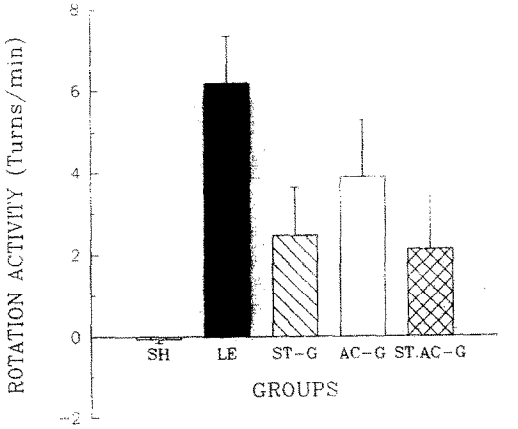

Fig. 3. Rotation activity induced by apomorphine (0.1 mg/kg). Results are expressed as mean rotation rate (turns/min, mean ± S.E.M.) over 1 h.

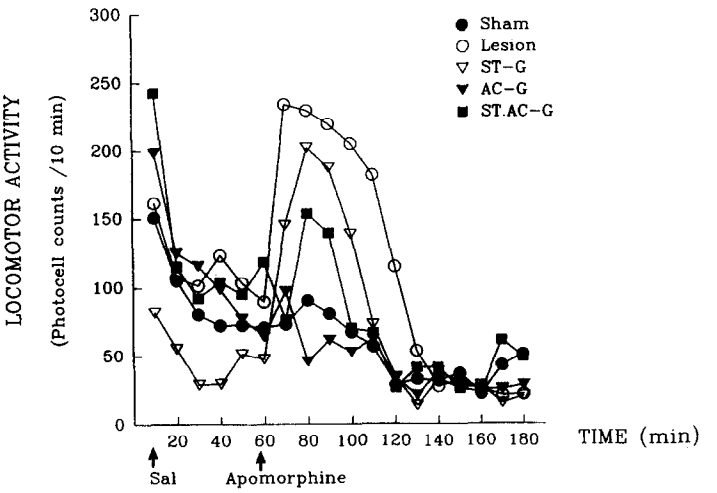

Fig. 4. Time course of the locomotor activity induced by apomorphine (0.1 mg/kg). Each point represents the mean score over 10 min.

rotation activity are shown in Fig. 2. The administration of amphetamine had no effect on the rotation activity of the sham group. In contrast, before grafting, amphetamine induced ipsilateral rotation in all four lesioned groups [Group effect: $F(4,39) = 30.38$, $P < 0.001$; Newman–Keuls: SH < LE at $P < 0.01$, LE = St-G = AC-G = St.AC-G].

After grafting, animals with grafts in the nucleus accumbens rotated toward the lesioned side as did the animals with lesions. In contrast, two months after grafting, the ipsilateral rotation observed after the lesion was significantly decreased in both the neostriatal (ST-G) and double (ST.AC-G) grafted rats. Four months later, an overcompensation was observed for ST-G and ST.AC-G groups as animals rotated contralaterally, i.e. away from the grafted side [Group × Time interaction: $F(8,78) = 17.82$, $P < 0.001$; SH, ST-G, ST.AC-G < LE at $P < 0.01$]. The time course of amphetamine-induced circling activity was similar for these two experimental groups [$F(24,336) = 0.60$, $P > 0.05$]

Apomorphine-induced rotation. The effect of apomorphine on circling activity after grafting is shown in Fig. 3. Sham operated animals displayed no significant rotational activity following the injection of drug. In contrast, apomorphine induced marked contralateral rotation in the lesioned animals, which was alleviated to varying degrees by the grafts. Grafts implanted in the nucleus accumbens slightly reduced contralateral rotation induced by apomorphine, whereas both neostriatal and double (ST.AC-G) grafts provided a greater reduction. However, the compensation was partial as the circling activity for ST-G and ST.AC-G groups was higher than that observed for the sham group [Group effect: $F(4,40) = 12.52$, $P < 0.001$; LE, SH > ST-G = ST.AC-G at $P < 0.01$; LE > AC-G at $P < 0.05$; ST-G = ST.AC-G > SH at $P < 0.05$; AC-G > SH, $P < 0.01$].

Apomorphine-induced locomotor activity. The results of the apomorphine-induced locomotor activity test are shown in Fig. 4. Locomotor activity after saline injection was similar in all experimental groups [$F(4,40) = 1.93$; $P > 0.05$]. Apomorphine increased the locomotor activity of lesioned rats, over a 1 h time period, compared with the effect of the drug in control animals. This locomotor activity was partially or totally compensated in the grafted groups. An analysis of variance indicates no significant effects in the last 60 min period [Group effect: $F(4,40) = 0.73$, $P > 0.05$; Group × Time bin interaction: $F(20,200) = 0.99$, $P > 0.05$]. However, in the 60 min immediately after apomorphine injection, marked differences between groups emerged [Group effect: $F(4,40) = 12.50$, $P < 0.001$; Group × Time bin interaction: $F(20,200) = 2.26$, $P < 0.01$]. Subsequent Dunnett tests indicated the lesion group to be more active than the shams at all time points between 10 and 60 min after apomorphine injection ($P < 0.01$). In contrast, the overall scores of all three graft groups indicated a significant compensation of hyperactivity. The nucleus accumbens graft group was fully compensated to control levels at all time points. The striatal graft group remained significantly hyperactive with respect to the shams over the first four time bins (bins 10 and 40, $P < 0.05$; bins 20 and 30, $P < 0.01$), and the double graft on bins 20 and 30 ($P < 0.05$).

Skilled paw-reaching

Spontaneous. The pre- and post-surgery performance on the staircase test, for both the experimental and intact paws, is shown in Fig. 5. During the training stage of the experiment, all animals learned the task quickly and reached asymptotic performance within five to 10 days. Subsequent analyses are based on performance levels during the last five days of testing once a stable level of performance was maintained. The five groups did not differ significantly in their reaching ability with either paw [Group × Paw interaction: $F(4,40) = 0.9$, $P > 0.05$].

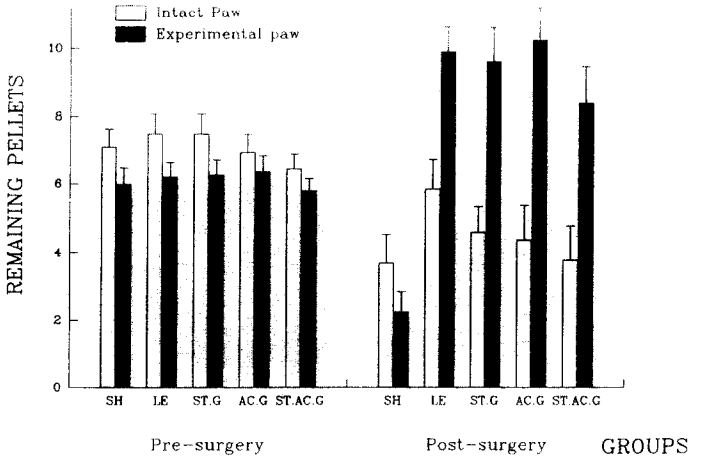

Fig. 5. Skilled paw-reaching before and after surgery. Results are expressed as the average number of pellets remaining in the staircase test over the last five trials.

After the lesion and the graft procedures, a clear separation in the performance of the two limbs was visible. Animals in all groups continued using their intact paw to reach the food with equal efficiency. Indeed, further improvements in performance were seen on the intact side in all rats after surgery, in spite of extensive pre-surgery training. In contrast the lesions induced a marked deficit in reaching with the contralateral paw. This deficit was not compensated by grafts implanted into the neostriatum and/or the nucleus accumbens.

Analysis of variance comparing the pre- and post-surgery conditions confirmed that these differences were highly significant [Group × Paw × Surgery interaction: $F(4,40) = 10.42$, $P < 0.001$]. Deficits in performance with the experimental paw during the

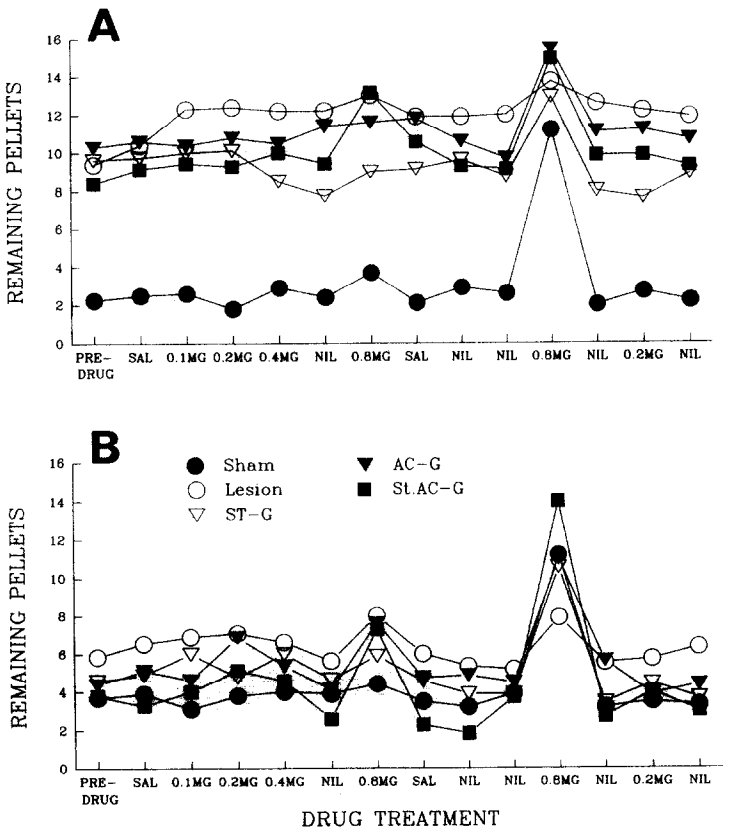

Fig. 6. Effect of D-amphetamine pretreatment on skilled paw-reaching. (A) Experimental paw. (B) Intact paw. Results are expressed as mean number of pellets remaining in the staircase test on each day.

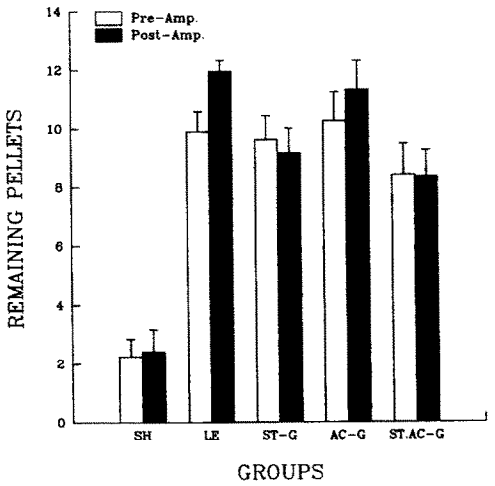

Fig. 7. Spontaneous performance with the experimental paw on the staircase test before and after D-amphetamine pretreatment. Results are expressed as the mean number of pellets remaining over the last five trials before and after amphetamine injections.

post-surgery phase were significant in the five experimental groups [Group effect: $F(4,40) = 26.93$, $P < 0.001$ with SH < LE = ST-G = AC-G = ST.AC-G at $P < 0.01$]. Moreover, when analysis was restricted to post-surgical performance of the lesioned and the grafted groups, no significant difference was observed. This indicates that the grafts had no significant effects on the reaching deficit induced by the lesions [Group × Surgery × Paw interaction: $F(3,31) = 0.40$, $P > 0.05$].

Amphetamine pretreatment. The effects of the different amphetamine doses on performance on each paw are shown in Fig. 6. Initially a dose–response regime was carried out, using an ascending series of doses. The performance of the sham group with either paw was unaffected by drug treatment with the exception of the second injection of 0.8 mg/kg of amphetamine, which did impair pellet retrieval. In contrast, the performances of the lesion and graft groups with the experimental, but not the intact, paw were influenced by the drug regime. There appeared to be a separation between the lesion and graft groups, especially the ST-G group, and the performance of the latter group was relatively improved. However, on subsequent days with either saline or no drug, this difference between groups outlasted the immediate drug action. High (0.8 mg/kg) and low (0.2 mg/kg) amphetamine doses were repeated, interspersed with days of no injections: the effects of amphetamine were found to be variable. The dose of 0.8 mg/kg induced a dramatic disturbance in all animals on a second application when compared to the first injection. In view of this variability, paw performance after amphetamine treatment has not been analysed further. On all drug-free days following the administration of amphetamine, a stable difference was observed between the lesion and grafted groups. Consequently, a restricted analysis was conducted, comparing the groups' performances during the last five baseline days—prior to amphetamine injections—to that on the five drug-free days following the introduction of amphetamine treatment (Fig. 7). This analysis indicated a significant Group × Condition interaction [$F(4,40) = 5.38$, $P < 0.001$] The effects of the amphetamine on each group were evaluated by first comparing performance before and after amphetamine treatment for each group separately. The lesioned animals performed worse after amphetamine than they had previously (Sidak test: $t = 4.46$, $P < 0.01$), whereas the performance of the other four groups was not altered significantly by amphetamine treatment. The five groups were then compared to each other to evaluate, for each group, the difference in the performances before and after treatment. This difference in the lesion group was higher than all other groups (Newman–Keuls test: LE > SH and LE > ST.AC-G at $P < 0.05$; LE > ST-G at $P < 0.01$) except the nucleus accumbens group. The difference before and after amphetamine in the other four groups was similar to each other.

DISCUSSION

The present experiment shows, firstly, that DA grafts implanted into the neostriatum and/or the nucleus accumbens can specifically influence drug-induced behaviours. The pattern of recovery on these tests was compatible with an additive effect of the grafts: implants in the neostriatum alleviated amphetamine-induced rotation, implants in the nucleus accumbens were particularly effective against apomorphine-induced hyperactivity and double grafts alleviated deficits on both classes of drug test. Secondly, the severe impairment in limb use induced by the lesion was not compensated when the striatum and the nucleus accumbens were simultaneously reinnervated by the grafted DA neurons.

Drug-induced behaviours

Initially, we evaluated the efficacy of the grafts using circular behaviour induced by direct or indirect dopaminergic agonists.[47,48] After lesion of the ascending mesotelencephalic pathway, the indirect DA agonist, amphetamine, induced an ipsilateral rotation which persisted for many months in the lesion group. Together with the TH immunohistochemistry, which demonstrates a clear destruction of TH-positive neurons in the substantia nigra and in the ventral tegmental area, the ipsilateral rotation induced by amphetamine indicates that the initial lesions were satisfactory. Previous studies have indicated that rotation rates and immunohistochemical cell losses comparable with those observed in the present experiment are associated with *post mortem* biochemical DA depletions of more than 99% in the neostratium and 96% in the nucleus accumbens.[3,25,41] DA neurons

implanted into the neostriatum compensated for this post-lesion ipsilateral rotation, as did the grafts implanted in both striatum and nucleus accumbens (double grafts). The nucleus accumbens-alone grafts had no effect. These results indicate, firstly, that the reinnervation of the neostriatum observed by immunohistochemistry was functional and, secondly, that the reinnervation of the nucleus accumbens alone was not sufficient to compensate the amphetamine-induced ipsilateral rotation.[11] It is noteworthy that both the double grafts and striatal grafts overcompensated the amphetamine-induced rotation, i.e. animals rotated away from the grafted side. This indicates that the restoration of a DA activation simultaneously within the nucleus accumbens and the neostriatum, structures functionally and anatomically linked, is not sufficient to alleviate this exaggerated rotation classically observed after amphetamine administration.[2,4,6,19,20,26,31]

Apomorphine, a direct DA agonist, induced contralateral rotation in lesioned animals as previously described.[47] This behavioural hyperactivity has been linked to the hypersensitivity of DA post-synaptic receptors within the denervated neostriatum.[15] Contralateral rotation was decreased in animals with grafts in the neostriatum or with grafts in the neostriatum and the nucleus accumbens together. The score of the AC-G group was intermediary between those of the other grafted groups and the lesioned groups. This was due to three of the 10 rats in this group which did not rotate after the administration of apomorphine, whilst the remaining seven animals turned at a similar rate to the lesion group. These three rats all exhibited TH-positive cells located in the neostriatum, reinnervating the medial–dorsal part of this structure, suggesting that reinnervation of neostriatal tissue was both necessary and sufficient to reduced apomorphine-induced circling. Consequently these results indicate that enough dopamine was released by the grafted neurons within the neostriatum to influence the functional state of the DA post-synaptic receptors within this structure and that the reinnervation of the nucleus accumbens alone does not influence compensation of the rotational asymmetry revealed by apomorphine.

Locomotor hyperactivity induced by apomorphine was selected as a second test in order to evaluate the functionality of DA neurons grafted into the nucleus accumbens.[11,32] Behavioural hyperactivity to apomorphine has been linked to the supersensitivity of deafferented post-synaptic DA receptors in the nucleus accumbens. Apomorphine-induced hyperactivity in the lesioned animals was reduced substantially only by grafts in the nucleus accumbens and by double grafts, whereas neostriatal grafts had only limited effects. Interestingly, the double grafts were not as effective as the nucleus accumbens grafts alone, which may be due to the fact that the intra-accumbens grafts were smaller in the double grafts than in the accumbens graft group. These data indicate that DA reinnervation within the nucleus accumbens was functional, and that, on this measure at least, a graft in the striatum alone did not have a beneficial effect.

Taken together, these results support previous works on the topographical organization of the striatal complex[19,20,35] and the functional additivity of multiple graft placements.[19]

Paw-reaching performance

While the grafts had a clear effect on drug-induced behaviours, none of the graft groups showed any substantial recovery on the paw-reaching task. Previous studies have also found skilled paw-reaching deficits to be unaffected by grafts in the striatum.[23,37] In view of this failure, Mandel *et al.* have suggested[36] that the DA innervation of the nucleus accumbens, which by itself is not critical in limb use,[50] could be important to the extent of the observed deficit. The failure of double grafts to restore skilled use of the contralateral limb in the present experiment contradicts such a hypothesis. The absence of an effect of the double graft was not due to a lack of reinnervation of host tissues, as the grafts did establish new fibre outgrowth. This was seen by TH immunohistochemistry, and was shown to be functional by drug-induced behavioural tests.

An explanation for the lack of influence of the graft on paw-reaching is the absence of graft reinnervation of other critical structures, denervated following the lesion, such as the prefrontal cortex, the septum and the amygdala. Whishaw and colleagues have shown that the destruction of the ventral tegmental area does not produce profound impairments in limb use.[50] However, in their experiment, the level of DA depletion in the cortex was not severe. Moreover, the role of other telencephalic structures in limb use has not yet been tested using lesion studies, let alone following transplantation.

Another possible explanation of the failure of the graft to improve paw-reaching is that in the present experimental paradigm, i.e. unilateral lesion and graft, the transplant cannot restore the brain symmetry to the level of ambidextrous performance. Consequently, unilateral grafts implanted in animals with bilateral lesions of the DA mesencephalic system[43,44] might improve limb use.

An alternative hypothesis is that the lack of influence of the graft might be linked to the absence of normal interneuronal regulation. Implanting grafts in an ectopic location, i.e. directly into the striatum, provides good terminal reinnervation, but the grafts remain isolated from the signals that modulate the activity of intact mesencephalic DA neurons *in situ*. Consequently, the activity of the grafted neurons may not be regulated by normal interneuronal stimuli.[29] The constant non-modulated basal level of dopamine released by grafted DA neurons may not be sufficient to restore more complex behaviours which require a continuous adjustment of dopaminergic activity by environmental stimuli.[10] In contrast to the effects of

DA-rich grafts on rats with unilateral 6-OHDA lesions, Dunnett and colleagues[24] found greater recovery in paw-reaching ability when embryonic neostriatal tissue was grafted into a neostriatum that had been lesioned with an excitotoxin. The major difference between these two paradigms is that in the latter the grafts are implanted in a homotopic location and the activity of the implanted cells may be modulated through the restitution of neuronal afferents.[14,40]

In the present study, we considered whether the efficiency of DA grafts could be potentiated by direct activation of the grafted cells by amphetamine. Herman and colleagues[27,28] have shown that DA grafts implanted bilaterally in the nucleus accumbens, previously deprived of its DA innervation, only compensated complex behavioural deficits in hoarding and exploration when the rats were pretreated with a low dose of amphetamine. This indicated that the grafts were potentially capable of ameliorating the deficits, but that pharmacological stimulation was needed to activate this capacity. In the same way, intrahippocampal cholinergic grafts that are below the threshold for behavioural effects can be primed with indirect cholinergic agonists such as physostigmine.[34] On the basis of these findings, the effect of pretreatment with amphetamine was examined. No improvement in the performances of the graft groups was observed. There are several possible explanations for the failure of the amphetamine to potentiate the efficiency of the grafts. Differences in the extent of the lesions might account for the discrepancy between the present results and those obtained by Herman and colleagues.[27,28] In that study, amphetamine pretreatment was effective in grafted rats with bilateral local lesions of the nucleus accumbens, whereas in the present experiment the DA mesotelencephalic system was totally destroyed on one side. In agreement with this hypothesis, Herman and colleagues[28] have demonstrated that for large lesions, consisting of the destruction of the DA mesencephalic neurons of the ventral tegmental area, pretreating the DA grafts with amphetamine had no effect on the deficits in hoarding or exploratory behaviours; the beneficial effect was observed only when the lesion was restricted to the nucleus accumbens.

Of more interest in the present experiment was the observation that the effects of the amphetamine treatment persisted into subsequent saline and drug-free trials. Snyder-Keller and colleagues[43,44] first suggested that amphetamine might enhance the efficacy of DA grafts in the striatum by "priming" the transplanted DA neurons. However, in the present experiment animals received a series of amphetamine injections for rotational and activity tests prior to the skilled paw-reaching test, and the effect of the drug appeared only when it was received within the training environment. This rules out a "priming" or sensitization process in the present situation, and instead suggests that some form of behavioural conditioning is involved. Although the basis of this mechanism is still unclear, a similar phenomenon has recently been established in other experimental conditions.[7]

CONCLUSION

The findings of this experiment have confirmed the fundamental effects of grafts in the dorsal and ventral striatum, also demonstrating the importance of the topographical placement of the grafts on the pattern of recovery observed. Whereas drug-induced behaviours such as rotation and locomotor hyperactivity were compensated by grafts implanted in appropriate locations, such grafts did not restore performance in the more complex reaching task. These results rule out the simultaneous restoration of a DA input within the neostriatum and the nucleus accumbens as being sufficient for the restoration of normal behavioural output.

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