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Björklund, A., Stenevi, U., Schmidt, R.H., Dunnett, S.B., Gage, F.H.: Intracerebral grafting of neuronal cell suspensions. II. Survival and growth of nigral cells implanted in different brain sites. Acta Physiol. Scand., Suppl. 522, 9-18, 1983.

Schmidt, R.H., Björklund, A., Stenevi, U., Dunnett, S.B., Gage, F.H.: Intracerebral grafting of neuronal cell suspensions. III. Activaty of intrastriatal nigral suspension implants as assessed by measurements of dopamine synthesis and metabolism. Acta Physiol.Scand., Suppl. 522, 19-28, 1983

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Gage, F.H., Björklund, A., Stenevi, U., Dunnett, S.B.: Intracerebral grafting of neuronal cell suspensions. VIII. Cell survival and axonal outgrcwth of dcpaminergic and cholinergic cells in the aged brain. Acta Physiol.Scand., Suppl. 522, 67-75, 1983.

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Intracerebral Grafting of Neuronal Cell Suspensions IV. Behavioural Recovery in Rats with Unilateral 6-OHDA Lesions following Implantation of Nigral Cell Suspensions in Different Forebrain Sites

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Single and multiple implants of nigral cell suspensions were grafted to the forebrains of rats with unilateral 6-hydroxydopamine-induced dopamine denervations. Control lesions alone induced a marked behavioural asymmetry, as assessed by amphetamine- and apomorphine-induced rotation, sensorimotor tests and side bias in an unbaited T-maze, and the animals were hyperactive to a low dose of apomorphine. Single suspension placements into different denervated striatal regions were capable of reversing the behavioural asymmetries dependent upon the specific placement for each test. Multiple suspension grafts were capable of reversing all behavioural asymmetries, and additionally abolished the supersensitive hyperactivity to apomorphine. By contrast, single suspension grafts placed into the substantia nigra or lateral hypothalamus had no detectable effect on any functional measure. The results indicate that nigral suspension grafts can be at least as effective as solid grafts in reversing the functional deficits induced by dopamine denervation, provided that placements are selected within appropriate dopamine terminal regions of the forebrain (e.g. caudate-putamen or nucleus accumbens).

INTRODUCTION

Solid grafts of embryonic substantia nigra in rats with dopamine (DA)-depleting 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal pathway can not only survive, but also provide functional recovery on many of the behavioural deficits induced by the lesions (1, 3, 4, 6, 7, 8, 11, 17). In these studies, the DA-rich grafts have been placed into either the lateral ventricle (11, 17) or a cortical cavity (1, 3, 6, 7, 8) adjacent to the DA-denervated neostriatum, and it has not proved possible to transplant viable solid grafts to deep sites. Consequently, it is unknown whether graft placement adjacent to denervated striatal terminals is important in sustaining functional recovery, or whether a more "natural" location, such as in the host substantia nigra, would prove even more effective.

Preliminary observations have indicated that nigral suspension injections into the DA-denervated neostriatum are as efficient as solid grafts in reversing the amphetamine-induced rotational asymmetry seen in rats with unilateral 6-OHDA lesions (2, 18). Suspension grafts have advantages over the solid transplantation procedure in that they do not require additional cortical lesions, they may readily be placed in any region of the host neuropil including deep forebrain and mesencephalic sites, and they permit the placement of multiple grafts with little additional trauma to the host (see Chapters I and II).

The present study investigates the functional competence of nigral suspension grafts in unilateral 6-OHDA lesioned rats, addressing four main questions. (i) Are suspension grafts as efficient as solid nigral grafts in ameliorating the rotational, locomotor and sensorimotor deficiencies induced by the 6-OHDA lesions? (ii) Can suspension grafts placed in different parts of the striatal complex affect different components of the dopamine deficiency syndrome? (iii) What is the relative functional competence of grafts placed in the host substantia nigra, lateral hypothalamus and neostriatum? These sites represent the location of the intrinsic DA cell bodies, major ascending fibre pathway, and area of termination, respectively, of the nigrostriatal neurones prior to lesion. (iv) Is it possible to improve the rapidity or range of functional recovery by the provision of multiple rather than single injections?

METHODS

Subjects

Eighty-four young adult female rats of the Sprague-Dawley strain were employed in the behavioural tests. They were housed together throughout the experiment in groups of 6-8 rats/cage under 12:12 hr light-dark cycle, and with *ad libitum* access to food and water. Suspension transplants were taken from 14-15 day embryos (crown-rump length 11-13 mm) of the same inbred strain.

6-OHDA lesions

All rats initially received a unilateral stereotaxic lesion of the right nigrostriatal pathway by injection of 8 µg 6-OHDA HCl (free-base weight) dissolved in 4 µl ascorbate solution (0.2 mg ascorbic acid per 1 ml 0.9% saline) as detailed in Chapter II.

Seven-10 days following the lesion, all rats were screened for 5 mg/kg amphetamine-induced rotation (see below). Any rat not satisfying a criterion net ipsilateral rotation rate of >7 turns/min over 1½ hour following the injection was rejected. Biochemical analyses of this lesion procedure have repeatedly shown that this screening procedure selects rats with an average of 99% depletion of DA content in the ipsilateral head of the caudate-putamen (19, see Chapter III).

Transplantation surgery

Two weeks following lesion nigral suspension transplants were prepared according to the protocol described in detail in Chapter I. The rats were subdivided into 8 groups, and received stereotaxic implantation of single or multiple suspension injections into various forebrain or midbrain sites, or remained as lesioned but non-transplanted controls. Table I and Fig. 1 give details of the 8 different groups, the number of rats in each, and the location and stereotaxic coordinates of each injection. For each injection two 2 µl deposits were delivered over 2 min each, the second 1-2 mm above the first, and the needle was left in place 3 min after the second injection. Finally, the needle was retracted and the wound sutured. No special postoperative care was required.

Behavioural Tests

Rotation. Rotation tests were conducted in a bank of 6 automated rotometer bowls, modelled on those described by Ungerstedt and Arbuthnott (20). The bowls were hemispheric and made of transparent perspex, with a handful of sawdust in each. A rat in the bowl was attached by a harness and tensile steel wire to a cam and pivot over the centre of the bowl. Half-turns of 180° in either direction were separately recorded by on-line connection to an ABC 80 microprocessor. Rotation tests were conducted over 90 min following intraperitoneal injection of 5.0 mg/kg methamphetamine, and over 40 min following subcutaneous injection of 0.05 mg/kg apomorphine in the neck. All rats received an initial amphetamine test 7-10 days following the 6-OHDA lesion, as a screen for completeness of the DA denervation, and

Group		n	A	L	V
C	Lesioned controls	18	–	–	–
SN	Substantia nigra	5	–5.5	1.5 & 2.5	7.0
LH	Hypothalamus	8	–1.0 & –2.0	1.5	7.5
La	Lateral striatum	10	+1.0	3.5	7.0 & 5.5
Do	Dorsal striatum	12	+1.0	2.5	5.7 & 4.0
x2	Double suspension	6			
	lateral striatum		+1.0	3.5	7.0 & 5.5
	dorsal striatum		+1.0	2.5	5.7 & 4.0
x3	Triple suspension	9			
	lateral striatum		+1.0	3.5	7.0 & 5.5
	dorsal striatum		+1.0	2.5	5.7 & 4.0
	nucleus accumbens		+2.8	1.9	6.5 & 5.0
x5	Multiple suspension	16			
	lateral striatum		+1.0	3.5	7.0 & 5.5
	dorsal striatum		+1.0	2.5	5.7 & 4.0
	posterior striatum		–0.3	3.8	6.5 & 4.5
	nucleus accumbens		+2.8	1.9	6.5 & 5.0
	amygdala		–2.0	4.0	7.3 & 6.3

Table I. The eight different control and suspension groups employed in the present experiment. For each is given the nomenclature employed to specify the group, the group name and number (n) of rats included. Each target placement involved two suspension injections, separated by 1-2.0 mm, and shown schematically in Fig. 1. The stereotaxic coordinates for each placement are given in mm anterior from bregma (A), lateral from the midline (L) and vertical below dura (V), with the incisor bar set at the level of the inter-aural line.

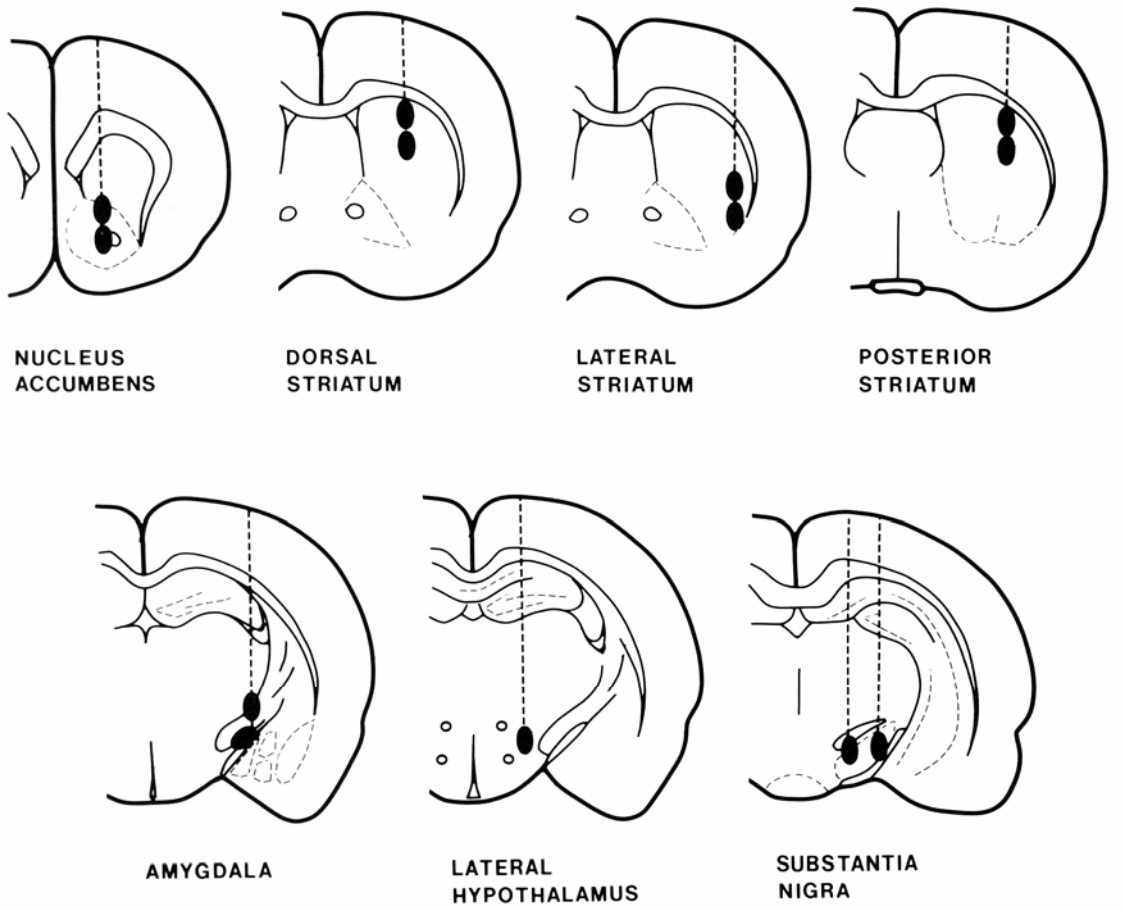

Fig. 1 Schematic diagram of the seven graft placements employed either singly or in combination (see Table I) in the behavioural studies.

then several further tests at 3-6 week intervals following transplantation. The apomorphine test was only administered to 4 of the groups (C, Do, x2, x5; cf Table I for nomenclature) prior to transplantation, but all groups were tested at 5-10 week intervals following transplantation.

Sensorimotor tests. The sensorimotor test battery was modelled on the tests of Marshall and Teitelbaum (15, 16) and has been fully described elsewhere (6, 9). Briefly, the test battery provides a separate rating score for the two sides of the body on an orientation component, involving orientation of the head to lateralized visual, olfactory and tactile stimuli, and a limb use component, involving the coordinated use of fore- and hind-limbs in placing, grasping and withdrawal responses. All rats received a single sensorimotor test 3-5 months after transplantation.

Activity tests. Activity tests were conducted in an Animex^{R} automated activity meter. Each rat received two tests, each of 5 min duration, separated by 1-2 hours. The animal was drug free for the first test ('spontaneous' activity), and received an injection of 0.05 mg/kg apomorphine sc in the neck 15 min prior to the second test.

T-maze bias. The T-maze was of conventional design and has been fully described elsewhere (6). Each animal received 3 tests, at approximately 1 month intervals, for spontaneous side bias during exploration of the unbaited maze. Each test comprised 3 trials at 15 min intervals, on which the rat was placed in the start arm and allowed to freely explore. The latency and side chosen to first reach one goal box was recorded.

Histology

The majority of rats from four groups (C, LH, x3, x5, cf Table I) subsequently received bilateral 6-OHDA lesions and further tests of aphagia, adipsia, akinesia and sensorimotor function (see Chapter V). All rats were finally taken for catecholamine histofluorescence according to the ALFA method, Procedure I (14; see Chapter II), with the exception of several rats in the Do group which were taken for biochemical analysis (see Chapter III).

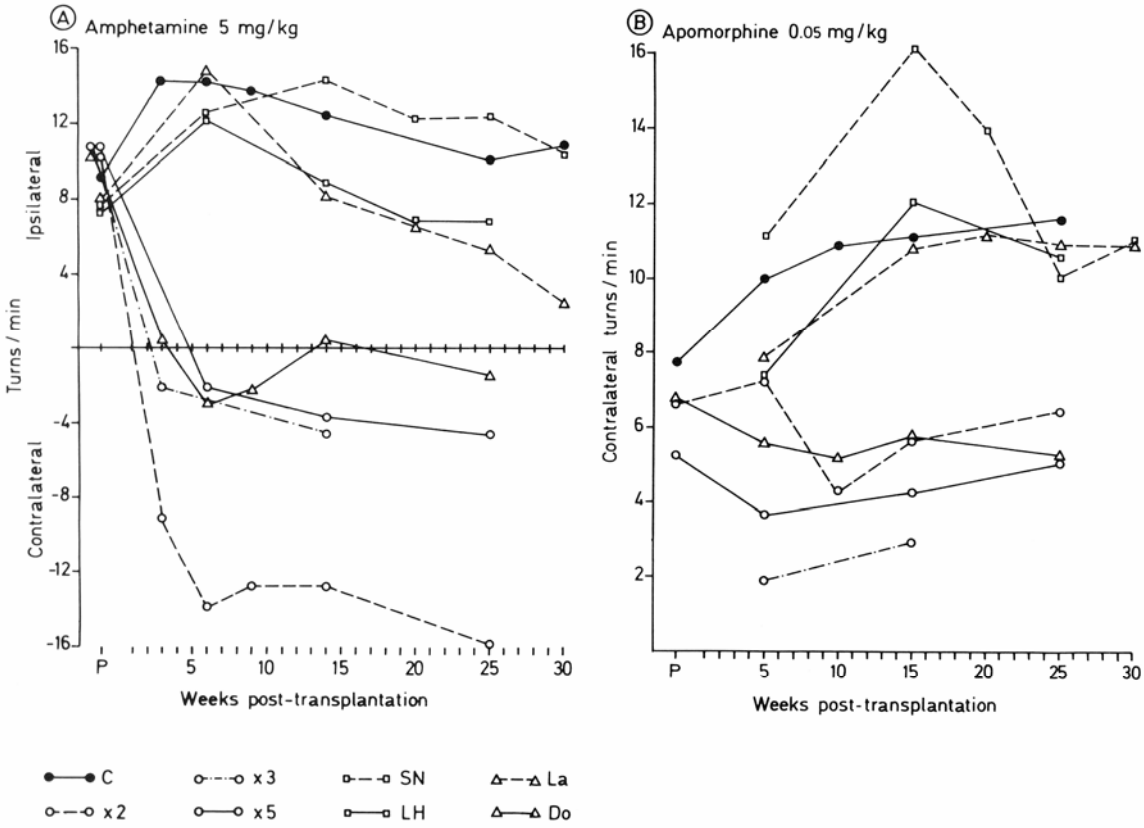

Fig. 2 Net rotation rates induced by 5.0 mg/kg amphetamine (A) and 0.05 mg/kg apomorphine (B) prior to (P) and up to 30 weeks following nigral suspension transplants. Scores are net 360° turns/min over 90 min (amphetamine test) or 40 min (apomorphine test). All rats received a unilateral 6-OHDA lesion 7-10 days before the initial (P) test and 2 weeks before transplantation. The 8 groups are as specified in Table I.

RESULTS

Behavioural tests

Rotation. The net amphetamine-induced rotation rates in the 8 groups prior to, and up to 30 weeks following transplantation are shown in Fig. 2A. All control rats continued to show high rates of ipsilateral turning for the full duration of testing (32 weeks after lesion), and a similar pattern was seen in the SN and LH groups. The La group initially showed an equally high rate of ipsilateral turning over the first two months following transplantation, but at longer survival times appeared to show some compensation in rotational bias. By contrast, the remaining four groups (Do, x2, x3, x5) showed a rapid compensation or overcompensation of rotational asymmetry from 3 weeks after transplantation. Many individual rats in these 4 groups manifested consistent moderate to strong contralateral rotation. In particular every rat in the x2 group showed strong contralateral turning, whereas a few rats in each of the Do, x3 and x5 groups failed to compensate. The biochemical analysis of the animals from the Do group (see Chapter III) indicated that the grafts had not survived in the uncompensated rats. All Do rats with surviving grafts did manifest strong contralateral rotation (mean = -7.7 turns/min).

Analysis of variance on the rotation scores at 14 weeks post-transplantation (the only time at which all groups were tested after a similar interval) revealed that the difference between groups was highly significant ($F = 11.59$ with 7,76 df, $p < 0.001$). Multiple comparisons between the groups by the Newman-Keuls test indicated that the groups fell into two discrete clusters: the C, SN, LH and La groups did not differ from each other, nor did the Do, x2, x3 and x5 groups, whereas each group in one cluster did differ significantly from all groups in the other cluster (critical values set at $p = 0.05$ in each

comparison). Further analyses of variance restricted to just those groups tested at each other time point each yielded a similar interpretation.

The net apomorphine-induced rotation rates in the 8 groups prior to and up to 30 weeks following transplantation are shown in Fig. 2B. Control unilateral lesioned rats showed a progressive increase in contralateral rotation in the weeks following the lesion, and the animals with SN, LH or La grafts showed an equivalent high rate of contralateral turning. By contrast, the Do, x2, x3 and x5 groups manifested a stable or slight decline in contralateral rotation following transplantation.

Analysis of variance on the apomorphine-induced rotation scores 15 weeks post-transplantation (the only time at which all groups were tested at a similar interval) revealed that the difference between groups was significant ($F = 11.46$ with 7,76, $p < 0.001$). Multiple comparisons revealed a similar clustering of the groups to that seen on the amphetamine test: the C, SN, LH and La groups did not differ from each other, nor did the Do, x2, x3 and x5 groups, whereas each group in one cluster differed from all in the other cluster ($p < 0.05$) with the single exception of the difference between the La and x2 groups ($t = 2.67$, $p < 0.1$). Analyses of all time points from 10 weeks post-transplantation onwards revealed a similar dissociation between the two clusters of groups.

Sensorimotor tests. By the time of sensorimotor testing, 6 rats with x5 suspensions had been taken for bilateral lesion, and one further x5 rat and one LH rat had died, and are not included in the present analysis. The ipsilateral and contralateral rating scores of each group on the orientation and limb use components of the sensorimotor battery are shown in Figs. 3A and 3B, respectively. Sensorimotor scores were analysed both by 2-factor analysis of variance, treating ipsilateral and contralateral scores as a within-subjects factor, and by a 1 factor analysis of variance between groups on the asymmetry scores (ipsilateral – contralateral ratings) of each rat. The main effect from the one factor analysis is equivalent to the interaction term of the two factor analysis, but facilitates comparisons between the groups using the Newman-Keuls test.

The results demonstrate a significant effect of grafts placed within the striatum, but not in the substantia nigra or lateral hypothalamus, on both the orientational and limb use components, and that the compensation was greater with multiple than single graft placements (Fig. 3A and B).

On the orientation components, there was no significant main effect due to group ($F = 0.40$ with 7,68 df, $p > 0.25$) but highly significant effects due to side ($F = 211.14$ with 1,68 df, $p < 0.001$) and group x side interaction ($F = 14.75$ with 7,68 df, $p < 0.001$). This appears to be attributable to a high ipsilateral bias in the C, SN and LH groups, which is markedly reduced in the La, Do and x2 groups and is abolished in the x3 and x5 groups. Multiple comparisons between the asymmetry scores of the different groups revealed that the C, SN and LH groups did not differ from each other, but that the La, Do, x2, x3 and x5 groups were significantly less asymmetrical than each of these three (critical value set at $p = 0.05$ for each comparison). Among the latter 5 groups only the x5 rats were significantly less asymmetrical than the La group. Of particular interest is the observation that the ipsilateral scores appear to be inversely related to the contralateral scores: the between-groups correlation between the ipsilateral and contralateral mean scores is significant ($r = -0.839$, $t = 4.37$ with 6 df, $p < 0.01$).

On the limb use component, again the main effect due to groups was not significant ($F = 0.91$ with 7,68 df, $p > 0.25$), whereas both the side effect and the group x side interaction were highly significant ($F = 114.47$ with 1,68 df and $F = 8.36$ with 7,68 df, respectively; $p < 0.001$ in each case). Multiple comparisons between the asymmetry scores of the different groups revealed that the C, LH and SN groups did not differ from each other, nor did the La, x2, x3 and x5 groups, whereas each group in the latter cluster was significantly less asymmetrical than each group in the former (critical value set at $p = 0.05$ for each comparison). The asymmetry of the Do group fell halfway between these two clusters, and was only significantly different from the SN group at one extreme and the x5 group at the other. In the limb use tests, the amelioration of sensorimotor asymmetry appears to be predominantly attributable to recovery of contralateral responding with little associated change in the ipsilateral scores.

Activity. Spontaneous and apomorphine-induced activity counts are shown for each

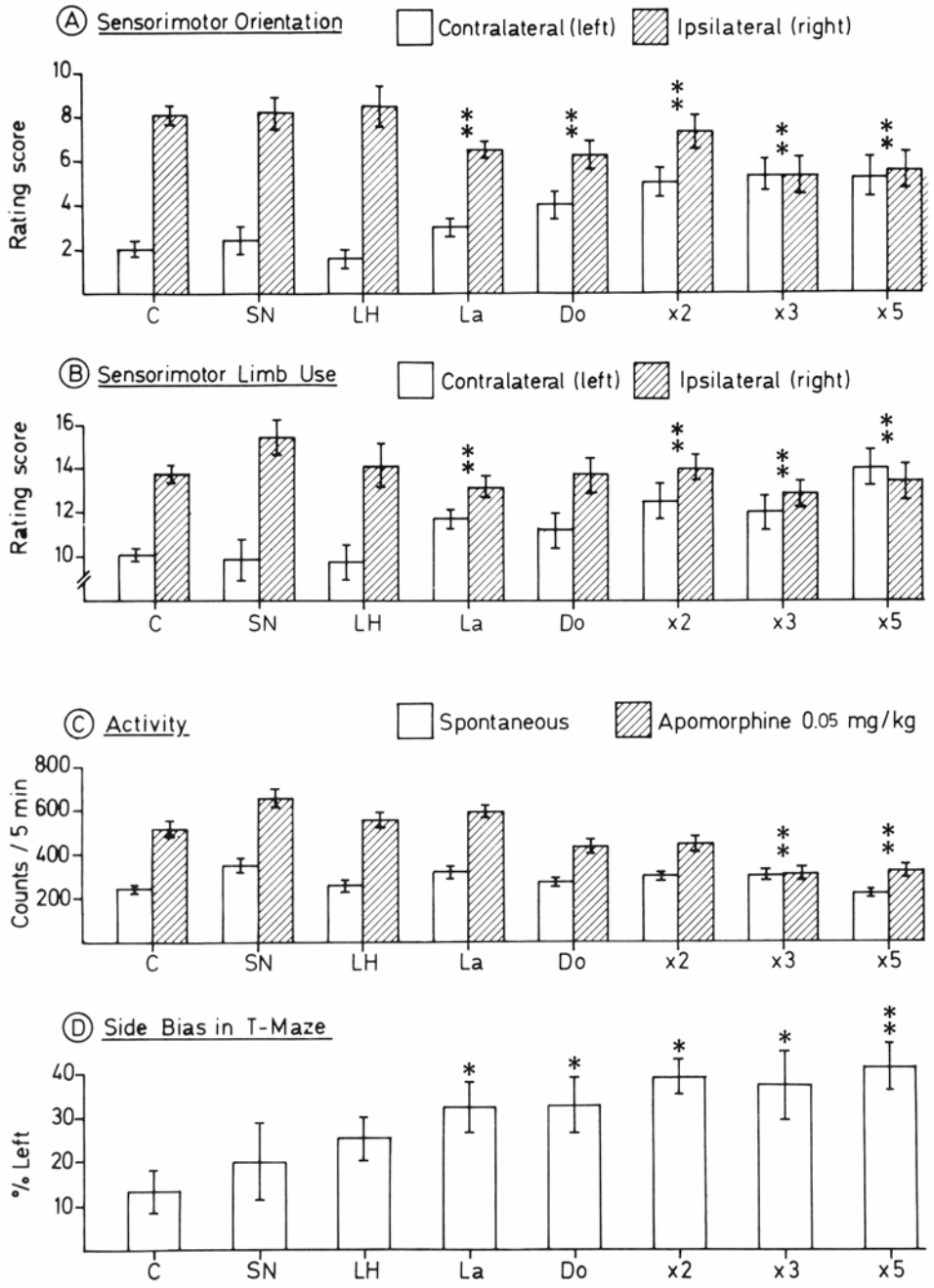

Fig. 3 A and B: Sensorimotor orientation and limb use ratings respectively on the left and right sides of the body, contralateral and ipsilateral to the 6-OHDA lesion. ** indicates groups in which the asymmetry is less than in the control group, $p < 0.01$. C: Mean activity counts recorded over 5 min tests in the Animex, both spontaneously and 15 min following the injection of 0.05 mg/kg apomorphine. ** indicates groups in which the difference between the apomorphine and spontaneous test is less than in the control group, $p < 0.01$. D: Mean percentage of total choices of the left arm, contralateral to the lesion, in the unbaited T-maze. * indicates groups in which the numbers of left choices is significantly greater than in the control group, $p < 0.05$; ** $p < 0.01$. The 8 groups are specified in Table I. Vertical bars on each column indicate the standard error of the mean.

group in Fig. 3C. Both main effects and the group x drug interaction were significant (group, $F = 12.00$ with 7,76 df; drug, $F = 187.36$ with 1,76 df; group x drug, $F = 6.75$ with 7,76 df; $p < 0.001$ in each case). In order to assess the activational effects of this low dose of apomorphine (0.05 mg/kg), multiple comparisons were conducted on the apomorphine-spontaneous differences between groups: the SN, LH, Do, La and x2 groups did not differ from controls ($p > 0.05$) whereas both the x3 and x5 groups showed a significantly reduced apomorphine activation with respect to controls ($p < 0.01$ in each case).

T-maze bias. Six of the x5 group were not tested in the T-maze, having already been taken for bilateral lesion. The spontaneous side bias in the T-maze was analysed in terms of the number of choices to the left arm, contralateral to the lesion (and transplant) out of a total of nine trials. The mean percentage of choices of the left arm by each group is shown in Fig. 3D. The difference between groups was significant ($F = 3.44$ with 7,70 df, $p < 0.05$). Multiple comparisons revealed that the C, SN and LH groups did not differ ($p > 0.05$ in each case). Conversely, the Do, La, x2, x3 and x5 groups each showed significantly more choices of the left arm than the controls (at least $p < 0.05$ in each case), but did not differ significantly from each other or from the SN and LH groups.

DISCUSSION

The results indicate that suspension grafts of embryonic nigral tissue can ameliorate behavioural impairments induced by dopamine-depleting 6-OHDA lesions, providing an appropriate graft placement is selected. The recovery from those impairments can be even more rapid than that seen with grafts of solid pieces of embryonic tissue. Thus, for example, complete compensation of amphetamine-induced rotation was seen in all groups with a suspension graft placed in the dorsal caudate-putamen over the first 3-6 weeks following transplantation, whereas a minimum period of 2-3 months has generally been found to be necessary with the solid graft procedures (1, 3, 6). Locomotor activity, side bias in the T-maze and sensorimotor orientation and limb use were tested only at relatively long intervals, after the transplants had become well established. Thus, on these measures, it was seen that suspension grafts can provide at least as efficient recovery from 6-OHDA-induced impairments as solid nigral grafts, but it is not possible to infer any difference between the two procedures in the time course of recovery.

Whereas solid grafts reinnervating dorsal or lateral neostriatum provide different profiles of functional recovery, suggesting that the field of graft-derived reinnervation is an important factor (5, 6, 7), the solid transplantation procedure is not sufficiently flexible in viable graft placements to allow a full investigation of this issue. The suspension graft procedure, however, does permit the selective placement of viable grafts into any chosen forebrain site (see Chapter II). It has been seen that, firstly, graft placement into deafferented DA terminal areas is a critical factor in promoting functional recovery. Whereas nigral suspension grafts placed into the substantia nigra or lateral hypothalamus survive equally well, they give rise to minimal outgrowth of DA fibres (see Chapter II) and no functional effects of the grafts could be detected in either the SN or LH groups. By contrast, rats with single or multiple graft placements in deafferented DA terminal areas did show substantial functional compensation, dependent on the particular test employed.

We have previously reported that solid nigral grafts innervating the dorsal, but not the ventrolateral, neostriatum compensate spontaneous drug-induced rotational asymmetries following unilateral 6-OHDA lesions. This may be related to the heavy projection from motor cortex to this region (12), although some discrepancy remains in that small intrastriatal 6-OHDA injections are no more effective into dorsal striatum than other striatal sites in producing low levels of rotation (10). Nevertheless, the present results support the previous results within animals with solid grafts in that only those groups receiving suspension injections into dorsal neostriatum (Do, x2, x3, x5) showed rapid and complete compensation of both amphetamine- and apomorphine-induced rotation. The group with ventrolateral striatal graft alone (La) was indistinguishable from controls. Even on the last amphetamine tests 6 months after transplantation, when the La group did show a partial compensation, this reduction in turning rate failed to achieve significance.

The earlier solid graft studies showed the opposite dissociation between graft placement and recovery of sensorimotor biases, namely

that reinnervation of the ventrolateral but not dorsal striatum is critical for restitution of sensorimotor function in unilaterally lesioned rats (7). A similar regional organization has been previously demonstrated by small injections of 6-OHDA into different neostriatal sites (9). The present data indicate that indeed all groups with a lateral striatal placement (La, x2, x3 and x5) were compensated on both the orientation and the limb use component of the sensorimotor tests. The rats with a single dorsal graft were, however, also compensated on the orientation component, which may be attributable to a long-term extension of outgrowth into ventral or lateral striatal segments. This is supported by the histofluorescence analysis (see Chapter II) which indicated a greater overlap in the central striatum from suspension grafts placed in dorsal and lateral aspects of the nucleus than had been seen previously from solid grafts placed in dorsal and lateral cortical cavities (1, 7).

The injection of multiple suspension grafts into the different sites within the same rat was found to be not only viable but to also provide a more extensive recovery both in the range of tests and in the degree of recovery on each individual test. Thus, not only were the x2, x3 and x5 groups well recovered on both rotation and sensorimotor tests but also they showed an even greater restitution on these and other tests, as compared to rats with only a single graft. Consequently, whereas the significant reduction in apomorphine-induced locomotor activity on the x3 and x5 groups alone is compatible with the view that the nucleus accumbens placement is the critical striatal focus for this function (13), this cannot be separated from the alternative interpretation that the present results are due to the additive consequences of multiple graft placements. Although this issue cannot be resolved from the present eight groups, further experiments with single suspension placements into the nucleus accumbens have been seen to provide an equally effective compensation of apomorphine-induced hyperactivity and amphetamine-induced hypoactivity following 6-OHDA lesions of the mesolimbic DA pathways (preliminary unpublished results).

In conclusion, the intrastriatal injection of DA-rich cell suspensions from the embryonic substantia nigra region has been found to be at least as effective as solid nigral grafts in providing recovery from a range of behavioural impairments induced by unilateral 6-OHDA lesions of the mesotelencephalic DA bundle. Suspension grafts are functionally effective only when placed into denervated DA terminal sites, and have no detectable effects when positioned in the substantia nigra or lateral hypothalamus. Striatal placement is of critical importance in determining whether recovery is achieved on any particular test whereas multiple placements permit a more extensive recovery in terms of both the number of test impairments ameliorated and the degree of recovery on each individual test. In particular, it has been possible with the multiple suspension grafts to restore functional equality between the intact and lesioned sides of the striatum on spontaneous, in addition to drug-induced, tests such as in the T-maze and with the sensorimotor battery.

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