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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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Intracerebral Grafting of Neuronal Cell Suspensions V. Behavioural Recovery in Rats with Bilateral 6-OHDA Lesions Following Implantation of Nigral Cell Suspensions

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Bilateral 6-hydroxydopamine-induced lesions of the ascending forebrain dopamine neurones induce a behavioural syndrome in rats which includes profound aphagia, adipsia, akinesia and bilateral sensorimotor neglect. Such animals will die unless maintained by intragastric feeding. Three experiments are reported in which we have attempted to ameliorate this syndrome with single or multiple placements of nigral cell suspensions into the forebrains of rats with bilateral dopamine depletions. Although the grafts were efficient in reversing the sensorimotor and akinetic impairments, and produced a significant increase in eating, the grafted rats remained hypophagic and adipsic. The results indicate that although many components of the bilateral dopamine denervation syndrome can be reversed by intrastriatal nigral suspension grafts, the severe eating and drinking deficits remain unameliorated.

INTRODUCTION

The rotational and sensorimotor asymmetries induced by unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal pathway in the rat can be reduced or completely abolished by grafts of embryonic substantia nigra to the forebrain of the lesioned host, either in the form of solid tissue pieces (1, 3, 5, 6, 8, 11) or as dissociated cell suspensions (2, 12; see Chapter IV). The same unilateral solid grafts induce a contralateral rotational and sensorimotor asymmetry if the rats receive a second bilateral lesion of the remaining intact nigrostriatal dopamine (DA) pathway, and there is also some amelioration of the spontaneous and drug-induced akinesia in the bilaterally lesioned rats (4, 6, 7). However, the severe body weight loss, aphagia and adipsia characteristic of bilateral 6-OHDA lesions (12, 14) were not appreciably influenced in these studies which employed solid nigral grafts reinnervating the dorsal or lateral striatum (1, 4, 6). Indeed, in one study the grafted animals were significantly more impaired than the corresponding bilaterally lesioned control group (1).

The present report investigates the functional effects of dopamine-rich nigral suspension grafts to various forebrain sites in rats with bilateral 6-OHDA lesions of the ascending mesotelencephalic dopamine projections. Multiple placements of nigral suspensions are seen to ameliorate the akinesia and to induce rotational and sensorimotor biases contralateral to the suspension grafts, but again no appreciable improvement has been achieved on the severe regulatory impairments.

METHODS

Subjects and Surgery

The bilateral lesion experiments were all conducted on subgroups of rats that had previously been tested following unilateral lesions (Chapter IV). All animals had received complete unilateral 6-OHDA lesions of the right nigrostriatal pathway prior to transplantation, and subgroups received single or multiple injections of nigral cell suspensions into different forebrain sites (see Chapter I and II) or remained as lesioned controls. Six to 9 months following the initial lesion and transplantation, the rats received a second 6-OHDA lesion of the contralateral, left nigrostriatal pathway (8 µg free base in 4 µl as before; Chapter II-IV). Provided that the first lesion was relatively complete, as determined by the amphetamine-induced rotation screen 7-10 days after the first lesion (see Chapter III), this serial bilateral lesion provides equally severe and permanent functional impairments as are obtained following single-stage bilateral lesions (compare refs. 1, 7 with 10, 14).

The rats were tested in 3 batches. *Batch I* comprised 8 rats with nigral suspensions placed in the right lateral hypothalamus (LH), 6 rats with suspensions in the right lateral caudate-putamen (La group) and 5 bilateral lesion controls. *Batch II* comprised 9 rats with triple suspensions into the right nucleus accumbens, dorsal and lateral caudate-putamen (x 3 group), and 8 bilateral lesion controls. *Batch III* comprised 8 rats with multiple suspension injections

into the right nucleus accumbens, dorsal, lateral and posterior caudate-putamen and a fifth injection aimed at the amygdala (but which generally fell dorsomedially into the internal capsule; see Chapter II and IV, x 5 group), 5 rats with 5 similar placements placed bilaterally into both the right and left forebrain (x 10 group), and 9 bilateral lesion controls. In the x 10 group the grafting was made on the side ipsilateral to the first 6-OHDA lesion 2 weeks after lesion, and the grafting on the contralateral side was made 3 months later into the non-denervated hemisphere. The second 6-OHDA lesion was made after a further 3 months.

Post operative care and regulatory measurements

Each batch of rats was housed in individual cages from 5-10 days prior to lesion and kept on a 12:12 hour light-dark cycle. Daily measurements were taken of body weight and weight of the water bottle, from which daily water consumption was computed. Dry lab chow was freely available in the cage food baskets throughout.

Following the second lesion, rats were daily provided with 5 new food pellets and 2 squares of palatable wholemeal bread (batches I and II only) or 2 chocolate chip cookies and a wet mash made of baby food, glucose and water (batch III only). Daily ratings were made of the fraction of each food that had been consumed (0.1 to 1.0 of each food item), with evidence of nibbling of a food attributed the minimum value of 0.1. All animals required additional maintenance by intragastric tube feeding of 20-30 mls of a high energy liquid diet (batches I and II: Biosorbin, Behring, GFR; Batch III: Vivonex, AB Vitrum, Stockholm, Sweden), administered in 3 daily feeds from the third post operative day.

Sensorimotor tests

The rats of batches II and III were tested on the sensorimotor test battery (see chapter IV) at 7 days following the second lesion.

Catalepsy tests

At the same time as the sensorimotor tests, the rats also received a range of catalepsy measures (see 7, 9). The rats were placed on the bench surface and the latency to move (a) any one and (b) all 4 paws was recorded. (c) The rats were perched with their forepaws resting on a 7 cm high wooden block, the hindpaws remaining on the bench surface. The latency to move both forepaws was recorded. (d) Rats were placed with hindpaws on the block, forepaws on the bench surface, and latency to move the hindpaws was recorded. (e) The rats were placed, head downwards, on a wire grid suspended 60 cm above the bench surface and inclined 60^0 below the horizontal. Normal rats rapidly turn so that the head is upwards. Latency to turn to the point where the nose was at the same horizontal level as the base of the tail was recorded. On all 5 measures, a maximum latency of 60 sec was allowed. Any rat which had not moved within the minute was assigned the maximum score; it then progressed to the next measure.

Activity tests

All rats of batches II and III were tested in an Animex^R activity meter, 11 days (batch II) or 13 days (batch III) following the second lesion. Each rat was initially tested over 15 min for spontaneous level of activity, then injected with 0.5 mg/kg methamphetamine i.p., and tested 30 min later for drug-induced activity over a further 15 min.

Rotation tests

All rats of batches II and III were tested for spontaneous and tail-pinch induced rotation 8 days following the second lesion, for 5 mg/kg (ip) methamphetamine-induced rotation on day 13, and for 0.05 mg/kg (sc in the neck) apomorphine-induced rotation on day 18. The spontaneous and tail-pinch measures were recorded by direct observation in hemispheric white perspex bowls. Each rat was placed in the bowl and spontaneous quarter turns in either direction were recorded over 3 min. A large paperclip, suspended by thread above the centre of the bowl was then attached to the tail 2-3 cm from the tip, and quarter turns in either direction were recorded for a further 3 min. Amphetamine- and apomorphine-induced rotation over 90 and 40 min, respectively, was recorded in automated rotometer bowls, as detailed in Chapter IV.

Histology

Animals were sacrificed for histogical analysis 9 days (batch I), 20 days (batch II) or 21 days (batch III) following the second lesion. Details of processing and analysis are presented in Chapter II.

RESULTS

Survival, Aphagia and Adipsia

All rats of all groups were aphagic and adipsic following the second lesion, and were dependent upon tube feeding for survival. In spite of these attempts at maintenance, several rats of each group died prior to the termination of each experiment (cf. Table I). Since within each batch the duration of survival varied between animals, whereas food and water intake values were relatively stable over time, body weights have been analyzed in terms of maximum weight loss by each rat, water intake in terms of mean daily weight reduction of the water bottles (1 gm = 1 ml), and food intake in terms of the mean daily consumption of the foods (100% taken as equivalent to 1 pellet, 1 biscuit, 1 square of bread and the whole dish of mash), over the period from the second lesion to death or sacrifice.

In no batch did the transplanted rats differ from their respective lesioned control groups in

TABLE I

BODY WEIGHT, FOOD AND WATER INTAKE

Batch/Group		n	Duration of Expt.	Survival	Max. Wt. Loss (gms)	Water Intake (ml/day)	Food Intake (% of provision) Pellet	Bread	Cookie	Mash
I	Control	5	9 days	4 of 5	37.0 ± 6.8	1.3 ± 0.1	3.3 ± 3.3	4.0 ± 4.0	–	–
	LH susp.	8		7 of 8	35.5 ± 2.9	1.2 ± 0.1	10.4 ± 4.3	13.4 ± 5.9	–	–
	La susp.	6		3 of 6	30.6 ± 2.2	1.4 ± 0.3	4.7 ± 3.9	8.0 ± 3.5	–	–
	F with 2,16 df				0.686	0.302	1.098	0.978		
II	Control	8	20 days	3 of 8	68.2 ± 3.4	1.5 ± 0.1	4.0 ± 1.3	9.0 ± 3.8	–	–
	x3 susp.	9		5 of 9	66.3 ± 3.6	1.3 ± 0.1	28.7 ± 9.5	37.1 ± 12.1	–	–
	t with 15 df				0.496		3.39**	2.36*		
III	Control	9	21 days	6 of 9	62.0 ± 5.9	2.2 ± 0.4	4.9 ± 2.9	–	46.0 ± 7.1	35.1 ± 7.7
	x5 susp.	8		7 of 8	51.8 ± 7.6	1.7 ± 0.2	7.8 ± 3.6	–	67.8 ± 6.3	62.3 ± 7.8
	x10 susp.	5		5 of 5	55.2 ± 8.6	1.7 ± 0.1	8.0 ± 8.0	–	69.8 ± 13.8	63.2 ± 9.4
	F with 2,19 df				0.612	0.499	0.150		2.675	4.077*

Regulatory impairments following bilateral mesotelencephalic DA lesions. Each entry represents group mean ± s.e.m. on each measure. The differences between the groups are compared by t test (batch II) or Analysis of Variance (F ratio, batches I and III). *, significant difference between groups $p < 0.05$, **, $p < 0.01$, all other differences not significant. –, not tested, n, number of rats per group.

either survival duration, numbers surviving until sacrifice, maximum body weight loss or water intake (cf. Table I). The values recorded for drinking by any rat can be fully attributed to 1-2 ml spillage at the time of removing and replacing the bottles for weighing.

By contrast to the measures of survival, weight loss and water intake, the multiple suspension grafts were seen to have a significant ameliorative effect on several of the food consumption measures (see Table I). In batch II, the rats with x3 suspensions showed an increased consumption of both chow pellets and wholemeal bread ($t = 3.39$, $p < 0.01$, and 2.36, $p < 0.05$, respectively, with 15 df in each case). In batch III, both the unilateral and bilateral x5 suspensions resulted in an increased consumption of both cookies and palatable wet mash although this was only significant in the latter case ($F = 2.68$ ns, and 4.08, $p < 0.05$, respectively with 2,19 df in each case), although in these groups the rats did not differ in consumption of the pellets. Moreover, neither of the single suspension injections into the lateral hypothalamus or lateral caudate-putamen (batch I) had a significant effect on consumption of pellets or bread. Even where the increase in food consumption was significant in the multiple suspension groups, it remained at too low a level to have any detectable effect on body weight or to promote rats' survival.

Akinesia

The rats of batches II and III were tested for both spontaneous and 0.5 mg/kg amphetamine-induced activity, and the data are shown in Fig. 1.

In batch II, the bilaterally lesioned controls were spontaneously hypoactive, showing approximately 10% of the counts registered in an identical test prior to bilateral lesion (compare Fig. 3C of Chapter IV), and failed to show any activational response to amphetamine. The rats with x3 suspension grafts showed a significant preservation of both spontaneous activity levels ($t = 2.15$ with 8 df, $p < 0.05$) and in the activational effect of amphetamine ($t = 2.05$ with 8 df, $p < 0.05$).

In batch III, the two multiple suspension

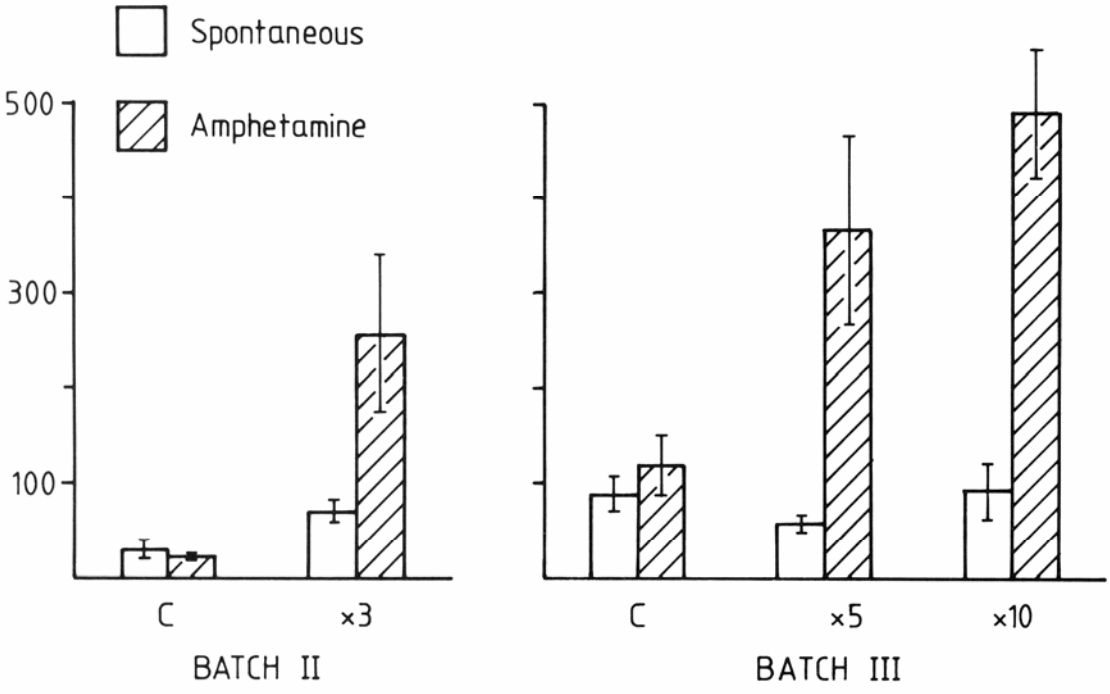

Fig. 1 Activity levels in the Animex activity cage. Histograms represent mean activity counts over 15 min on the spontaneous (open columns) and 0.5 mg/kg amphetamine (hatched columns) tests. Vertical bars indicate the s.e.m. of each column. *, significantly different from the respective control value, $p < 0.05$; **, $p < 0.01$. C, control groups, x3, x5 and x10, the respective multiple suspension groups.

TABLE II

CATALEPSY TESTS

Batch/Group		Activity 1 paw	Activity 4 paws	Catalepsy Forepaws	Catalepsy Hindpaws	60⁰ Grid
II	Control	17.4 ± 7.3	28.5 ± 5.8	49.1 ± 5.0	47.3 ± 6.8	45.7 ± 6.8
	x3 susp.	27.43 ± 9.0	45.7 ± 6.2	40.6 ± 9.5	56.1 ± 3.8	48.8 ± 8.3
	t with 12 df	-0.862	-2.027	0.797	-1.133	-0.294
III	Control	13.3 ± 2.3	39.9 ± 5.5	47.3 ± 5.6	55.1 ± 3.2	59.4 ± 0.5
	x5 susp.	8.8 ± 3.7	46.2 ± 6.20	53.2 ± 3.8	42.9 ± 9.4	47.9 ± 8.0
	x10 susp.	22.2 ± 9.6	43.6 ± 10.0	41.0 ± 11.8	58.2 ± 1.8	37.8 ± 13.6
	F with 2,19 df	0.868	0.209	0.833	1.499	2.098

Catalepsy tests following bilateral mesencephalic DA lesions. Each entry represents mean latency ± s.e.m. in seconds (maximum = 60 sec) on each of the five subjects. Differences between groups are compared by Student's t test (batch II) or Analysis of Variance (F ratio, batch III) and are not significant on any test.

groups did not differ from controls in spontaneous activity level ($F = 0.93$ with 2,18 df, ns), although control levels were somewhat higher than seen previously (compare batch II and ref. 7). Again controls failed to show any activational response to injection of amphetamine whereas both the x5 and x10 groups showed significantly increased levels ($F = 6.36$ with 2,18 df, $p < 0.01$).

Catalepsy

The scores of the rats of batches II and III on the 5 catalepsy tests are shown in Table II. The transplanted rats did not differ significantly from the control groups on any test ($p > 0.05$ in each case).

Sensorimotor tests

The scores of the rats of batches II and III for both orientation and limb use components of the sensorimotor test battery are shown in Fig. 2.

On the orientation component, the overall scores of the rats in the x3 group did not differ from their respective controls (batch II), but the grafted rats manifested a marked bias towards the left, contralateral to the side of the graft (main group effect, $F = 0.076$ with 1,12 df, n.s.; group x side effect, $F = 10.021$ with 1,12 df, $p < 0.01$). The suspension grafted rats in the x5 group also showed a strong bias to the left, contralateral to the side of the transplant, which was markedly reduced in the x10 group with grafts on both sides of the brain, and was absent in the lesioned controls (batch III; group x side effect, $F = 7.17$ with 2,19 df, $p < 0.01$). In this case the grafted rats also showed significantly higher overall orientation scores in comparison to their control group (main group effect $F = 24.09$ with 2,19 df, $p < 0.001$).

On the limb use component, the rats in the x3 group showed higher overall scores and a significantly greater bias to the left, away from the side of the graft, than their control group (batch II; main group effect, $F = 13.30$ with 1,12 df, $p < 0.01$; group x side interaction, $F = 4.64$ with 1,12 df, $p < 0.05$). The x5 rats similarly showed a marked bias to the left which was absent in the rats of the control and the bilaterally grafted x10 groups (batch III; group x side interaction, $F = 6.87$ with 2,19 df, $p < 0.01$; main group effect $F = 2.46$ with 2,19 df, n.s.).

Rotation

The rotation scores on the 4 tests for the rats of batches II and III are shown in Table III.

The unilateral multiple suspension grafts on the right side of the brain (x3 and x5 groups) tended to increase spontaneous turning to the left, but in neither case did this achieve significance.

Tailpinch-induced activation of the rats increased turning rates in all rats (main treatment effect: batch II, $F = 5.65$ with 1,11 df, $p < 0.05$; batch III, $F = 27.30$ with 2,19 df, $p <$

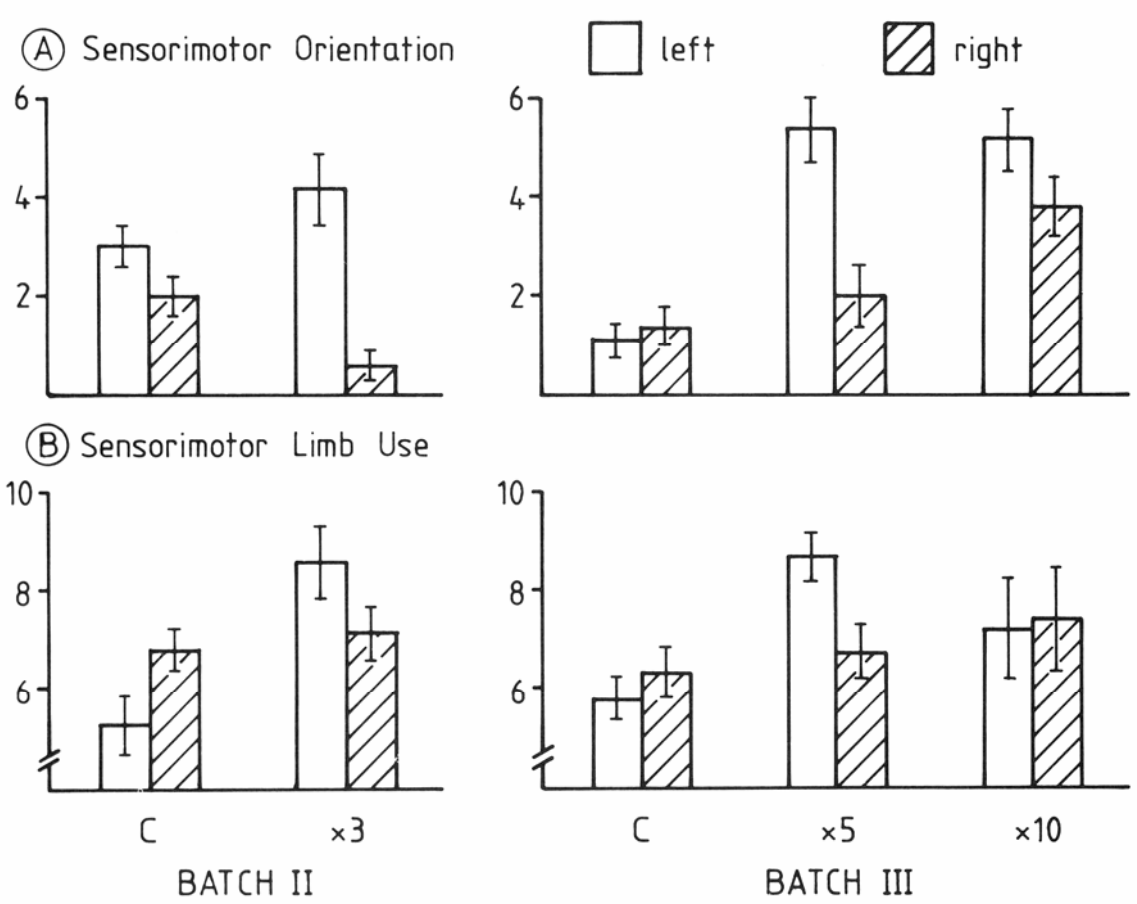

Fig. 2 Sensorimotor test scores on the orientation component (upper) and limb use component (lower) of the test battery. The mean scores on the left (open) and right (hatched) sides of the body are presented separately for each group of rats. Vertical bars indicate the s.e.m. of each column. C, control groups; x3, x5 and x10, the respective multiple suspension groups.

0.001). Again, the rats with multiple suspensions showed higher rates of turning than the lesioned controls, in particular away from the grafted side, but this only achieved significance in the batch III groups ($F = 3.83$ with 2,19 df, $p < 0.05$).

The lesioned control rats did not turn in response to amphetamine, whereas rats with multiple suspension grafts in the x3 and x5 groups turned predominantly to the left, away from the side of the grafts. However, rates were higher in the x3 group, and the effect only reached significance in this group (batch II) ($t = 3.15$ with 11 df, $p < 0.01$).

Control rats turned strongly to the left under apomorphine, presumably reflecting DA receptor supersensitivity on the right side, i.e. the side which had sustained the long-term dopaminergic denervation. This was non-significantly reduced to approximately zero net turns in the x5 group (batch III; $F = 3.07$ with 2,19 df, $p < 0.1$) and significantly reversed in the x3 group (batch II, $t = 4.06$ with 11 df, $p < 0.001$).

DISCUSSION

Previous studies have indicated that embryonic substantia nigra grafted as solid tissue pieces to the borders of the neostriatum of adult rats with bilateral 6-OHDA denervations of the ascending DA pathways is capable of reducing some but not all of the impairments induced by the lesions. In particular, whereas such grafts can reduce the akinesia of bilaterally lesioned rats and induce rotational or sensorimotor bias away from the side of the transplant (4, 6, 7), the catalepsy, body-weight loss and adipsia have remained as severe and debilitating as was seen in control lesioned rats (1, 6, 7). Although a significant reduction in the aphagia, as manifested by nibbling of palatable

TABLE III

SPONTANEOUS, TAIL-PINCH and DRUG-INDUCED ROTATION

Batch/Group		n	Spontaneous	Tail Pinch	Amphetamine	Apomorphine
II	Control	6	1.56 ± 0.74	3.89 ± 0.72	1.11 ± 0.66	12.50 ± 4.27
	x3 susp.	7	3.91 ± 2.33	7.47 ± 2.50	14.26 ± 3.80	-9.04 ± 3.28
	t with 11 df		-0.896	-1.302	-3.149**	4.063**
III	Control	9	1.89 ± 0.88	8.04 ± 1.17	-0.09 ± 0.47	7.17 ± 1.58
	x5 susp.	8	6.66 ± 2.23	14.67 ± 2.69	5.44 ± 1.87	0.35 ± 2.60
	x10 susp.	5	2.87 ± 0.88	13.00 ± 4.45	5.96 ± 6.13	6.18 ± 2.60
	F with 2,19 df		2.387	3.827*	1.905	3.069

Rotation rates on the 4 rotation tests following bilateral mesencephalic DA lesions. Each entry represents net left turns/min ± s.e.m. A negative value indicates net right bias on that measure. The differences between groups of a batch are compared by Student's t test (batch II) or analysis of variance (F ratio, batch III).*,$p < 0.05$;**,$p < 0.01$; n, number of rats tested.

foods, was seen in rats with laterally-placed solid grafts (6), this effect was slight and not sufficient to enable the animal to maintain itself without additional intragastric feeds.

Consequently, the present studies with bilaterally 6-OHDA lesioned rats have focussed upon an attempt to reverse some of the persistent and most severe impairments of the bilateral lesion syndrome, using primarily multiple placements of nigral suspension grafts, rather than an investigation of the effect of different striatal placements *per se*. The results have closely paralleled those seen following the single solid grafts; in particular in the x3 group which showed the most consistent graft survival and extensive outgrowth (see Chapter II). Thus, the rats with x3 and x5 graft placements have shown marked rotational and sensorimotor biases in a manner similar to that seen in unilateral lesioned rats with one intact mesotelencephalic DA system (compare with Figs. 2 and 3A,B of Chapter IV). Likewise, the rats in the x3 group were spontaneously more active than their respective lesioned control group, and both x3 and x5 groups showed a marked activational response to amphetamine which was absent in the control groups.

The x10 group received serial x5 transplants on the two sides of the brain in addition to bilateral lesions. The later implantation in the left hemisphere, 3 months after the right lesion and grafts, and their placement into an intact rather than DA-denervated forebrain are both liable to contribute to lesser fibre outgrowth and reinnervation of the left hemisphere than the right by the time of the second, bilateral lesion. These animals consequently manifested a partial rotational bias away from the first grafted side, in particular under tail-pinch activation. Nevertheless, on several tests, they showed improved performance on both sides of the body with respect to the controls, as in the sensorimotor tests. This provides evidence that suspension grafts implanted into a non-denervated target can become functional almost to the same extent as grafts implanted into a previously denervated target (for further discussion, see Chapter VIII).

By contrast, none of the multiple suspension grafted groups, nor the groups with nigral suspension grafts placed in the lateral hypothalamus or lateral neostriatum, showed any substantial recovery of the regulatory impairments, which are the most severe component of the bilateral 6-OHDA lesion syndrome. None of the transplant groups tested had improved in terms of survival rate, body weight loss or water intake. The palatability of available foods has been reported to be an important factor in the recovery from aphagia in bilaterally lesioned rats (13) and in the present experiments the more palatable mash and biscuits were more extensively consumed by all rats than the less palatable dry pellets and bread. Several of the transplanted groups with multiple suspensions showed significant improvement in the consumption of the various foods with respect to controls, and in particular of the more

palatable varieties. Nevertheless, the degree of recovery was slight in the context of the profound impairments induced by the bilateral lesions. Throughout the experiments the rats remained dependent upon intragastric feeding for survival, and body weight remained as reduced as the control lesioned rats.

Thus, at present, no single or combined forebrain graft placement has been identified which will sustain a substantial recovery of the severe aphagia, adipsia and body weight decline induced by forebrain DA denervation. It is plausible that some other critical forebrain DA region has not yet been provided with an essential reinnervation, or that the overall magnitude of DA recovery in the denervated forebrain has been insufficient. The biochemical data in Chapter III showed that the average DA content restored by a 3 x 3 µl nigral suspension graft represented between 5 and 13% of the total forebrain DA content in one hemisphere. The reinnervations achieved in the present x3 and x5 groups are probably not much higher than that. Also, it should be pointed out that since no protective treatment (e.g. by desipramine) was used, the 6-OHDA lesion employed in the present studies produced severe depletions (80-99%) also of forebrain noradrenaline. This additional defect was of course not compensated for in the present set of experiments.

An alternative explanation for the failure of the grafts to compensate for the ingestive impairments is that the ectopic placement of the grafts, remote from the normal location of intrinsic DA neurones in the ventral mesencephalon, may result in the failure of the grafted neurones to establish afferent connections which normally regulate their function. In this respect the role of the DA neurones in the neural circuitry involved in the mediation of consummatory behaviours may be organized differently to those underlying other behavioural phenomena associated with dopaminergic function, such as rotation, sensorimotor attention or akinesia. These issues can only be resolved when a clearer understanding has been achieved of the role of DA neurones in the regulation of food and water intake. Indeed, it is plausible that the nigral transplantation paradigm may itself contribute to the development of such deeper understanding of dopaminergic functions in the brain.

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