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BEHAVIOURAL RECOVERY FOLLOWING TRANSPLANTATION OF SUBSTANTIA NIGRA IN RATS SUBJECTED TO 6-OHDA LESIONS OF THE NIGROSTRIATAL PATHWAY. I. UNILATERAL LESIONS

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SUMMARY

The ability of embryonic substantia nigra transplants to compensate for behavioural deficits induced by unilateral destruction of the nigrostriatal dopamine pathway has been investigated in adult rats. Six days following unilateral 6-OHDA lesions of the nigrostriatal pathway, the adequacy of the lesion was assessed by measurement of the intensity of ipsilateral amphetamine-induced rotation. All rats then received surgical cavities in the cortex overlying the head of the caudate-putamen on the lesioned side. In 51 rats, transplants of embryonic substantia nigra were placed on the dorsal surface of the caudate-putamen, and the remaining 19 rats served as unilateral lesioned controls. Behavioural testing was conducted approximately 3 months after transplantation: (a) the transplant animals alone showed a marked reduction in ipsilateral rotation induced by 5 mg/kg amphetamine ('compensation'); (b) although both transplanted and control rats expressed equal contralateral rotation at a dose of 0.25 mg/kg apomorphine, the transplant animals alone showed a marked reduction in rotation at a lower dose of 0.05 mg/kg; (c) the transplanted rats showed less asymmetry in spontaneous rotational behaviour than controls, and the asymmetry was further reduced by mild tailpinch; (d) when tested for spontaneous choice behaviour in a T-maze, control rats showed 97% selection of the arm ipsilateral to the 6-OHDA lesion, whereas the transplanted rats that were well compensated on the amphetamine rotation test turned to the contralateral side on 30–40% of choices; (e) no transplant-induced changes were found in contralateral sensory inattention on a sensorimotor test battery, whether tested spontaneously or under mild tailpinch-induced activation. The results support the conclusion that dopaminergic reinnerva-

tion of the dorsal neostriatum is capable of inducing functional recovery in many, but not all, behavioural tests which involve side choice or bias, not only after pharmacological activation but also in the spontaneously behaving animal.

INTRODUCTION

Unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal pathway, which cause substantial dopaminergic denervation and profound reduction in dopamine (DA) content in the striatum ipsilateral to the lesion, produce a long-lasting behavioural syndrome that has been well-characterized in the rat. Firstly, injections of the DA-releasing agent, amphetamine, induce intense rotational behaviour in the direction of the lesion[10,23,25], whereas the DA receptor agonist, apomorphine, induces contralateral rotation[10,24]. Secondly, Marshall et al.[15,17] have developed a neurological test battery which reveals impairments in the orientation to sensory stimuli and coordination of motor responses on the side contralateral to the lesion. These deficits may be characterized as a failure in sensorimotor integration, i.e. the organization of coordinated responses directed towards external stimuli[13,15,16,20,22].

Glick has reported that injections of amphetamine will induce stable rotation even in intact rats, and that this reflects imbalances in endogenous concentrations of striatal DA[9]. Not only do rats tend to rotate towards the side contralateral to the striatum with higher DA content, but both paw-preference in a lever-press operant task and the arm of choice in a T-maze escape task also reflected enhanced DA levels in the contralateral striatum[8,26]. It may therefore be predicted that a unilateral nigrostriatal lesion would result in a decline in choices of the contralateral side in spontaneous spatial choice behaviour.

Previous experiments[4,5] have demonstrated that dopamine-rich tissue, taken from the substantia nigra region of rat embryos and transplanted to a cortical site in 6-OHDA-lesioned rats, can extensively reinnervate the denervated striatum of adult host rats. Perlow et al.[19] have demonstrated a similar reinnervation from nigral transplants placed in the lateral ventricle. Furthermore, the extent of ingrowth into the striatum in unilaterally lesioned rats was found to be paralleled by a marked reduction in amphetamine-induced rotation. The ability of the transplant to reduce apomorphine-induced rotation is uncertain: whereas Perlow et al.[19] obtained a partial reduction in turning in rats bearing intraventricular nigral transplants, Björklund et al.[4], using a higher dose, failed to replicate this finding.

In the previous study[4,5], transplant-induced restoration of function was dependent upon amphetamine activation, whereas no recovery was found in the sensorimotor test battery under a spontaneous level of activation. In order to determine whether the activation level or the particular test underlies this difference, the present experiment investigated in more detail the effects of nigral transplants on several tests of nigrostriatal function, following 6-OHDA lesion. Tests were conducted not only under spontaneous and pharmacological activation, but also under tailpinch, since this stimulus has an activating effect in many respects similar to amphetamine[1,

[2,11]. Moreover, tailpinch is known to provide a partial reversal of some deficits following 6-OHDA lesions[16,21]. The present results show that nigral transplants can reverse functional impairments not only after pharmacological activation but also in the spontaneously behaving rat.

MATERIALS AND METHODS

Subjects

Young adult female rats of the Sprague–Dawley strain were used as transplant recipients and operated controls. They were 180–200 g at the start of the experiment, and were housed in cages of 3–8 rats with ad libitum food and water available throughout. The donors were 16–17-day-old foetuses of the same inbred strain.

Lesions

All animals were given unilateral right nigrostriatal 6-OHDA lesions at the start of the experiment. The rat was anesthetized by i.p. injection of 40 mg/kg Brietal (Lilly). 8 μg 6-OHDA HCl (free-base weight) was dissolved in 4 μl ascorbate solution (0.2 mg ascorbic acid per 1 ml 0.9% saline) and injected stereotaxically over 4 min. Injection coordinates were anterior 4.4 mm behind bregma, lateral 0.9 mm from midline, and vertical 7.5 mm below dura, with the incisor bar set 2.3 mm below the intra-aural line. This placement is at the rostromedial aspect of the substanita nigra, where the DA axons of the nigrostriatal, mesolimbic and mesocortical pathways assemble. Previous biochemical analysis of identical lesions in other animals has generally shown greater than 97% depletions of DA content in the ipsilateral caudate-putamen.

Transplantation surgery

One week following the unilateral 6-OHDA lesions 70 rats (which all satisfied the amphetamine-induced rotational criterion below) were again anaesthetized and a 2 $\times$ 3 mm cavity was made by suction under visual guidance through the right anterior parietal cortex and corpus callosum, ipsilateral to the 6-OHDA lesion, in order to expose the dorsal surface of the head of the caudate-putamen. The wound was filled with gel-foam and sutured close. Approximately 3 weeks later, when a vessel-rich pia had formed over the walls of the cavity, the wound was reopened in 51 animals, and they received transplants comprising the ventral mesencephalic tegmentum from 16–17-day-old rat embryos (crown–rump length 15–20 mm) which contain the developing dopamine neurones of the bilateral substantia nigra–A10 system. The remaining 19 rats were not subjected to this second stage of surgery and provided unilateral lesion plus cavity controls.

Behavioural testing

All behavioural tests, apart from the initial and final amphetamine test, were conducted between 4 and 5 months after the 6-OHDA lesion (3–4 months after transplantation). The entire experimental material comprised 51 transplanted and 19 lesioned control rats. Half way through the behavioural test period 14 transplanted

and 10 control animals were taken for bilateral lesion[7], and the remainder of the tests on unilateral lesioned animals were conducted with reduced groups of 37 transplanted and 9 control rats. The following tests were conducted on the full groups unless otherwise stated.

Rotation testing

All rotation tests were conducted, after Ungerstedt and Arbuthnott[25] in hemispheric perspex bowls supported on metal rings, as described previously[3,4].

The initial amphetamine test was conducted 6 days after the 6-OHDA injection. Rats were injected with 5 mg/kg Met-amphetamine (dissolved in 5 mg/ml isotonic saline) and immediately placed in the rotometer bowls. Rotation was measured by sampling a 1-min interval every 10 min over 1 h and recording the number of full turns in each direction separately. Only animals that achieved a net score of $\geq$ 50 ipsilateral turns (after subtraction of any contralateral turns) were used in subsequent testing or underwent transplantation surgery.

Approximately 4 months after the initial test (3 months after transplantation), a second identical amphetamine-induced rotation test was carried out. Apomorphine-induced rotation was tested 1 week later. Rats were injected s.c. with 0.25 mg/kg apomorphine (dissolved in 0.25 mg per 1 ml isotonic saline), and quarter-turns in each direction were recorded in 2-min sample intervals every 10 min over a 30-min period following injection. The reduced groups were tested again under apomorphine (0.05 mg/kg) two weeks later, and finally, 7 months after the initial lesion (6 months after transplantation), they received a third amphetamine (5 mg/kg) test.

Spontaneous and tailpinch-induced rotation were recorded in a single test session, separated by at least two days from either pharmacological test. The rat was placed in the rotometer bowl and quarter-turns in each direction were recorded over a 3-min period. The animal was returned to its home-cage for 10 min, after which a paper-clip was attached to its tail, 2–3 cm from the tip. The paper-clip had plastic adhesive tape bound around each prong, so as to provide mild, even pressure. The clip was suspended from above the centre of the bowl by a strong thread, which served to lift the rat's tail off the floor and inhibit tail-directed biting behaviour. The rat was immediately placed back in the rotometer bowl and quarter-turns recorded for a further 3 min.

Sensorimotor testing

The sensorimotor test battery was based on the neurological test battery of Marshall and Teitelbaum[15]. It involved a series of tests measuring both the orientation towards external stimuli applied to either side of the body, and the coordinated use of each of the limbs in a variety of situations.

Orientation tests. In each of the following tests the stimulus was applied first to one side of the body and then the other. The orientation of the head to the stimulus was rated on a 3-point scale: absent (0), weak (1) or strong (2). (a) Whisker touch — the vibrissae were lightly touched, using a wooden probe approached from behind and below the rat to reduce visual cues. (b) Wooden probe — the probe was lightly brushed

against the snout of the animal, approaching from the side. (c) Olfaction — a swab of cotton soaked in ammonia solution was brought slowly towards the nose of the animal from either side and below. (d) Somaesthesis — a light pin-prick was applied to 6 different sites on the lateral surface of the body, in a rectangular array of dorsal and ventral points at rostral, middle and caudal levels.

Limb use tests. In each of the following tests coordinated limb use was rated for each limb as appropriate on a 3-point scale: ineffectual (0), poor (1) and good (2). On tests involving a latency measure the times were divided into 3 ranges: < 3 sec (2), 3–10 sec (1), and > 10 sec (0). (a) Catalepsy — the animal was placed with both forelimbs on a wooden block 7 cm above the bench surface, and the latency to move each forelimb was recorded. The procedure was then repeated with the hindlimbs placed on the block. (b) Forelimb placement — the rat was held, head downwards, and slowly lowered towards the surface of the bench. The speed and accuracy with which the forelimbs were extended and placed on the approaching surface were recorded. (c) Forelimb support — the experimenter grasped the rat by one forepaw and raised the animal off the bench. The latency until the animal used its free limbs to pull itself up and climb onto the hand was recorded. (d) Climbing grid — a metal grid of horizontal rungs was clamped with its upper edge flush with the bench surface. The rat was placed on the grid, facing upwards, and the ability to grasp the rungs, accurately place, and support the body weight was rated for each of the limbs. (e) Limb withdrawal — the animal was held vertically, head upwards, and each paw lightly pinched with forceps. The speed and strength of limb withdrawal was rated for each fore- and hindlimb. (f) Wooden probe — following the orientation measure to the wooden probe, the probe was gently inserted into the side of the animals mouth. Grasping of the probe with the ipsilateral forepaw, and biting, were recorded. (g) Olfaction — following the orientation measure to the ammonia swab, which is highly noxious for a normal animal, the speed and strength of head and body withdrawal were rated.

In addition to the above tests, the battery was preceded by observation of the animal on the open bench surface, and any spontaneous asymmetry or rotation noted. The test battery provided a total of 9 orientation measures and 10 measures of limb-use for each side of the body. Scores on the various tests were summed to provide, for each rat, an overall score of orientation (maximum = 18) and of limb-use (maximum = 20), for each side of the body.

All 70 transplant and control rats were given the initial sensorimotor test battery, approximately 3 months after the transplantation. The reduced groups were retested on the sensorimotor battery one week later under tailpinch, and again after two days under a low dose of amphetamine (0.5 mg/kg injected i.p., 10 min prior to testing).

Maze testing

A T-maze of conventional design, with aluminium walls and a varnished wooden floor, was used throughout testing. At the end of each side arm a further short right-angled turn constituted the goal box. Three tests of spontaneous alternation, separated by at least two days between each test, were given to each rat. Each test consisted of

two trials separated by a 5–10-min interval, during which time the rat was returned to its home-cage. On each trial, the rat was placed at the beginning of the start arm and immediately released. It was allowed to move freely around the maze until it entered one goal box, when the trial was terminated, and the side and latency recorded. If the rat had not left the start alley within 2 min it was given a gentle push, which in all cases proved sufficient to initiate locomotion. All animals were first tested in the absence of any manipulation; on the second test tailpinch was applied by affixing the paper-clip immediately prior to each trial; on the third test 0.5 mg/kg metamphetamine was injected 10 min before the first trial.

RESULTS

Amphetamine-induced rotation

Seventy of the 6-OHDA-lesioned rats achieved the criterion of at least 50 ipsilateral (right) turns during the sampled 6 min in the initial amphetamine rotation test (equivalent to a mean rotation score of > 8 turns/min). This criterion signifies an almost complete ($> 97\%$) dopaminergic denervation of the ipsilateral neostriatum[4]. All these animals received surgical cavities one week later, and 51 subsequently received transplants, while the remaining 19 rats served as operated controls.

Fig. 1 presents the mean number of ipsilateral turns in the transplant and control groups on the initial amphetamine rotation test, conducted before transplantation, and on the second test performed 3 months after transplantation. In all cases any turns to the contralateral side were subtracted from the ipsilateral total to produce each animal's final score. The overall mean rotational response was reduced by 75% in the transplanted group ('compensation'), whereas strong rotation remained in the non-transplanted control animals. Both the main effects due to group and to test, and their interaction, were significant ($F = 17.27$, $F = 117.43$, $F = 73.77$ respectively, with 1,68 df, $P < 0.001$ in each case.) Although the transplant group showed slightly

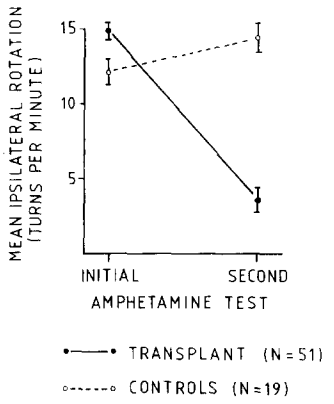

Fig. 1. Net ipsilateral rotation counts (after subtraction of contralateral turns) in the transplant and control groups, on the two amphetamine rotation test, 6 days ('initial') and 4 months ('second') after the unilateral 6-OHDA lesions. The transplants were inserted after the initial test. Values are means ± S.E.M.

higher rotation scores on the initial test, the major effect is due to a considerable reduction in ipsilateral rotation between the two tests in the transplant group, in contrast to a slight increase in rotation among controls. In fact, 49 out of the 51 animals in the transplant group showed lower net ipsilateral turning on the second test, whereas only 7 out of the 19 controls showed any decrease ($\chi^2 = 25.93$ with 1 df, $P < 0.001$).

Since in our earlier experiments[4] reduction in amphetamine-induced rotation was found to be directly proportional to the degree of dopaminergic reinnervation of the caudate-putamen by the transplant, the present transplant group was divided into 4 subgroups on the basis of rotation rates (ipsilateral turns/min) on the second test:

uncompensated ('U' < rate > 10, mean = 12.6, n = 9)
partially compensated ('P', rate 5–10, mean = 7.3, n = 9)
compensated ('C', rate 0–5, mean = 1.8, n = 20)
overcompensated ('C+', rate < 0, mean = —2.7, n = 13)
and for comparison purposes:
control group ('Cont.', mean = 14.3, n = 19)

It can be seen that a considerable degree of compensation was found in the majority of transplanted animals over the 3-month post-transplantation period, and in only 18% of the rats was the transplant ineffective in producing a substantial reduction in the amphetamine-induced rotation. The 4 subgroups did not differ in their rotation scores on the initial test.

A third amphetamine test, 7 months after the initial lesion, was conducted on the reduced groups. No control rats showed any change in rotation between the second and third tests. By contrast the degree of compensation of the transplanted rats continued to increase between the second and third tests. Table I presents the subgroups (defined as above) into which the rats were categorized on the second and third tests. Whereas 19 of the 36 animals fell into a category representing a higher degree of compensation on the third test, only 3 animals showed less compensation (sign test, $P < 0.001$). In particular, whereas 9 of the 51 transplanted rats were still

TABLE I

Subgroups (see text for details) into which the transplanted rats fell on the second and third amphetamine tests (3 months and 6 months post-lesion respectively)

No control rats showed any change in compensation between the second and the third tests. C+ = overcompensated, C = compensated, P = partially compensated, U = uncompensated.

	Third test				
	n	*C+*	*C*	*P*	*U*
Second test					
C+	7	6	1	–	–
C	12	6	4	2	–
P	9	–	6	3	–
U	9	1	4	2	2

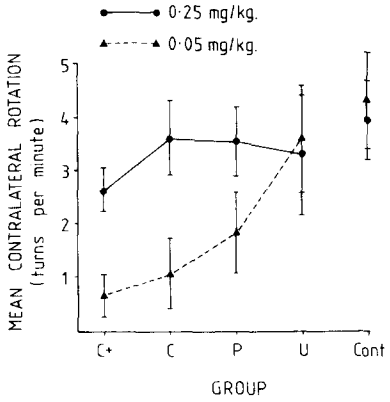

Fig. 2. Net contralateral rotation counts (after subtraction of ipsilateral turns) in the reduced transplant subgroups and unilateral lesioned controls under the two doses of apomorphine (0.25 and 0.05 mg/kg). The 4 transplant subgroups are defined in terms of the degree of compensation of amphetamine-induced rotation: C+, overcompensated; C, compensated; P, partially compensated; U, uncompensated, as described fully in the test. Cont, non-transplanted unilateral lesion controls. Values are mean 360° turns ± S.E.M.

uncompensated 3 months after transplantation, all but two had developed some compensation by the final test 6 months after transplantation.

Apomorphine-induced rotation

On the first apomorphine test (0.25 mg/kg), conducted on the full groups, all animals showed contralateral rotation and the transplanted rats did not differ from controls (mean net contralateral turns = 3.42 and 3.52 full 360° turns/min respectively, $t = 0.15$ with 67 df, $P > 0.8$). The results for the reduced group on the second apomorphine test (0.05 mg/kg) are shown in Fig. 2, together with their results on the initial test for comparison. Those animals that show the highest degree of compensation on the amphetamine test manifest correspondingly greater reduction in contralateral rotation in response to the lower dose of apomorphine (analysis of variance reveals a significant difference between subgroups, $F = 4.94$ with 4, 39 df, $P < 0.005$). In the C+ and C groups the rotational response to the low dose was thus reduced by as much as 75–80%.

Spontaneous and tailpinch-induced rotation

The spontaneous and tailpinch-induced rotation of the various subgroups is shown in Fig. 3. The probability of a left turn (contralateral to the lesion) in the spontaneous test is higher in the transplant than the control group ($F = 5.27$ with 4, 65 df, $P < 0.0002$) which was due to a greater number of turns contralateral to the lesioned side in the transplanted rats. Whereas tailpinch had little effect on the asymmetry of the controls, it reduced the side bias in the transplant animals ($F = 17.49$ with 1, 65 df, $P < 0.0002$), by further enhancing the number of contralateral turns. In fact, under tailpinch, the transplanted animals approach an asymmetrical score of 50% left turns (as in normal unoperated rats), whereas the control rats remain below the 25% level.

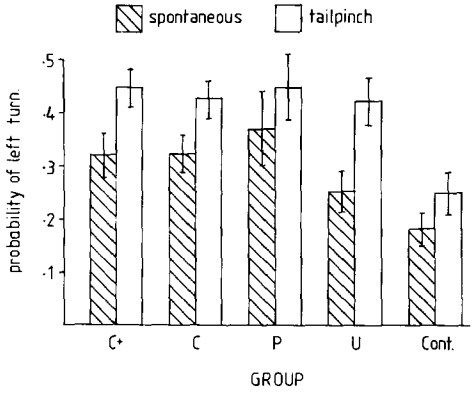

Fig. 3. Proportion of turns to the left recorded from the different transplanted subgroups and non-transplanted controls in the spontaneous (shaded columns) and tailpinch-induced (open columns) rotation tests. The 4 transplant subgroups are defined in terms of the degree of compensation of amphetamine-induced rotation: C+,'overcompensated; C, compensated; P, partially compensated; U, uncompensated, as described fully in the text. Cont, non-transplanted unilateral lesion controls. Values are means ± S.E.M.

This behavioural asymmetry was also apparent when observing the freely-moving rat on a bench surface. Non-transplanted controls assumed a characteristic curved postural asymmetry, and when activated (e.g. by tailpinch) they showed a clear preference to move towards their right side. These signs of asymmetry were less pronounced in the transplant group, and many of the rats that showed good compensation in the amphetamine test manifested quite normal posture and locomotion on the bench.

Arm-preference in T-maze

The number of animals showing left (contralateral) choices in each of the maze tests is presented in Table II. Sixteen of the control animals chose the ipsilateral arm on every possible occasion, and the remaining 3 rats chose the contralateral arm only once each, out of 6 possible opportunities. By contrast the transplanted rats frequently

TABLE II

Arm preferences in the T-maze, giving number of rats in each group manifesting each possible combination of right (R) and left (L) turns on the two trials for each test

Tpl = transplant group; Cont. = controls.

		RR	*LR*	*RL*	*LL*	*T*
Spontaneous	Tpl.	32	8	5	6	51
	Cont.	18	0	1	0	19
	Total	50	8	6	6	
Amphetamine	Tpl.	33	5	8	5	51
	Cont.	18	0	1	0	19
	Total	51	5	9	5	
Tailpinch	Tpl.	34	3	9	5	51
	Cont.	18	1	0	0	19
	Total	52	4	9	5	

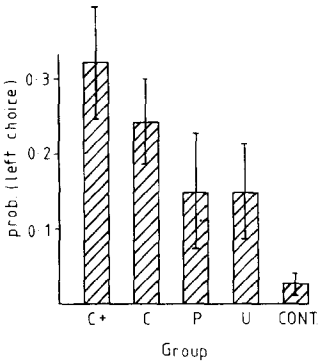

Fig. 4. Proportion of total choices to the left arm by the transplant subgroups and controls in the 3 maze tests combined. The 4 transplant subgroups are defined in terms of the degree of compensation of amphetamine-induced rotation: C+, overcompensated; C, compensated; P, partially compensated; U, uncompensated, as described fully in the text. Cont, non-transplanted unilateral lesion controls, Values are means ± S.E.M.

chose the arm contralateral to the lesion, whether tested under spontaneous levels of arousal, or activated by tailpinch or low doses of amphetamine. In all 3 tests the transplant group showed a significantly higher proportion of left turns than controls (spontaneous: $2\hat{I} = 11$; amphetamine: $2\hat{I} = 10$; tailpinch: $2\hat{I} = 10.27$; with 3 df, $P < 0.025$ in each case, by the Information statistic, distributed as χ^2–ref. 12).

Since the proportion of left choices in either group did not differ between the 3 tests, the total number of left choices for each rat over all 3 tests were combined, in order to compare maze-choice performances with the amphetamine rotation scores. The mean number of left turns (maximum = 6) of rats in each subgroup is shown in Fig. 4. The difference between groups is significant ($F = 3.50$ with 4,65 df, $P < 0.02$), which is due to an increasing proportion of left choices (contralateral to the lesion), the greater the degree of compensation in amphetamine-induced rotation.

The results in the T-maze are supported by qualitative differences noted in the rats' behaviour in the maze. The unilateral lesioned controls directed their attention and sniffing almost exclusively to the right wall, and kept their body close to this wall when traversing the maze. The transplanted rats directed their attention to both walls of the maze and ran down the centre of the alley. On reaching the choice point, the controls would bend their body, almost automatically, round the right-hand corner, whereas the transplant rats frequently stopped and sniffed in both directions before deciding which arm to enter.

Sensorimotor tests

The orientation and limb-use scores in the sensorimotor test battery are shown in Fig. 5. Although highly significant differences were found between ipsilateral and contralateral scores on both the orientation and limb use components ($F = 139.35$ and 118.85 respectively, with 1, 67 df, $P < 0.001$ in each case), no differences at all were found between the controls and the transplanted animals. Although tailpinch pro-

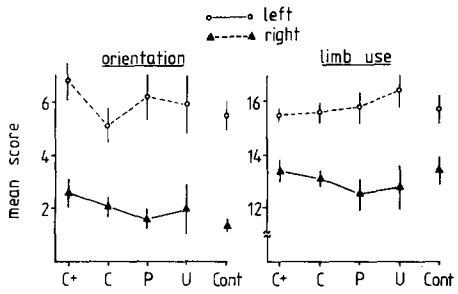

Fig. 5. Mean scores of the transplant subgroups and the non-transplanted controls on orientation (left panel) and limb-use (right panel) components of the sensorimotor test battery. The two sides of the body are represented separately. The 4 transplant subgroups are defined in terms of the degree of compensation of amphetamine-induced rotation: C+, overcompensated; C, compensated; P, partially compensated; U, uncompensated, as described fully in the text. Cont, non-transplanted unilateral lesion controls. Values are means ± S.E.M.

duced an overall increase in orientation scores between the groups. The injection of 0.5 mg/kg amphetamine in these animals induced marked hyperactivity. As a consequence the test scores were highly unreliable, and were not analyzed further.

DISCUSSION

In previous studies it has been demonstrated in rats that transplants of embryonic substantia nigra can reinnervate substantial portions of the dopaminergically denervated neostriatum[3,4,19]. Histological analysis of the present transplant is not available, since most of the animals were subsequently included in a further experiment[7]. However, the compensation of the amphetamine induced rotation response has been found to be directly correlated with the density and extent of the dopaminergic reinnervation from the transplant into the dorsal caudate-putamen[4]. As the operative procedures here were identical to those employed in the previous experiments, we have taken the degree of compensation of amphetamine-induced rotation as a good index of the extent of the dopaminergic reinnervation for each animal.

The analysis of rotational compensation under amphetamine in transplanted rats has been extended in the present study by the inclusion of a unilaterally lesioned control group. In agreement with previous reports, no compensation was found in amphetamine-induced ipsilateral rotation of the non-transplanted control rats over the 6 months of testing, indicating that the unilateral 6-OHDA lesions of the nigrostriatal DA pathway do indeed produce, in the absence of a transplant, long-lasting amphetamine-induced rotational asymmetry[10,23,25]. Of the 19 unilateral-lesioned control rats, 7 showed a decline in number of ipsilateral turns on the second test, and of these only two fell within the 'partially compensated' range. These two animals had relatively low scores on the first test, and the actual size of the decline in counts was small in each case. By contrast, all but two of the 51 transplant animals manifested a decline in net ipsilateral rotation scores between the first and second tests,

and about 60% of the animals achieved the operational criterion of 'compensaton' (C and C+ groups) by 3 months after transplantation. The compensation of amphetamine-induced rotation continued to develop between 3 and 6 months after transplantation, such that by the final test less than 20% of transplanted rats had failed to achieve good compensation, and only two animals remained in the uncompensated category. This not only confirms previous results[3–5], but also demonstrates that the majority of animals in the present experiments had well-functioning transplants.

Apomorphine-induced contralateral rotation was found to be reduced in those animals which similarly compensate in the amphetamine rotation test only at the lower dose of 0.05 mg/kg. In agreement with our previous results, the higher dose of 0.25 mg/kg apomorphine failed to differentiate between the transplant and lesioned control rats. This can be interpreted in terms of a partial reduction in apomorphine supersensitivity following dopaminergic reinnervation of the neostriatum, producing a shift in the apomorphine dose–response curve to the right[18]. In particular, with the low dose of apomorphine the degree of reduction of the rotational response was proportional to the degree of compensation seen in the amphetamine test, indicating that the reduction in DA receptor sensitivity in the denervated neostriatum correlates with the extent of reinnervation from the transplant. This observation is therefore compatible with a partial restoration of normal dopaminergic transmission in the reinnervated neostriatum of the transplanted rats, and resolves the descrepancy between our initial report[4] and the results of Perlow et al.[19].

The present study is the first to show a reversal of lesion-induced deficits by nigral transplants in spontaneous behaviour. This was demonstrated for spontaneous rotational asymmetry as well as in spontaneous choice behaviour in a T-maze.

Ungerstedt[23] has previously reported that rats subjected to a unilateral 6-OHDA lesion of the nigrostriatal pathway exhibit long-lasting spontaneous turning towards the lesioned side. This is further supported by the observations of Glick et al.[8] that rotational bias in non-lesioned rats reflects an imbalance in dopaminergic transmission in the two nigrostriatal pathways. Consistent with these findings, the non-transplanted control animals in the present experiments expressed a persistent, strong ($> 80\%$) bias in turning towards the lesioned side. This bias was reduced in the transplanted rats, which was due to an increase number of turns to the left side (contralateral to the lesion), i.e. to the side under the influence of the reinnervated caudate-putamen. Thus the transplant is able to boost turning activity on the contralateral side of the body in a spontaneously active rat almost up to the level maintained by the intact side, and substantially reduce the asymmetry that remains in the unilaterally lesioned controls. Tailpinch served to further decrease the asymmetry in the transplant rats.

Thus both tailpinch and amphetamine served to reduce the rotational asymmetries in transplanted rats, in agreement with other reports of the similarity of their behavioural effects in normal rats[1,2,11]. It is not at present understood exactly how tailpinch is able to modify brain function. Chiodo et al.[6] have recently shown electrophysiologically that tailpinch activates nigrostriatal neurones, therefore one possibility is that tailpinch activates the transplant via an afferent innervation from the host brain. Nothing is yet known about such connections, although anatomical studies

are currently in progress to characterize such inputs. Alternatively, the DA transplant could serve a 'permissive' function which is not related to its afferent connections. Such a mechanism would imply that DA release in the neostriatum facilitates tailpinch induced behaviours mediated at other brain sites.

In addition to rotational compensation, the transplant animals showed a clear reduction in bias in the arm-choice experiments in the T-maze. Normal, unstressed rats generally alternate their responses given two unreinforced trials in such a situation. In view of the sensorimotor inattention and side preferences shown after unilateral 6-OHDA or lateral hypothalamic lesions it may be expected that the lesions employed here would induce a marked bias towards selection of the side of the body ipsilateral to the lesion, although it is not believed that this has been previously demonstrated. This was confirmed for the control rats, and appears to provide another reliable test for unilateral DA deficiency. Moreover, observation of the rats in the maze suggested that the deficit was not simply due to motor asymmetry but to lack of attention to contralateral stimuli. The transplant rats showed increased attention to both sides of their environment, and a corresponding increase in the selection of the left as well as the right arm. Because the different manipulations in the 3 tests influenced maze choice behaviour in the same way, the results were combined, and it was demonstrated that the number of contralateral choices was proportional to the degree of compensation in the amphetamine rotation test. Therefore, it may be inferred that the degree of restitution of contralateral choice behaviour in the T-maze was proportional to the extent of DA reinnervation of the denervated striatum. From these results, we propose that spontaneous choice behaviour in the T-maze may provide another sensitive measure of recovery from 6-OHDA lesions.

By contrast to the rotation and T-maze data, the presence of the transplant had no significant effect on any aspect of performance in the sensorimotor test battery. In a previous experiment we found that the presence of the transplant resulted in worse performance than found in the control animals[4], which was interpreted in terms of a block of spontaneous recovery in the transplant group. This has not been replicated in the present experiment: the control animals were as impaired as the transplanted rats, and it is likely that the 6-OHDA lesions in several of the rats of the previous small control group were suboptimal. The surgical and selection procedures now employed generally ensure greater than 97% DA depletion in the ipsilateral striatum. It appears that recovery of the contralateral sensory neglect phenomenon in unilateral 6-OHDA-lesioned rats only takes place with incomplete lesions, and Marshall[14] has recently reported that the severity and duration of the syndrome correlates with the extent of the striatal DA depletion.

The failure to find recovery in the sensorimotor tests may in part be due to the test battery used being a less sensitive measure of recovery in sensory inattention than is the choice behaviour in the T-maze. The difference in recovery between the T-maze and sensorimotor tests may, however, also reflect the degree of specificity of information to be processed by dopaminergic mechanisms in the performance of any particular task, or else the particular subpopulation of denervated neostriatal cells which become reinnervated.

In conclusion, the present results provide for the first time evidence that intracerebral transplant of DA-rich embryonic tissue can compensate not only for the asymmetries found in pharmacologically-induced rotational tests following unilateral 6-OHDA-induced lesions of the ascending dopamine systems, but also for the asymmetries induced by 6-OHDA lesions under physiological conditions of activation, most notably in spontaneous rotation and choice behaviour. Tailpinch served to further reduce the asymmetry in rotation. Nevertheless, not all the deficits induced by the 6-OHDA lesion are reduced by the present transplant technique, most notably the sensory inattention as measured by the sensorimotor test battery. In view of these limitations, caution must therefore be exercised in drawing any conclusion about the imminent applicability of intracerebral transplant methods in the treatment of human clinical disorders.

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