900 mg dl$^{-1}$ before absorption to 550 mg dl$^{-1}$ without diminishing the anti-IFN titre. Preincubation of RITA cells with the absorbed serum did not inhibit HuIFN-$\beta$ activity.

To demonstrate that the HuIFN-$\beta$ neutralizing activity was in fact due to antibodies, activity was determined after sucrose density-gradient centrifugation of FS-4 absorbed serum from S.R. Total protein content ($E_{280}$) and concentrations of IgG in sucrose density-gradient fractions of serum are shown in comparison with anti-HuIFN-$\beta$ titres in Fig. 1. The peak of anti-HuIFN-$\beta$ activity was found in fraction 4 (Fig. 1*a*) which correlates with the IgG (Fig. 1*b*) and the IgA peak (not shown). The IgM peak (fraction 8) showed no measurable HuIFN-$\beta$ neutralizing activity. In addition, serum IgG from patient S.R. was purified by ion-exchange chromatography to demonstrate that the anti-HuIFN-$\beta$ activity was due to IgG antibodies. FS-4 cell-absorbed serum was precipitated with saturated ammonium sulphate, *p*H 6.5, and the precipitate dialysed against 0.02 M phosphate buffer, *p*H 8.0. IgG was prepared from the precipitate by chromatography on DEAE-Sephacel column (Pharmacia, Uppsala, Sweden) equilibrated with the same buffer. The protein peak passed through with the first buffer volume contained most of the anti-HuIFN-$\beta$ activity. Immunodiffusion showed that there was no IgA or IgM contamination of the purified IgG. Immunoelectrophoreses of 0.7 mg IgG ml$^{-1}$ against rabbit anti-human serum protein (Medac, Hamburg) revealed only one component in the IgG preparation.

The first successful demonstration of antiserum to an IFN was reported by Paucker and Cantell[3]. However, studies on the antigenicity of IFN have shown that they are not antigenic in homologous systems[4]. In a number of clinical trials no neutralizing activity could be demonstrated in the sera of any patients, even after treatment with several millions of human IFN units[5,6].

This study presents a strong case for the presence of antibodies to HuIFN-$\beta$ in the serum of patient S.R. treated successfully with $\sim 5 \times 10^8$ HuIFN-$\beta$ units. A number of other patients in the Department of Pediatrics in Tübingen were treated with the same preparation of HuIFN-$\beta$—three of these had a nasopharyngeal carcinoma[7]. Total remission of the carcinoma after HuIFN-$\beta$ therapy was shown only in S.R. who was the only patient in which HuIFN-$\beta$ neutralizing antibodies could be demonstrated, although several others were treated with equally high doses.

We are now attempting to determine whether the patient's cells lack the genes for HuIFN-$\beta$ and further experiments are being done to clarify this phenomenon of anti-IFN production and its possible clinical significance.

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# Functional reactivation of the deafferented neostriatum by nigral transplants

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**Functional deficits following brain lesions can be due not only to the disruption of conduction in specific input and output pathways passing through the site of injury, but also to the loss of important regulatory systems controlling the functional state of neuronal circuitries in areas distant from the lesion. For example, the behavioural disturbances that result from lesions of the nigrostriatal dopamine (DA) pathways[1–3] can be reversed by administration of dopamine receptor-activating drugs, such as L-dopa or apomorphine[4–6]. This suggests that the lesioned dopaminergic system, rather than conveying specific input and output signals, is normally acting on neuronal machineries whose activity levels are set by the activity at the dopaminergic synapses. Thus the neurological deficits resulting from these lesions are due to functional inactivation of otherwise intact neostriatal circuitries. Previous studies have shown that intracerebral transplants of embryonic substantia nigra can compensate for drug-induced[7–9] as well as spontaneous asymmetric motor behaviour[11] (expressed as a tendency to move in circles towards the lesioned side), whereas the sensorimotor asymmetry, which is pronounced in rats with a unilateral lesion of the nigrostriatal DA pathway[4,10], was unaffected by the transplant[11]. We report here that restoration of striatal dopaminergic neurotransmission by nigral transplants in animals with bilateral, complete lesions of the nigrostriatal DA pathways can reinstate not only certain aspects of spontaneous motor behaviour, but also sensorimotor orientation and sensory attention on the side of the body contralateral to the graft.**

Young female Sprague–Dawley rats (180–200 g at the time of surgery) initially received a unilateral right lesion of the nigrostriatal DA pathway, produced by an intracerebral injection of 8 μg 6-hydroxydopamine (6-OHDA; Hässle) into the pathway[12]. The completeness of the lesion was tested by assessing the intensity of the amphetamine-induced rotational response according to Ungerstedt and Arbuthnott[13]. When injected intraperitoneally with 5 mg per kg metamphetamine 1 week after the 6-OHDA lesion, all the rats included in the study showed a mean of >8 complete 360° turns per min. This turning rate has previously been found to correspond to a reduction in striatal DA of 97% or more[9].

About 4 weeks after the 6-OHDA lesion the rats received transplants of the substantia nigra region taken from 16–17-day rat embryos (crown to rump length 15–20 mm) as described elsewhere[7,9]. The transplants were placed on the dorsal surface of the denervated neostriatum, within a suction cavity made through the anterior parietal cortex and the corpus callosum. Eight control rats received the cavity lesion but no transplants. Six months after transplantation, 14 of the transplanted rats which showed complete compensation of their initial amphetamine-induced turning response (Table 1) were selected for further behavioural testing. (The compensation of amphetamine turning has previously been shown to be a good criterion for animals with surviving transplants and with significant dopaminergic re-innervation of the dorsal part of the denervated caudate-putamen[9]. This was confirmed in the final fluorescence histochemical analysis which demonstrated DA-rich surviving transplants and significant DA fibre ingrowth in each case.)

Seven months after the first 6-OHDA lesion, all 22 rats received a second 6-OHDA lesion to the contralateral nigrostriatal DA pathway. They were maintained by intragastric tube-feeding for 8 weeks, giving 25–40 ml per day of a high-energy liquid diet preparation (Vivonex, Vitrum). All surviving rats were finally killed and the brains taken for fluorescence microscopical analysis using the ALFA method of Lorén *et al.*[14]. The rats underwent behavioural testing before and after the second lesion, which involved observations on amphetamine-induced and spontaneous asymmetric motor behaviour, arm

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**Table 1** Behavioural recovery in nigra-transplanted rats

| | Unilateral (right) lesion | | Bilateral lesion | |
|---|---|---|---|---|
| | No transplant | Right transplant | No transplant | Right transplant |
| | ($n = 8$) | ($n = 14$) | ($n = 8$) | ($n = 14$) |
| Amphetamine-induced rotation (net ipsilateral turns per min) | | | | |
| Before transplantation (1 week after 1st lesion) | 14.0 ± 2.5 | 14.0 ± 0.7* | — | — |
| 3 months after 1st lesion | 16.3 ± 1.4 | 2.7 ± 1.0† | — | — |
| 2 months after 2nd lesion | — | — | 0.9 ± 1.4 | −11.6 ± 0.8† |
| Spontaneous rotation (probability of left turn) | | | | |
| Without activation | 0.17 ± 0.04 | 0.32 ± 0.05† | 0.57 ± 0.12 | 0.92 ± 0.04† |
| Tail-pinch activation | 0.24 ± 0.06 | 0.46 ± 0.05† | 0.61 ± 0.11 | 0.91 ± 0.03† |
| Arm preference in T-maze (probability of left choice) | 0.03 ± 0.02 | 0.32 ± 0.07† | 0.41 ± 0.09 | 0.79 ± 0.06† |
| Sensorimotor asymmetry (right–left scores) | | | | |
| Without activation | 3.00 ± 0.82 | 4.00 ± 0.72* | 0.29 ± 0.52 | −4.33 ± 0.67† |
| Tail-pinch activation | 4.17 ± 1.05 | 3.23 ± 0.63* | 1.14 ± 0.77 | −4.67 ± 0.50† |

Amphetamine-induced rotation was recorded in plastic bowls over 60 min after intraperitoneal administration of 5 mg per kg metamphetamine, after the method of Ungerstedt and Arbuthnott[13]. Scores are net ipsilateral (right–left) turns per min. Spontaneous rotation was recorded in the same bowls, and quarter-turns in each direction were recorded over a 3-min period. Ten min later, a paper clip was attached to the rat's tail to provide mild activation, and again quarter-turns were recorded over a 3-min period. Maze testing: rats were tested for arm preference in an unbaited T-maze of conventional design, with and without tail-pinch activation, as described in detail elsewhere[11]. Each test consisted of two trials separated by a 5–10-min interval. Three tests were carried out, each separated by several days, and the scores for each animal combined as no differences were found between tests. Sensorimotor asymmetry was tested in a battery modified from Marshall and Teitelbaum[10,20], and described in detail elsewhere[11]. Briefly, the orientation to a light pin-prick at different points on the body surface, to touch of the whiskers and snout, and to the odour of an ammonia-soaked swab, were each rated on a three-point scale (0 = absent, 1 = weak, 2 = normal). The asymmetry scores are the difference between total scores on the two sides of the body (right–left). All tests were performed 3–4 months after the initial unilateral lesion and transplantation in the unilateral animals. They all received bilateral lesions at 7 months, and were retested 6–8 weeks later. The amphetamine rotation and sensorimotor tests indicate net (right–left) scores, with positive values denoting bias towards the right (transplant/initial lesion) side. The spontaneous rotation and maze tests indicate the probability of a left turn, scores $<0.5$ denote a right bias, and $>0.5$ a left bias. All values are means ±s.e.m. and comparisons by unrelated Student's $t$-test.

* Not significant, $P > 0.01$

† $P < 0.001$.

preference in a T-maze, and asymmetry in sensorimotor responding, as summarized in Table 1.

After the second 6-OHDA lesion both the controls and the transplanted rats became severely akinetic. Both groups of rats also became aphagic and adipsic, and they had to be maintained by intragastric tube-feeding throughout the 8-week test period.

The akinetic state of the animals was partially reversed by tail-pinch activation. When activated in this way, the non-transplanted control rats showed no side bias in their spontaneous turning behaviour and no asymmetric posture. In contrast, the transplanted rats showed a high degree of turning away from the transplant side (towards the left) (right-hand panels in Table 1) and asymmetric posture, with the head, body and tail describing a curve concave on the side of the second lesion. Without tail-pinch activation the transplanted rats showed an equally pronounced bias for direction of turning in the spontaneous rotation test, although the turning rate was much lower. Before the second lesion (left panels in Table 1), the lesioned, non-transplanted controls showed a strong rotational bias towards the lesioned side; this bias was significantly attenuated in the transplanted rats, both with and without tail-pinch activation.

When tested for arm preference in the T-maze, which can be taken as a measure of the relative degree of sensory attention on the two sides of the rat's body, before the second lesion the non-transplanted controls showed a strong bias (97%) for the right arm contralateral to the intact nigrostriatal pathway. This bias was significantly reduced in the transplanted rats. After the second lesion the transplanted rats showed a marked preference (79%) for the left arm, contralateral to the transplant, whereas the bilaterally lesioned controls showed no such asymmetry (Table 1).

When performed before the second lesion, the sensorimotor orientation test demonstrated an equally marked sensory inattention on the left side of the body in both the transplanted and non-transplanted rats. This deficit was unaffected by mild tail-pinch activation. After the second lesion the controls became equally unresponsive on both sides of the body, whereas the transplanted rats showed a marked asymmetry, with significantly greater response towards stimuli applied on the left side of the body, contralateral to the transplant (Table 1). Thus, with respect to both motor asymmetry, choice behaviour in the T-maze and sensorimotor orientation, the bilaterally lesioned rats with unilateral nigral transplants showed a performance similar to that of rats with a unilateral lesion of the nigrostriatal system.

Thus, our results support the conclusion that an ectopic, intracortical nigral transplant, without any external (that is, drug-induced) activation, can induce recovery in certain aspects of motor behaviour, and in sensory attention and sensorimotor orientation specifically on the side contralateral to the transplant, in the spontaneously behaving animal. In contrast, the severe akinesia, aphagia and adipsia of the bilaterally lesioned rats were unaffected.

Figure 1 shows in diagrammatic form a possible model for the mechanism of action of the ectopic nigral transplant, based on the one previously proposed by Stricker and Zigmond[2]. In this model the DA systems regulate the behavioural responsiveness through modulation of an inhibitory control mechanism. In the intact animal the nigrostriatal DA pathway seems to act as a tonic regulatory system which sets the level of activity in the neostriatal machinery. Removal of this dopaminergic regulatory control function results in inhibition of neostriatal function. Reinstatement of dopaminergic neurotransmission by drugs[4–6],

or—as in our experiments—by transplantation of DA neurones, can thus be interpreted as a reactivation of an inhibited, but otherwise intact, neuronal machinery. If we assume that the nigrostriatal DA system is inhibitory in nature (see ref. 15 for discussion), then the likely action of the nigral transplant would be to disinhibit elements of the nigrostriatal circuitry.

In the model in Fig. 1 the nigral transplant acts by reducing the threshold for behavioural response (R) to diverse sensory inputs (I). An interesting implication of this model is that recovery of responsiveness to sensory stimuli in the transplanted rats can occur without any access of sensory information to the transplant. In the intact animal various types of sensory stimuli have been shown to activate the nigrostriatal DA neurones[16,17]. However, in the transplanted animal such activation would not be necessary for behavioural recovery provided that the transplanted neurones have a sufficiently high spontaneous activity. Other parallel observations indicate that this indeed is the case. First, in biochemical determinations of DA and its metabolite, dihydroxyphenylacetic acid[18], the turnover of DA in both the transplant and the re-innervated neostriatum has been found to be as high as that of the intact nigrostriatal system. Second, $^{14}$C-2-deoxy-D-glucose autoradiography has revealed a significant glucose uptake in the nigral transplants, amounting to about two-thirds of that of the intact substantia nigra[18], and third, measurements of the rotational response to the DA receptor agonist, apomorphine, in nigra-transplanted rats indicate that re-innervation of the neostriatum by nigral transplants is accompanied by a partial reduction in the supersensitivity of the neostriatal DA receptors[11]. This observation is compatible with a partial restoration of dopaminergic neurotransmission in the re-innervated neostriatum.

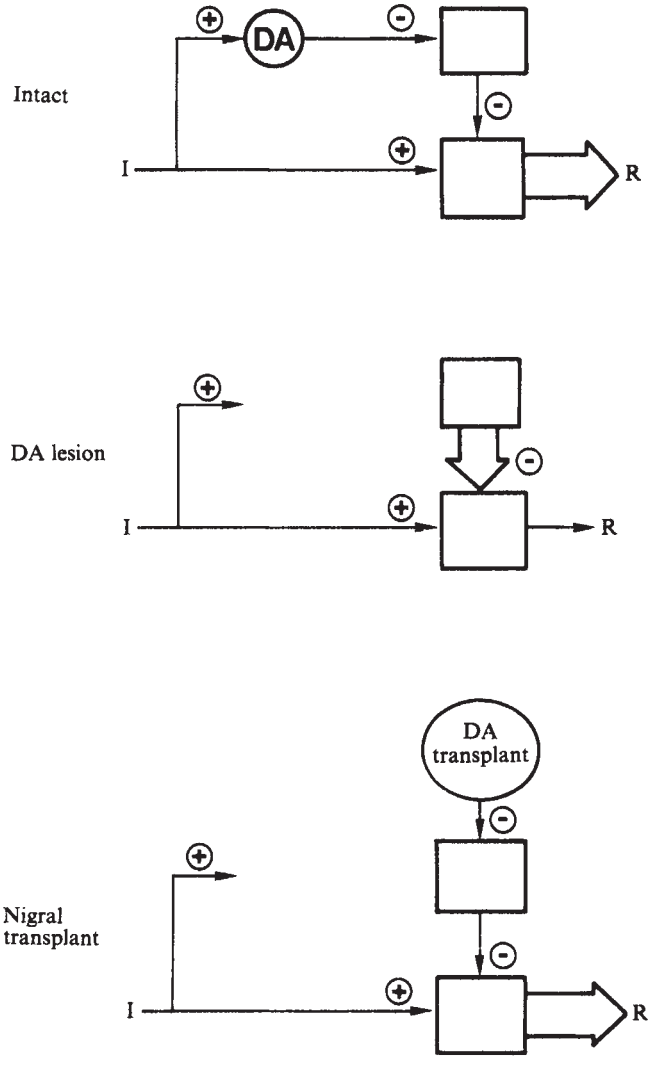


**Fig. 1** Proposed mode of action of the DA-producing intracortical nigral transplants in the promotion of behavioural recovery in rats with unilateral or bilateral lesions of the nigrostriatal DA pathway. The behavioural response, R, evoked by an activating sensory input, I, is modulated by the transplant through an inhibitory control mechanism.

An important feature of the behavioural effects of the nigral transplants was the specificity of the transplant-induced recovery. Thus, in the bilaterally lesioned rats the transplants did not induce a diffuse, partial recovery in all aspects of the DA deficiency syndrome. Rather, the recovery appeared discrete and complete, such that in those behaviours where recovery did occur (motor asymmetry and choice behaviour) the transplanted animals performed as well as an intact rat, whereas in those behaviours where recovery did not occur (akinesia, aphagia and adipsia) they performed as badly as the lesioned control rats. This can be explained if one assumes that different parts of the striatal complex subserve, at least partly, different types of function (see ref. 19). If so, the transplant-induced recovery would depend on which parts of the striatal complex are re-innervated from the transplant. With the present intracortical transplantation site the ingrowing DA fibres are confined to the dorsal parts of the head of the caudate-putamen, whereas the ventral and lateral parts, as well as nucleus accumbens, are left denervated. The possibility that this regional restriction of the transplant-induced dopaminergic re-innervation determines the pattern and specificity of behavioural recovery is now being investigated.

These results demonstrate that intracerebral transplants can be functional not only after pharmacological activation but also in the spontaneously behaving animal, and that ectopic nigral transplants, which probably lack their normal afferent inputs, can nevertheless compensate for the loss of the rat's own substantia nigra, at least in certain types of behaviour. Functional reactivation of denervated central nervous system circuitries by neural transplants should thus offer an interesting approach to the analysis of mechanisms of neurological and behavioural recovery after brain or spinal cord damage as well as in animal models of neurodegenerative disorders, such as Parkinson's disease.

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