Brain Research, 177 (1979) 555–560

Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants

ANDERS BJÖRKLUND and ULF STENEVI

Departments of Histology and Ophthalmology, University of Lund, Lund (Sweden)

(Accepted August 9th, 1979)

Surgical or neurotoxic lesions of the nigrostriatal dopamine (DA) pathway produce a denervation of the striatum that leads to severe and long-lasting motor disturbances[2,3,5,6,11,12] and, if the lesion is bilateral and complete, fatal deficits in consummatory behaviour[1,11,13]. With substantial lesions of the nigrostriatal DA pathway the denervation will persist and functional recovery is very limited[10]. We here report that transplants of embryonic substantia nigra, implanted into the parietal cortex in adult rats, are able to establish a new dopaminergic input to the previously denervated neostriatum, and that this newly-formed 'nigrostriatal' DA pathway may compensate for at least some aspects of the lesion-induced motor disturbances.

The ventral mesencephalic tegmentum (containing the developing dopamine-containing-neurones of the substantia nigra — A10 cell system) was dissected out from 16–19-day-old rat embryos (crown rump length 15–33 mm) and transplanted into a cavity in the parietal cortex, using a modification[8] of a previously published transplantation technique[9]. The recipients were adult, female, Sprague–Dawley rats (180–200 g body weight) that underwent the following sequence of operations. First, a unilateral destruction of the DA neurones of the substantia nigra was made by two injections of 6-hydroxydopamine (6-OHDA) into the right substantia nigra. Each injection contained 8 μg of 6-OHDA (free base) in 4 μl of saline. 0.2 mg/ml of ascorbic acid was added as an anti-oxidant. After 1–2 weeks, a 2 × 3 mm wide cavity was made through the anterior parietal cortex and the corpus callosum ipsilateral to the 6-OHDA lesion, exposing the dorsal surface of the head of the caudate-putamen. The cavity was made by suction under visual control; it was filled with gel-foam and the wound was closed. Some weeks later the transplant was inserted into the cavity, as shown in Fig 1. The rats were allowed to survive for 1.5 (8 rats), 3.5 (10 rats) and 7 months (5 rats). The animals in the 3.5 month group were tested repeatedly, before and after transplantation, for amphetamine-induced circling behaviour (5 mg/kg metamphetamine, i.p.) according to Ungerstedt[10,12]. All rats in this group responded to the amphetamine injection with at least 10 turns/min in the direction of the lesion side before transplantation. After sacrifice, all brains were processed for monoamine fluorescence histochemistry[7]. Microscopy was made in serial sections of the mesencephalon and the

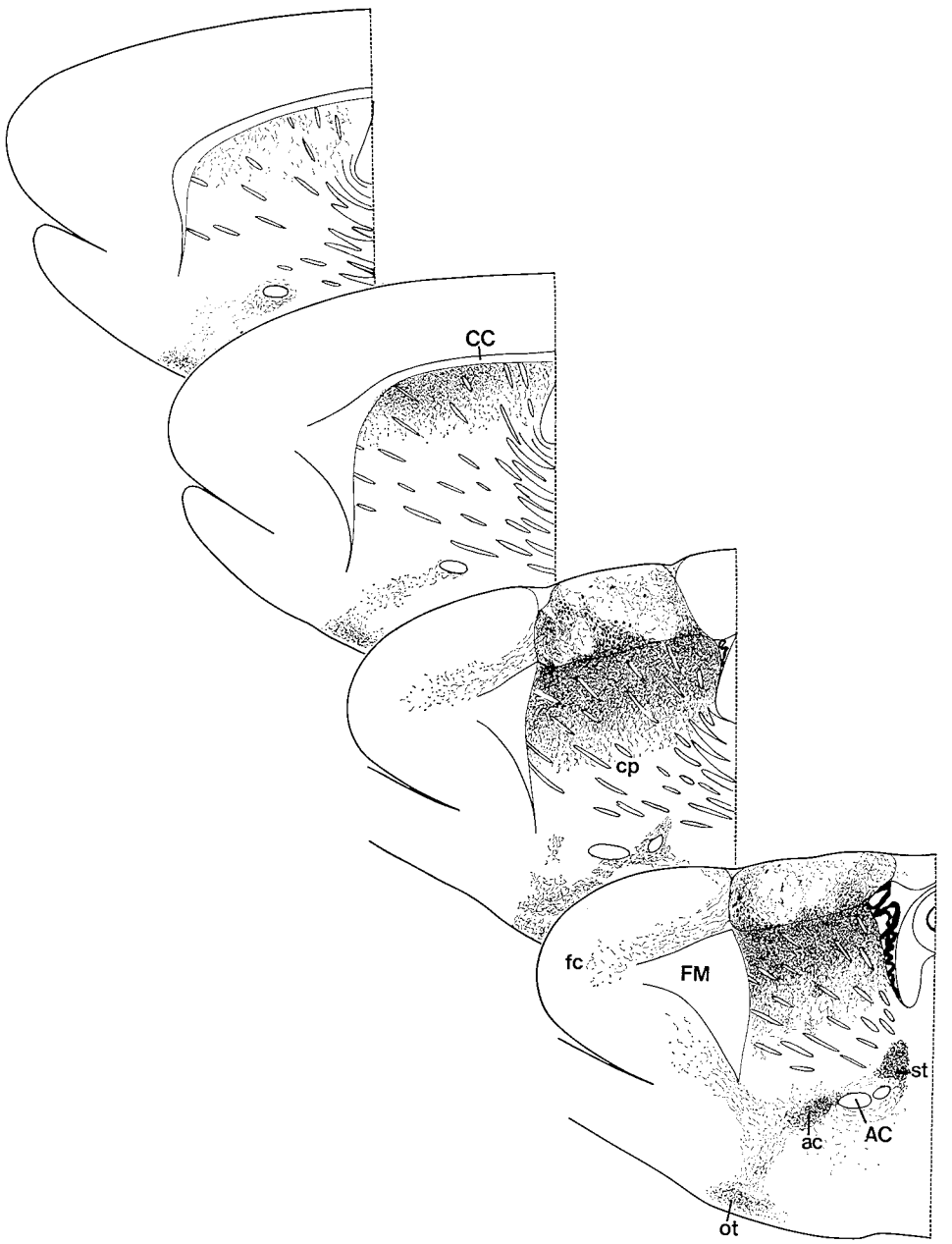

Fig. 1. Drawings illustrating the outgrowth of DA-containing fibres from an intracortical nigral implant, 3.5 months survival, represented in 4 sagittal planes spaced by about 0.5 mm. Bottom-right is medial and top-left is lateral. The DA neurones in the implant had grown extensively ventrally into the previously denervated caudate-putamen (cp), and rostrally into the anteromedial frontal cortex (fc). The fibres seen in the bed nucleus of the stria terminalis (st), nucleus accumbens (ac), and the olfactory tubercle (ot) were spared by the 6-OHDA lesion. FM, forceps minor; CC, corpus callosum; AC, anterior commissure.

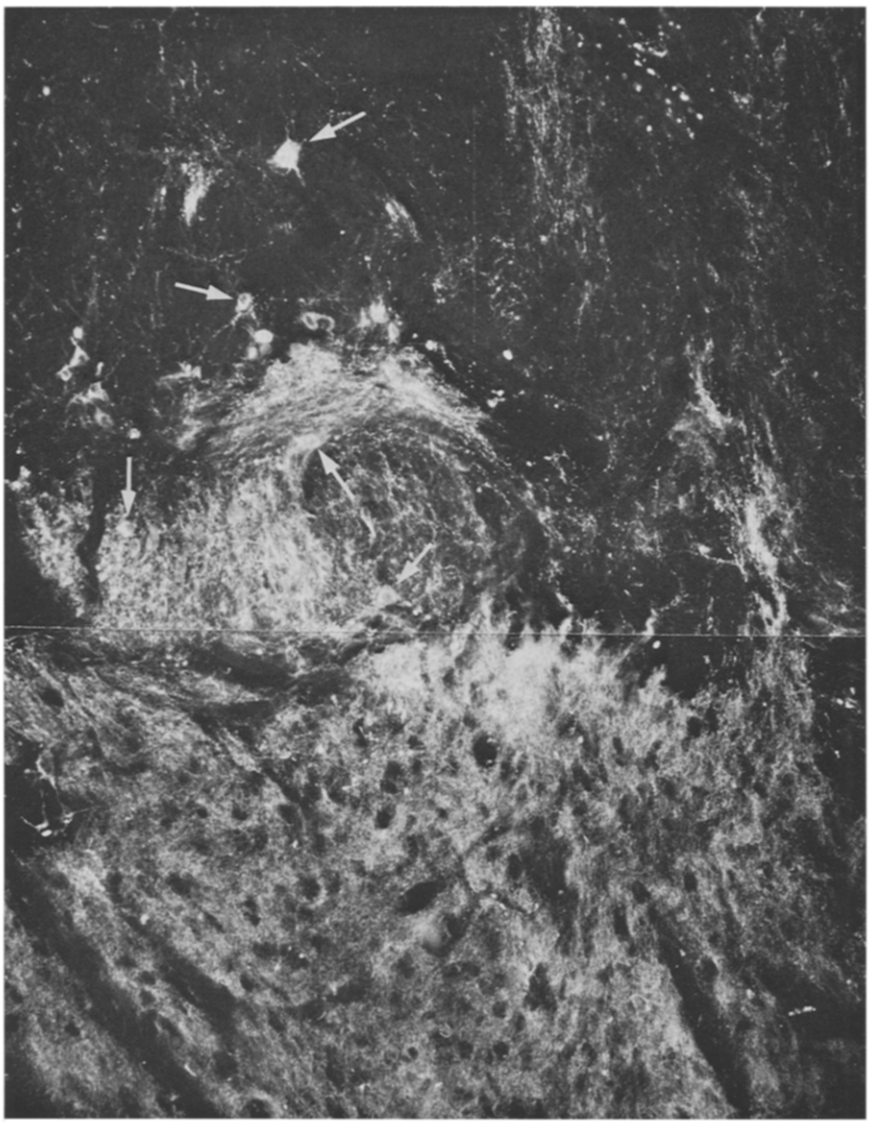

Fig 2 Fluorescence photomicrograph, from the specimen shown in Fig 1, illustrating the extension of DA fibres from the transplant (top) into the nucleus caudatus-putamen of the host brain (bottom) Arrows denote DA-containing nerve cell bodies 120

basal ganglia in order to establish. (a) the localization of the 6-OHDA injections and the degree of destruction of the substantia nigra DA neurones; (b) the survival and appearance of the transplant; and (c) the extent of growth of DA-containing axons from the transplant into the caudate-putamen and the surrounding neocortex.

Of the 23 transplants, 19 survived. The survival was as good in the 3.5 or 7-month groups as in the 1.5 month group, indicating that the conditions for the transplants in their new environment are excellent for long-term survival. The

transplants were well demarcated, separated from the adjacent neostriatum and cortex by a thin glial layer, and covered by a new pia-arachnoid. The size of the transplants as well as the number of surviving DA-containing neurones varied widely: the maximum cross-sectional area varied from about 0.2 to about 6 sq. mm and the total number of DA neurones from very few up to about 4000. The DA cell bodies were usually arranged in clusters or dense aggregates.

DA fibre outgrowth was demonstrable in all transplants, and the total amount of fibres formed was grossly proportional to the number of surviving DA neurones. The newly-formed fibres formed terminal networks within the transplant itself and they could be traced, across the limiting glial layer, ventrally into the dorsal neostriatum, and rostrally into the anterior cingulate and frontal neocortex, i.e. into areas normally receiving a dopaminergic innervation. Such DA fibre growth into the host brain occurred in 16 of the 19 specimens that had surviving transplants. The outgrowth was abundant in those specimens having many surviving DA neurones, such as the one illustrated in Figs. 1 and 2. Maximally about one-third of the head of the nucleus caudatus-putamen was reached by the newly-formed fibres. There was a clear-cut gradient in the fibre network so that the highest fibre density was found in a 1–2 mm wide zone close to the transplant. In the most successful cases the fibre density was close to normal in this dorsal zone. Further ventrally, the terminal plexus became gradually more sparse. Interestingly, it was precisely confined to the grey matter of the caudate-putamen.

The completeness of the 6-OHDA lesion was ascertained through serial section analysis of the substantia nigra in the fluorescence microscope. In most animals no DA-containing neurones remained in the substantia nigra on the lesion side, whereas the medial part of the A10 cell group had been spared. Moreover, in the animals with no or very little ingrowth from the transplant, no or maximally some single scattered DA-containing terminals remained in the ipsilateral nucleus caudatus-putamen, with the exception of the most ventral portions bordering on the bed nucleus of the stria terminalis and the nucleus accumbens where more fibres were left. The accumbens and the bed nucleus themselves were only partially denervated. In the 3.5 month group the completeness of the nigral lesion was confirmed by the intensity of the amphetamine-induced turning response (10–30 turns/min) prior to transplantation (cf. refs 2,5 and 12).

In the 3.5-month group the growth of new DA fibres into the caudate-putamen from the transplant was monitored by repeated measures of the intensity of the amphetamine-induced ipsilateral turning. This turning response reflects the imbalance in the amphetamine-induced release of DA from the intact and lesioned nigrostriatal pathways[4,12], and its intensity has been shown to be related to the degree of DA depletion in the denervated ipsilateral striatum[2,4,5]. The results suggested a very good correlation between the magnitude of ingrowth of DA-containing fibres into the denervated neostriatum and the attenuation of the turning response. Of the 4 cases illustrated in Fig. 3, 2 rats (triangles and dashed line) had no DA ingrowth into the caudate-putamen and the turning response remained high. In the third case (open circles), having a small transplant containing about 320 surviving DA neurones, there

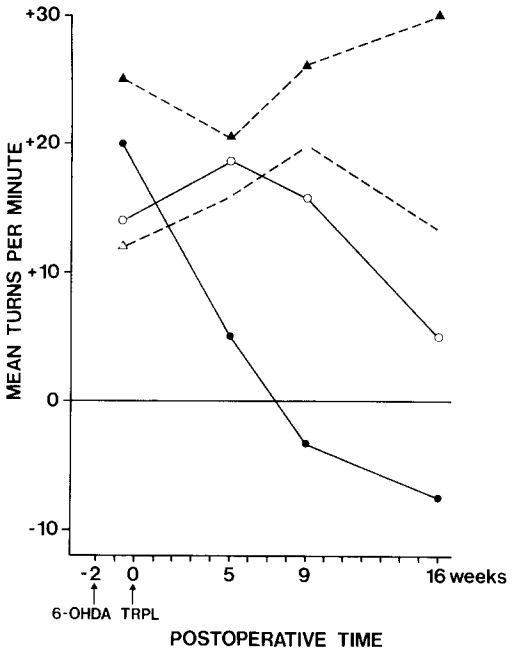

Fig 3 Amphetamine-induced turning responses in 4 representative cases before and after nigral transplantation. The right substantia nigra was lesioned with 6-OHDA 2 weeks before transplantation Turning was registered every 10 min for 60 min after injection The x-axis gives the mean of the 6 registrations, with positive values indicating complete, 360° turns towards the lesion side, and negative values turns towards the intact side Filled circles give the responses in the specimen illustrated in Fig 1, having extensive DA ingrowth into the neostriatum Open circles represent a rat with a small implant and a limited DA ingrowth into the neostriatum Filled triangles give the responses in a specimen with few surviving DA neurones and no DA ingrowth into the host brain Open triangles represent a rat in which the transplant did not survive

was a limited ingrowth into the rostral portion of the head of the caudate putamen, and the turning response was reduced to about one-third of the pre-transplantation value The fourth case (filled circles), which is the specimen illustrated in Fig. 1, had a large transplant with a large number of surviving DA neurones and an extensive ingrowth into the head of the caudate-putamen. In this rat the ipsilateral turning was not only eliminated, but gradually even reversed. Further experiments, yet in progress, have shown that the amphetamine-induced rotational response recorded prior to transplantation can be reinstated by surgical removal of the transplant (Björklund, Dunnett, Lewis, Stenevi and Iversen, in preparation).

In conclusion, the results show that the intracerebral implantation technique can successfully be used to restore a new nigral–DA input to the denervated neostriatum from an ectopic site. The observed growth patterns indicate that the newly-formed DA projections are specific in the sense that they are made preferentially into cortical and striatal areas normally receiving a dopaminergic innervation. The amphetamine data, furthermore, suggest that the new 'nigrostriatal' connection is functional and may compensate for at least some aspects of the functional deficit resulting from the normal nigral–DA innervation. In the long perspective we believe that the present approach

could offer an interesting experimental strategy for studies on behavioural and functional recovery in neurodegenerative extrapyramidal disorders, such as Parkinson's disease.

We thank Ulla Jarl and Gertrude Stridsberg for expert technical assistance.

The study was supported by grants from the Swedish MRC and from Segerfalk's and Kock's foundations.

While this paper was under submission to *Science*, a related, independent study by M. J. Perlow et al. appeared (*Science*, 204 (1979) 643–647).

1 Breese, G R , Smith, R. D., Cooper, B. R. and Grant, L D., Alterations in consummatory behavior following intracisternal injection of 6-hydroxydopamine, *Pharmacol. Biochem Behav.*, 1 (1973) 319–328.
2 Costall, B., Marsden, C. D., Naylor, R. J and Pycock, C. J., The relationship between striatal and mesolimbic dopamine dysfunction and the nature of electrolytic lesions of the ascending dopamine systems of rat brain, *Brain Research*, 118 (1976) 87–113
3 Creese, I. and Iversen, S. D., The pharmacological and anatomical substrates of the amphetamine response in the rat, *Brain Research*, 83 (1975) 419–436.
4 Glick, S D., Jerussi, T P. and Fleisher, L. N , Turning in circles: the neuropharmacology of rotation, *Life Sci* , 18 (1976) 889–896
5 Kelly, P. H , Unilateral 6-hydroxydopamine lesions of nigrostriatal or mesolimbic dopamine-containing terminals and the drug-induced rotation of rats, *Brain Research*, 100 (1975) 163–169
6 Kelly, P. H., Seviour, P. W. and Iversen, S. D., Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum, *Brain Research*, 94 (1975) 507–522.
7 Lorén, I., Björklund, A., Falck, B. and Lindvall, O., An improved histofluorescence procedure for freeze-dried paraffin-embedded tissue based on combined formaldehyde-glyoxylic acid perfusion with high magnesium content and acid pH, *Histochemistry*, 49 (1976) 177–192
8 Stenevi, U. and Björklund, A., In preparation.
9 Stenevi, U , Bjorklund, A. and Svendgaard, N A , Transplantation of central and peripheral monoamine neurons to the adult rat brain· techniques and conditions for survival, *Brain Research*, 114 (1976) 1–20
10 Ungerstedt, U., Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behaviour, *Acta physiol. scand.*, Suppl. 367 (1971) 49–68.
11 Ungerstedt, U., Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigrostriatal dopamine system, *Acta physiol. scand.*, Suppl. 367 (1971) 95–122.
12 Ungerstedt, U. and Arbuthnott, G. W , Quantitative recording of rotational behavior in rats after 6-hydroxydopamine lesions of the nigrostriatal dopamine system, *Brain Research*, 24 (1970) 485–493.
13 Zigmond, M. J. and Stricker, E M., Deficits in feeding behavior after intraventricular injection of 6-hydroxydopamine in rats, *Science*, 177 (1971) 1211–1213