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

Dunnett, S.B., Björklund, A., Schmidt, R.H., Stenevi, U., Iversen, S.D.: Intracerebral grafting of neuronal cell suspensions. IV. Behavioural recovery in rats with unilateral implants of nigral cell suspensions in different forebrain sites. *Acta Physiol.Scand.*, Suppl. 522, 29-38, 1983.

Dunnett, S.B., Björklund, A., Schmidt, R.H., Stenevi, U., Iversen, S.D.: Intracerebral grafting of neuronal cell suspensions. V. Behavioural recovery in rats with bilateral 6-OHDA lesions following implantation of nigral cell suspensions. *Acta Physiol.Scand.*, Suppl. 522, 39-48, 1983.

Björklund, A., Gage, F.H., Stenevi, U., Dunnett, S.B.: Intracerebral grafting of neuronal cell suspensions. VI. Survival and growth of intrahippocampal implants of septal cell suspensions. *Acta Physiol.Scand.* 522, 49-58, 1983

Björklund, A., Gage, F.H., Schmidt, R.H., Stenevi, U., Dunnett, S.B.: Intracerebral grafting of neuronal cell suspensions. VII. Recovery of choline acetyltransferase activity and acetylcholine synthesis in the denervated hippocampus reinnervated by septal suspension implants. *Acta Physiol.Scand.*, Suppl. 522, 59-66, 1983.

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

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Intracerebral Grafting of Neuronal Cell Suspensions

I. Introduction and General Methods of Preparation

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The steps involved in the grafting of mesencephalic and septal embryonic tissue in the form of dissociated cell suspensions are described in detail. This includes dissection of the donor embryos, incubation in trypsin, mechanical dissociation, and stereotaxic injection into the brains of adult recipient rats. Some of the technical problems and limitations are discussed.

INTRODUCTION

The transplantation of neuronal tissue to the CNS of mammals has been attempted periodically since the end of the last century. Although earlier studies achieved only limited success, over the last decade the conditions for attaining reliable graft survival have been characterized and the transplantation of pieces of CNS tissue to various sites within the adult nervous system can now be achieved with a high degree of reliability.

Several important factors have been identified for viable CNS grafting. Firstly, although adult PNS tissue partly survives transplantation to the mature host brain, transplantation of CNS tissue is viable only if taken from suitably-aged embryonic, or in certain circumstances, neonatal donors. In adult recipients, the best survival and growth of CNS grafts is consistently seen with embryonic donors, although the optimum donor age seems to vary for different parts of the neuraxis (13, 17). The reasons for these time-constraints are not entirely clear. Foetal CNS tissue may be able to survive periods of anoxia better than mature CNS tissue, and proliferating and early postmitotic neurons may benefit from less surgical trauma due to extensive axotomy during dissection. Embryonic tissue also has the capacity for continued neurogenesis after transplantation. Secondly, the adult brain has been characterized as an "immunologically privileged" site for the receipt of transplants of different types of tissue, and problems of immunological rejection are rare in rats when employing donors and recipients of the same strain. In our experience, graft failures under such conditions are more generally attributable to bleeding or infection at the time of surgery

rather than to specific rejection of the tissue.

However, perhaps the most important reason for failure in earlier studies of intracerebral CNS grafts was a failure to achieve sufficiently rapid incorporation of the graft into the host blood and cerebrospinal fluid circulation. In the anterior eye chamber, which is a classical site for tissue grafting (see ref. 17 for review), favourable conditions for graft survival are created by the direct contact with the highly vascularized iris surface and the access to the chamber fluid. Similar conditions are available at least at certain places within the ventricular system in the brain, particularly in association with the choroidal plexuses or the richly vascularized median eminence (18, 19), CSF-bathed highly vascularized pial surfaces can also be created surgically in the brain or spinal cord, and such sites have proved just as favourable as the anterior eye chamber for the support of CNS tissue survival (12, 22, 23). The features of the transplantation site appear to be more critical in adult than in neonatal, developing host CNS. This is probably because the neonatal rat brain generally provides better support for survival, richer sources for vascularization and easier access to CSF. Thus, in contrast to adult rat recipients, direct intraparenchymal placement of pieces of embryonic or neonatal CNS tissue has given excellent results in several areas of the neonatal rat brain (7, 8, 9, 24, 25).

The necessity of a suitable pial bed for grafts in the brain of adult recipient rats permits only a limited number of naturally available transplantation sites. This in turn limits the ability to reinnervate many targets in the host brain because growth from such grafts tends to

be limited to a few mm. This limitation has in certain places, notably the neostriatum, been overcome by the adoption of a two-stage grafting procedure (4, 22). In this approach a small suction cavity is made in the cortex, which heals with a new highly vascularized pial lining over a period of 3-6 weeks. This prepared cavity is then, in a second operation, reexposed and used for grafting.

Nevertheless, even with such modifications, many regions of the brain remained out of reach of suitable transplantation sites, in particular in more ventral areas of the forebrain, diencephalon and mesencephalon. To overcome this limitation we have recently developed a quite different transplantation procedure, which involves the intracerebral injection of dissociated suspensions of graft tissue (3, 20). The preparation procedure is modified from standard tissue culture techniques and potentially allows culturing, or use of cell separation and combination techniques, prior to grafting, to achieve greater specificity of cells for transplantation. However, the particular advantage of this technique is that the dissociated cells can be implanted, in direct contact with the host neuropil, and will survive without the provision of special vascular support or access to CSF spaces. Consequently, we have been able to achieve good survival of many types of embryonic tissue suspensions (e.g. from cortex, hippocampus, cerebellum, neostriatum, septum, substantia nigra, locus coeruleus, raphe nuclei) into all sites so far investigated. Placements into the diencephalon, mesencephalon and spinal cord have proved to be as viable as placements into deep and superficial sites in the telencephalon, such as the cortex, hippocampus or basal ganglia.

The flexibility of the tissue suspension graft procedure has permitted the design of more comprehensive experiments, investigating the role of cell type and substrate in the survival, anatomical growth and functional consequences of grafts in models of neuronal development, regeneration and recovery from brain damage. Several additional advantages have accrued from utilization of suspension grafts. Firstly regeneration generally takes place with the same reliability but greater rapidity than that seen following grafting of solid tissue pieces. Secondly, the suspension injections involve less trauma to the host brain than, for example,

involved in the creation of transplantation cavities. This has further permitted the study of graft growth and functional changes in animals without extrinsic lesions, such as in the aging brain. Furthermore, the suspension technique also permits multiple grafts to be placed within a single target of the host brain.

Several previous reports have introduced the suspension transplantation procedure (3, 20), and used suspension grafts in parallel with solid grafts in functional studies (10). The present monograph provides the first comprehensive account of the methodology, together with anatomical, biochemical and functional accounts of suspension grafts of two particular populations of CNS tissue: the dopamine-rich cell groups of the ventral mesencephalon, and the cholinergic cells of the developing septal-diagonal band area in ventral forebrain.

METHODS

The principal steps in the preparation of cell tissue suspensions are shown schematically in Figure 1. It involves dissection of the embryonic donor, collection of tissue pieces, incubation in trypsin, washing, mechanical dissociation of the tissue, and stereotaxic injection of the resultant suspension into the host brain.

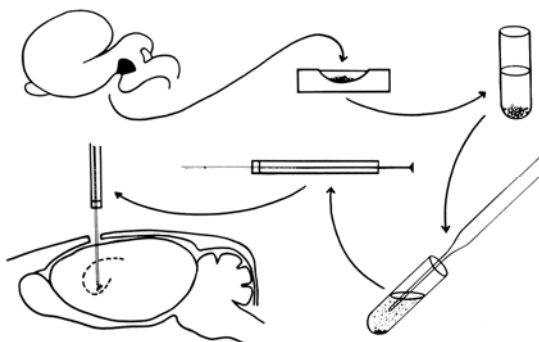


Fig. 1 Principal steps in the suspension grafting procedure, as described in the text.

Preparation

All glassware, dissection instruments and syringes are sterilized either by heat or in an autoclave. The basic medium comprises about 50 ml glucose-saline (0.6% D-glucose in sterile 0.9% saline), and the incubation medium comprises 5-10 ml trypsin solution (0.1% of a crude trypsin preparation, Sigma type II, dissolved in the basic medium). The staged pregnant female is anaesthetized by intraperitoneal injection of a barbiturate (e.g. 1 ml 60 mg/ml Brietal, Lilly), and prepared for Caesarian section. The mother is kept anaesthetized until all foetuses have been dissected.

Dissection

Embryos are removed from the mother, one at a time from the distal end of the uterine horn. Embryos are taken at about 13-15 days of gestation (crown-rump length, CRL, 10-14 mm) for dopaminergic ventral mesencephalic grafts, and at about 14-16 days of gestation (CRL 12-16 mm) for cholinergic septal tissue. In all cases, the gestational days are given with the day after mating as embryonic day (ED) 0. Although the most exact dating of the donor foetuses will be obtained by using timed pregnant mothers, we have found it fully satisfactory to use the CRL measure to estimate the age of the embryos in non-timed mothers. With some practice, the CRL size of the foetuses in a litter can be assessed by palpation of the mother under ether anaesthesia.

The dissection begins by trimming away the placenta and snipping the amniotic sac so that the embryo drops with a little amniotic fluid (to keep the tissue moist) onto a glass microscope slide. A clean sterile slide is used for each embryo. Dissection is performed under an operating microscope using fine iridectomy scissors and Dumont No. 5 dissecting forceps. The embryonic brain is removed intact by peeling the skin and cranial cartilage away from the brain surface, and ensuring the complete removal of all attached meninges. Avoid pulling or squeezing the brain tissue itself. The embryonic brain is then laid on its dorsal or ventral surface, or on its side, for the dissection of the specific pieces of tissue for transplantation. For the dopaminergic cells of the developing substantia nigra - ventral tegmental region, this involves taking the entire ventral mesencephalon bilaterally, centered at the mesencephalic flexure, as indicated in Figure 2. For the cholinergic cells of the developing septum-diagonal band region this involves taking the ventral

forebrain, from the rostral border of the hypothalamus, caudally, to the olfactory bulb, rostrally, with the lateral cuts placed just medial to the medial border of the striatal eminence (see Figure 2). This dissection most likely includes also the precursor cells of the nc. basalis. Care must be taken not to tear or lacerate the tissue to be grafted and all cuts and peeling should be away from this area. Following dissection, each tissue piece, approximately 1-1½ mm³ in size, is transferred with the forceps to a small collecting dish containing 3-5 ml of the basic medium at room temperature. Between 8 and 13 pieces are usually obtained from one litter over a period of 30-45 min.

Suspension preparation

The tissue pieces are gently transferred with forceps to a glass micro test tube (400 µl capacity, with a scratch mark indicating the 100 µl level) containing 200-300 µl of the trypsin incubation solution. The pieces are incubated for 20 min at 37°C in a small tissue incubator (e.g. Eppendorf). This time includes the warm-up of the pieces from room temperature to +37°C. Following incubation the trypsin is washed off by replacing the fluid with a sterile disposable syringe 4 times with 300-350 µl of the basic medium, and finally brought to a volume equivalent to 10 µl per dissected tissue piece (i.e. 100 µl for 10 pieces). The tissue pieces are finally dissociated into a suspension of single neurones by repeated pipetting through a Pasteur pipette with a fire-polished opening (usually some 20-25 strokes, not too forcefully). Care must be taken not to introduce air bubbles into the suspension at this stage. The resulting suspension is a milky fluid containing wisps of connective tissue, which does slowly deposit and reaggregate so that gentle repipetting is necessary (we usually suspend at 30-60

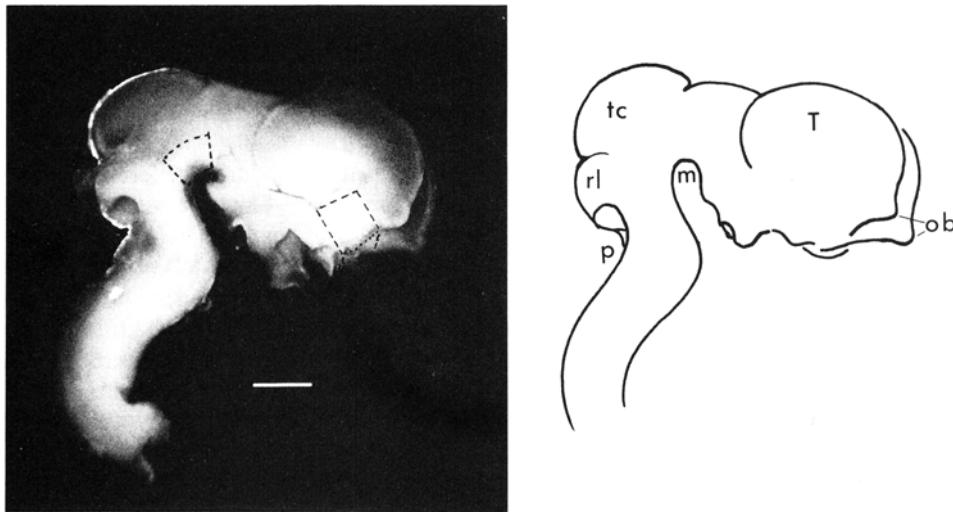


Fig. 2 Lateral view of a dissected brain from a foetus of approx. embryonic day 15 (CRL 13 mm). The "nigral" graft was taken from the bottom of the mesencephalic flexure (left dashed outline), and the "septal" graft was taken from the ventral forebrain (right dashed line). The septal piece was taken bilaterally on both sides of the midline (dotted line). Bar indicates 1 mm. *Abbreviations:* m = mesencephalic flexure; p = pontine flexure; rl = rhombic lip; tc = developing tectum; T = telencephalic vesicle; ob = olfactory bulb primordia.

min intervals). The suspension is kept at room temperature and can be used for grafting over at least 3-3½ hours following preparation.

Alternative Procedure

A modification of the above procedure has recently been developed (21). The glucose-saline basic medium is replaced by a calcium free Earles basic salt solution (EBBS)* during tissue collection. Incubation is conducted with the addition of a more purified trypsin in lower concentration (0.025%; Sigma type III) in EBBS and washing and suspension take place in EBBS with addition of 0.004% DNase (Sigma, type I), 0.0125% soybean trypsin inhibitor (Sigma, type I-A) and 15 mM MgSO₄. All other stages of the modified procedure are identical to the basic procedure. DNase, in particular, is included in order to minimize reaggregation of cells in the suspension due to DNA released from damaged cells.

Suspension Injection

Injections are made with sterilized 10 µl Hamilton syringes, with 23-27 gauge needles. It is advantageous to use removable needles since this permits frequent exchange of needles (to minimize infections) and repeated sterilization can damage the seal of fixed-needle syringes. The needle tip is ground down to a 45° bevel. The syringe is mounted directly into the head of a stereotaxic frame and the tissue suspension is drawn gently up into the syringe taking care to avoid blockages or vacuum pockets. Young adult host rats are anaesthetized with methyl hexital (Brietal^R, Lilly, 40 mg/kg; Chapter II to VII) while older rats or rats likely to be sensitive to the anaesthesia are anaesthetized with a mixture of ketamine (Ketalar^R, Parke-Davis, 10 mg/kg) and xylazine (Rompun^R, Hoechst, 5 mg/kg) (Chapter VIII). They are placed in the stereotaxic frame and the injection needle lowered to predetermined coordinates through small burr holes in the cranium. 2-5 µl of suspension are deposited at the rate of 1 µl/min, and a further 2-5 min are allowed for diffusion prior to slow retraction of the needle. Two separate deposits are frequently made, separated by 1-3 mm, along the same needle penetration, and up to 5 deposits scattered over the target area can readily be made in the same operation. At the completion of surgery and retraction of the needle the animal is removed from the frame and the wound is sutured. No additional antibiotic or antiimmune agents are administered.

In most studies reported here (Chapters II-VII, but not Chapter VIII), the host rats received additional denervating lesions, either by intracerebral injection of 6-hydroxydopamine into the mesotelencephalic dopamine bundle several weeks prior to transplantation (Chapters II-V), or by an aspirative lesion of the fimbria-fornix during the same surgical operation immediately prior to transplantation (Chapters VI and VII). Specific details of the lesions are given in the Methods sections of each Chapter.

*calcium-free EBSS contains 5.36 mM KCl (0.4 g/l), 116.3 mM NaCl (6.8 g/l), 26.2 mM NaHCO₃ (2.2 g/l), 1.0 mM NaH₂PO₄·H₂O (0.14 g/l), 0.8 mM MgSO₄·7H₂O (0.2 g/l), 0.1% D-glucose and 0.001% phenol red.

COMMENTS ON THE PROCEDURE

Age of donor embryos

One of the most critical parameters in the suspension grafting technique is the developmental age of the donor tissues. The relationship between donor age and neurone survival has so far been investigated in two different experimental situations. Dopaminergic cells of the substantia nigra survive most reliably and produce the best reinnervation of the caudate nucleus when taken from donors on the 14th or 15th gestational days (CRL 11-14 mm). When donors only some 12-36 hours older (CRL 15-20 mm) were tested, graft survival was markedly reduced, and fewer dopaminergic cells and outgrowing axons were seen in the surviving grafts (ref. 3, and unpublished observations). The optimal age for taking dopaminergic nigral cells corresponds to an interval late in the period between gestational days 11-15 when the nigral neurones differentiate and undergo their last cell division (14, 15). A similar relationship was observed for cerebellar Purkinje cells (21). Large numbers of these cells were seen in grafts prepared from 15 day old foetuses, i.e. at a stage when these neurones are still being generated (1). When 18 day old foetal donors were used only an occasional Purkinje cell was seen to have survived, and none survived from older donors. By contrast, cerebellar microneurones, which continue to be generated up into the postnatal period, survive suspension grafting when taken from late embryonic or early postnatal donors.

These results indicate that optimum neuronal survival in the suspension grafting method coincides with the period of proliferation, migration and early differentiation for each neurone type. This is consistent also with the observations made so far on septal cholinergic neurones (Chapter VI), which show good survival when taken during the period of active neurogenesis in the medial septum and the diagonal band area (6). It seems likely that the decline in survival seen after this period is due to the inability of neurones with long developed processes to sustain the dissociation procedure. This explains also why the time constraints are greater in the suspension grafting procedure than when the same regions are grafted as solid pieces. As a consequence, the optimum donor age must be determined independently for each neuronal subpopulation, using both generally available information on neurogenesis from

[³H]-thymidine autoradiographic studies, and empirical information about survival of neurones in suspension grafts prepared from different donor ages.

Cell Suspensions

The dissection of embryos and the preparation of cell tissue suspensions need to be conducted in such a way as to minimize both mechanical trauma and bacteriological infection of the graft tissue. These appear to be the two major causes of cell death in non-surviving grafts, rather than immune reactions *per se* within the host brain following transplantation. It is advisable to monitor cell viability in the prepared suspensions by means of vital stains. The procedure routinely used in our laboratories consists of mixing 3 μ l of suspension with 9 μ l of a solution of acridine orange and ethidium bromide (1 μ g/ml of each) (5).

When viewed in a haemocytometer under a fluorescence microscope, "viable" cells accumulate acridine orange and show a green nuclear fluorescence, whereas non viable cells (with leaky membranes) are characterized by an orange cytoplasmic fluorescence due to ethidium bromide passing into the cells. In fresh suspensions prepared from the ventral mesencephalon as above, the cells appear uniformly spherical and cell counts typically range from 30,000 to 40,000 per microlitre of the original suspension. Usually, 80-95% of the cells are "viable" in freshly prepared suspensions and this percentage remains stable over several hours (5). A greater proportion of damaged cells probably reflect mechanical damage due to excessive or too rigorous pipetting. Since the suspended cells reaggregate and slowly deposit at the bottom of the tube, resuspension is necessary in order to maintain a reasonable cell density in the injected suspensions, unless the preparation protocol using DNase is employed. However, since re-pipetting also reduces the viability of the cells (5) resuspension should be gentle and not too often. The middle course we have adopted is to resuspend every 30-60 min in the 3-3½ hours during which the intracerebral injections are made.

Although a wide variety of neurone types have been found to survive grafting with the present suspension procedure, provided the optimal donor age is used (see ref. 21), we have recently observed that the noradrenergic neurones of the

developing locus coeruleus are sensitive to trypsin, and that these neurones will not survive suspension grafting unless the trypsin incubation step is omitted (2). Therefore, for central noradrenergic neurones (and perhaps other neurone types as well) a modified procedure without trypsinization should be used.

Alternative Procedure

The experience with the more elaborate alternative suspension procedure is as yet limited. Although it is likely that the use of a more complete medium, a purer trypsin preparation, and DNase should be generally favourable for neuronal survival and reproducibility in the grafting procedure, no systematic comparison of the results obtained with the original and the modified procedures has so far been performed. Nevertheless, the modifications included in the alternative procedure are not critical; indeed, all the studies of the present monograph (Chapter II-VIII) have been conducted using the standard procedure.

Injection Procedure

Bacteriological infections can be reduced by maintaining sterile conditions not only during preparation of the suspension itself, but also during stereotaxic injection. Rats which have previously received surgical operations for denervation of intrinsic pathways are particularly likely to have residual bacterial activity within the partially-healed scalp wound, and such infections readily become transferred between rats if the same non-sterile injection needle is used. This speaks in favour of using syringes with removable needles, so that the needle can be exchanged frequently.

Multiple injections can readily be made within the same rat, but care must be taken to limit the total volume administered, preventing increases in intracranial pressure which can extrude the grafts back up the injection tract into ventricular spaces, the choroidal fissure or out onto the cortical surface. The observations on multiple nigral grafts in Chapter II indicate that when as much as 20 μ l (5 x 4 μ l) was injected into the same hemisphere in one session the survival rate and volumes of the individual grafts were reduced. Total volumes of 9 μ l (3 x 3 μ l) gave clearly better results in that respect. Furthermore, grafts can

show extensive subsequent growth and even single placements can expand to distort the host tissue considerably. This was frequently seen with the 5 μ l implants of septal suspensions into the hippocampus in Chapter VI. The injected volume has therefore to be adjusted according to the proliferative capacity of the dissected region, which is a factor that will vary markedly also according to the age of the donor foetuses. For nigral and septal suspensions injection volumes of 1-3 μ l (typically containing a total of 30,000-120,000 cells; see above) are adequate.

CONCLUSIONS

The suspension transplantation procedure provides a number of distinct advantages over previous procedures involving grafting solid pieces of embryonic tissue to the adult brain. In particular, the technique provides new possibilities to graft tissue from any neuronal

subpopulation within the neuraxis to deep as well as superficial sites in the adult CNS, at least within all combinations so far investigated. This permits a more flexible and balanced design of experiments as exemplified in the following chapters. First, the results are presented of nigral grafts placed into various forebrain and midbrain sites, studied anatomically (Chapter II), biochemically (Chapter III) and functionally (Chapter IV and V). Next, anatomical (Chapter VI) and biochemical (Chapter VII) results of septal suspension grafts to the hippocampus are presented. Preliminary functional studies of septal suspension grafts compared with solid grafts, also placed into the hippocampus, have already been published (10). Finally, a detailed description is presented of the anatomical survival and growth of both nigral and septal suspensions implanted in the depth of the intact brain in aged rats (Chapter VIII). Some aspects on the functional competence of these grafts in aged rats has also been presented elsewhere (11).

REFERENCES

1. Altman, J., Autoradiographic and histological studies of postnatal neurogenesis. II. Dating the time of production and onset of differentiation of cerebellar microneurons in rats. *J. Comp. Neurol.* 136 (1969) 269-294.
2. Björklund, A., Nornes, H., Dunnett, S. B., Gage, F. H. and Stenevi, U., Intracerebral and intraspinal implants of locus coeruleus cell suspensions: deleterious effect of trypsin in the suspension medium. *Soc. Neurosci. Abstr.* 9 (1983) in press.
3. Björklund, A., Schmidt, R. H. and Stenevi, U., Functional reinnervation of the neostriatum in the adult rat by use of intraparenchymal grafting of dissociated cell suspensions from the substantia nigra. *Cell Tiss. Res.* 212 (1980) 39-45.
4. Björklund, A. and Stenevi, U., Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. *Brain Res.* 177 (1979) 555-560.
5. Brundin, P. and Isacson, O., Monitoring of neuronal survival in suspensions of embryonic CNS tissue used for intracerebral grafting. *Neurosci. Lett. Suppl.* 14 (1983) 544.
6. Creps, E. S., Time of neuron origin in preoptic and septal areas of the mouse: an autoradiographic study. *J. Comp. Neurol.* 157 (1974) 61-244.
7. Das, G. D., Transplantation of embryonic neural tissue in the mammalian brain. I. Growth and differentiation of neuroblasts from various regions of the embryonic brain in the cerebellum of neonate rats. *T.I.T. J. Life Sci.* 4 (1974) 93-124.
8. Das, G. D., Hallas, B. H. and Das, K. G., Transplantation of neural tissue in the brains of laboratory mammals: technical details and comments. *Experientia* 35 (1979) 143-153.
9. Das, G. D., Hallas, B. H. and Das, K. G., Transplantation of brain tissue in the brain of rat. I. Growth characteristics of neocortical transplants from embryos of different ages. *Amer. J. Anat.* 158 (1980) 135-145.
10. Dunnett, S. B., Low, W. C., Iversen, S. D., Stenevi, U. and Björklund, A., Septal transplants restore maze learning in rats with fornix-fimbria lesions. *Brain Res.* 251 (1982) 335-348.
11. Gage, F. H., Dunnett, S. B., Björklund, A. and Stenevi, U., Aged rats: recovery of motor coordination impairments by intrastriatal nigral grafts. *Science* 219 (1983) 416-418.
12. Kromer, L. E., Björklund, A. and Stenevi, U., Intracerebral implants: A technique for studying neuronal interactions. *Science* 204 (1979) 1117-1119.

13. Kromer, L. F., Björklund, A. and Stenevi, U., Intracerebral embryonic neural implants in the adult rat brain. I. Growth and mature organization of brainstem, cerebellar and hippocampal implants. *J. Comp. Neurol.* (1984) in press.
14. Lauder, J. M. and Bloom, F. E., Ontogeny of monoamine neurons in the locus coeruleus, raphe nuclei and substantia nigra of the rat. I. Cell differentiation. *J. Comp. Neurol.* 155 (1974) 469-482.
15. Marchand, R. and Poirier, L. J., Isthmic origin of neurons of the rat substantia nigra. *Neurosci.* 9 (1983) 373-381.
16. Mishell, B. B. and Shiigi, S. M., *Selected Methods in Cellular Immunology*, W. H. Freeman and Co., San Francisco, 1980, pp. 21-22.
17. Olson, L., Seiger, Å. and Strömberg, I., Intraocular transplantation in rodents. A detailed account of the procedure and example of its use in neurobiology with special reference to brain tissue grafting. In S. Federoff (ed.) *Advances in Cellular Neurobiology*, Vol. 4. Academic Press, New York, 1982.
18. Perlow, M. J., The brain and subarachnoid space as possible sites for endocrine tissue transplantation. *Brain Res. Bull.* 6 (1980) 171-176.
19. Rosenstein, J. M. and Brightman, M. W., Intact cerebral ventricle as a site for tissue transplantation. *Nature* 275 (1978) 83-85.
20. Schmidt, R. H., Björklund, A. and Stenevi, U., Intracerebral grafting of dissociated CNS tissue suspensions: a new approach for neuronal transplantation to deep brain sites. *Brain Res.* 218 (1981) 347-356.
21. Schmidt, R. H., Björklund, A., Stenevi, U. and Dunnett, S. B., Intracerebral grafting of dissociated CNS tissue suspensions, in F. J. Seil (ed.) *Nerve, Organ and Tissue Regeneration: Research Perspectives*, Academic Press, N.Y., 1983, in press.
22. Stenevi, U., Björklund, A. and Dunnett, S. B., Functional reinnervation of the denervated neostriatum by nigral transplants. *Peptides* 1 (Suppl. 1) (1980) 111-116.
23. Stenevi, U., Björklund, A. and Svendgaard, N.-A., Transplantations of central and peripheral monoamine neurons to the adult rat brain: Techniques and conditions for survival. *Brain Res.* 114 (1976) 1-20.
24. Sunde, N. Aa. and Zimmer, J., Cellular, histochemical and connective organization of the hippocampus and fascia dentata transplanted to different regions of immature and adult rat brain. *Dev. Brain Res.* 8 (1983) 165-191.
25. Sunde, N. Aa. and Zimmer, J., Transplantation of central nervous tissue. An introduction with results and implications. *Acta Neurol. Scand.* 63 (1981) 323-335.