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Neural Transplantation in Mammals: Our Experience

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Neural tissue transplantation was carried out at various sites in rodents and rhesus monkeys using different regions of the fetal brain to establish the technique. Whereas there was 80–85% success in rodents, the success rate in monkeys was approximately 20%, that too when the donor embryonic tissue was cryo-preserved for few days prior to transplantation. The significance of this observation is not clear. Further, fetal substantia nigra and adult adrenal medulla were transplanted in the anterior chamber of the eye, the lateral ventricle and the striatum of adult rats for long term morphological studies. Most available studies have been restricted to 3 to 7 months and detailed morphological investigations are lacking. These studies showed that, though nigral cells differentiated to express phenotypic features, there was a gradual "ageing or degenerative" changes observed in transplants of more than six months. A peculiar feature, not seen in the normal substantia nigra was frequent occurrence of intimately opposed neurons. Transplanted adrenal medullary cells did not show any neuronal differentiation and they degenerated at a faster rate.

These observations reveal that the transplanted aminergic cells survive in the host's brain for limited duration and manifest several morphological abnormalities. The neurobiological significance of these findings needs to be explored further. Moreover, in nonhuman primates survival rate of the transplant is poor. These factors need to be taken into consideration before using this procedure for treatment of human diseases.

Key Words: Neural Transplant, Ageing of Transplants, Aminergic Cells in Transplant

Introduction

Nearly three years ago Department of Science & Technology, Government of India, established a National Facility for Neural Transplantation to study neurobiological and behavioural consequences of neural transplants in mammals. A multidisciplinary group working in this Unit has standardized the technique of fetal neural transplantation in rats and monkeys. A variety of sites, e.g. different parts of the brain parenchyma, lateral ventricle and the anterior chamber of the eye, have been utilized in the hosts for transplantation of fetal tissue obtained from different regions of the brain. Besides, autografts and allografts of adrenal medulla have also been similarly transplanted. These studies have interesting implications both from the standpoint of basic neurobiology and also for ultimate use of this technique for therapy of neurological disorders in man.

Material and Method

Initially a variety of sites in the host brain were used to transplant donor tissue from different regions of the developing brain of 17-18 days old rat fetuses. The technique used was one standardized by Das et al. (1979). Successful transplantation could be achieved in 80-85% cases. Having standardized the technique, it

was decided to concentration transplantation of dopaminergic neurons from fetal substantia nigra and adult adrenal medulla.

Simultaneously, work was started on rhesus monkeys to ascertain if the results so consistantly obtained by workers all over the world in rodents could be duplicated in sub-human primates. As a prelude to this, monkey fetuses of varying periods of gestation, (50-90 days) were studied to determine the stage of neurogenesis most suitable for purposes of transplantation in the light of experience obtained from rodents (Das et al. 1980 and Gopinath et al. 1987). It is now generally recognised that the donor tissue should have enough growth potential to multiply, differentiate and mature so that it can integrate with the host brain. Mixture of dividing neuroepithelial cells and neuroblasts without mesenchymal contamination is ideally suited for this purpose. Host monkeys were treated with MPTP to produce a model of Parkinsonism (intramuscular injection for bilateral effect and intracarotid for hemiparkinsonism). These monkeys were then transplanted with fetal substantia nigra in their striatum unilaterally.

Both groups of animals were sacrificed at varying intervals and submitted to detailed morphological studies utilizing the following techniques:

(i) Nissel stain for cytoarchitecture
(ii) Electronmicroscopic studies for cytological details
(iii) Golgi stain for details of individual neurons
(iv) Fluorescence for catacholamines
(v) HRP for neuronal tracing and connectivity
(vi) Immunocytochemical studies by gold labelling
(vii) Quantitative morphometry

While several other studies, e.g. transplantation following kainic acid lesions in preoptic and caudate region or transplantation of fetal neocortex in anterior chamber of eye and tectal region have been carried out in rats, the present communication deals with our experience with dopaminergic neurons only. The same applies to our studies in monkeys. Table 1 summarises the sites and nature of donor tissue utilized by us. Details of study in various groups will be published separately. Only a brief summary is being provided here.

Table 1 *Neocortical grafts in rat (A) and Substantia nigral grafts in intact adult rats (B)*

Host site	Total transplants	Successful transplants
Cerebellum	45	25
Caudate	31	20
Lateral ventricle	15	8
Tectum (New born host)	20	16
Intraocular	84	78
Intraventricular	62	53
Intrastriatal	55	46

Results

Rats

Neocortex, substantia nigra, and adrenal medullary transplants in the anterior chamber of the eye, lateral ventricle or the striatum could be successfully established in 70 to 85% adult host rats. These were followed for a period until 1 year after transplantation. To the best of our knowledge such detailed morphological studies, for such long periods are not available in the literature.

As already demonstrated by a host of workers, neural tissue survived, grew, developed organotypic maturation, sent out processes, established synaptic connections and produced appropriate neurotransmitter. While majority of cells remained in clusters, some at the approptiate sites showed migration. Axonal transport could also be demonstrated with the help of HRP labeling. The surrounding brain sent out processes towards the graft and probably into it. It must be mentioned that while all these features were observed in varying degrees in fetal substantia nigra transplants, irrespective of the sites of transplanatation, the anterior chamber of the eye, lateral ventricle or the striatum, the adrenal medullarly transplants did not transform into neurons but retained the characteristics of aminergic cells. This was obviously exciting and held great promise for therapeutic application of the technique.

However, a more critical study of the transplants revealed a number of features which appear to have important implications, if its integration with the host

with development of appropriate circuitry and long term survival of the graft are a pre-requisite for recovery of lost functions. Some of these features are summarized below:

i) While the transplanted neurons developed processes, at times indeed very impressive, these seldom extended more than 2-3mm into the host tissue.

ii) These processes manifested some branching but seldom the degree of arborizations seen in normal neurons (figures 1, 2).

iii) The processes, occasionally seen to be growing from the host parenchyma into the transplant, could not definitely be demonstrated to develop synapses with the neurons of the transplant.

iv) Most of the synapses observed were between the neurons of the transplant itself (figure 3).

v) Close approximation of two neurons, not seen in normal substantia nigra, was a common feature in the transplants and gap junctions could be seen between neurons without any associated vesicles. The significance of this observation is not clear (figure 3).

vi) Even more important than the above observations, are the changes observed in these neurons with passage of time. From 60th day of transplantation a number of neuronal soma and a few dendrites started showing clear spaces in the cytoplasam (figure 5). Frequency of such neurons increased as the age of the transplant advanced. As time progressed, one could observe shunken neurons with hyperchromatic nuclei. Thus, the proportion of such neurons to normal neurons was at 1:3 180 days and 1:2 at 360 days.

vii) Similarly there was increasing amount of lysosomal bodies with increasing age of the transplant. This may be indicative of early ageing in these neurons.

It may be emphasized that such changes were observed in transplants at all the three sites, e.g. anterior chamber of the eye, the ventricle and the striatum.

viii) Besides these morphological changes, very likely indicative of degeneration, there was a concurrent decrease in the total number of neurons and fluorescing neurons in the transplant.

ix) Similarly features suggestive of progressive degeneration were observed in the transplanted aminergic cells from adrenal medulla also. (figure 6).

Rhesus Monkeys

Successful transplant in monkeys could be achieved only in 20% of cases, as opposed to 80-85% in rats. It is interesting to note that the fetal tissue used for transplantation in the first two successful transplants in monkeys was preserved in culture medium for 4 days before injecting in the host while all transplants using fresh tissue failed. Following demonstration of successful transplants in these monkeys (figure 7), experimental model of Parkinsonism using parenteral or unilateral intracarotid MPTP injection, was established. Once the syndrome was stabilized, fetal (50 days gestation period) substantia nigra was transplanted in the caudate. Two monkeys (one with generalized and other with hemiparkinsonism) survived long enough for study. There was considerable improvement in tremor, rigidity and akinesia in 2 weeks time in one, even though the movements of the limbs were still slow and clumpsy. This animal was sacrificed 100 days after transplantation, and showed diffuse aminergic fluorescence at the site of transplant.

Discussion

These results confirm, what has already been well established that the fetal neural transplants into the brain of adult hosts, with or without pre-existing lesion, survive, grow, develop organotypic maturation, produce appropriate neurotransmitter and result in varying degree of recovery of lost function. In the present study we have primarily concentrated on morphological aspects using a battery of techniques, light and electonmicroscopy, Golgi, HRP labelling, immunohistochemistry and immunofluorescence. While on the face of it, it appears that in a large measure these investigations are confirmatory in nature, a more careful review of our observations reveal some interesting findings summarized above. These have important implications both from basic neurobiological standpoint as also for the clinical application of the technique.

It is obvious from the present study that while successful transplantation is a rule rather than an excep-

tion in rodents, the same is not true for rhesus monkeys. This is not just our experience but others have the same high failure rate. The reason for this failure is not obvious. Though routine studies failed to show significant lymphocytic infiltration , more detailed studies using specific antibodies will be necessary to determine if this is due to immunological rejection. It may be mentioned that in lesser evolved sub-human primates like African green monkeys, cynomolgus monkeys, squirrel monkeys or bonnet monkeys the failure rate is not as high. At the time of this presentation only a few reports of successful transplantation in rhesus monkeys are available (Bakay et al. 1987, Morihisa et al. 1987). Furthermore, detailed morphological studies are still lacking which makes it difficult to evaluate, if the behavioural recovery was really due to the establishment of neuronal circuitry between the host and the transplant.

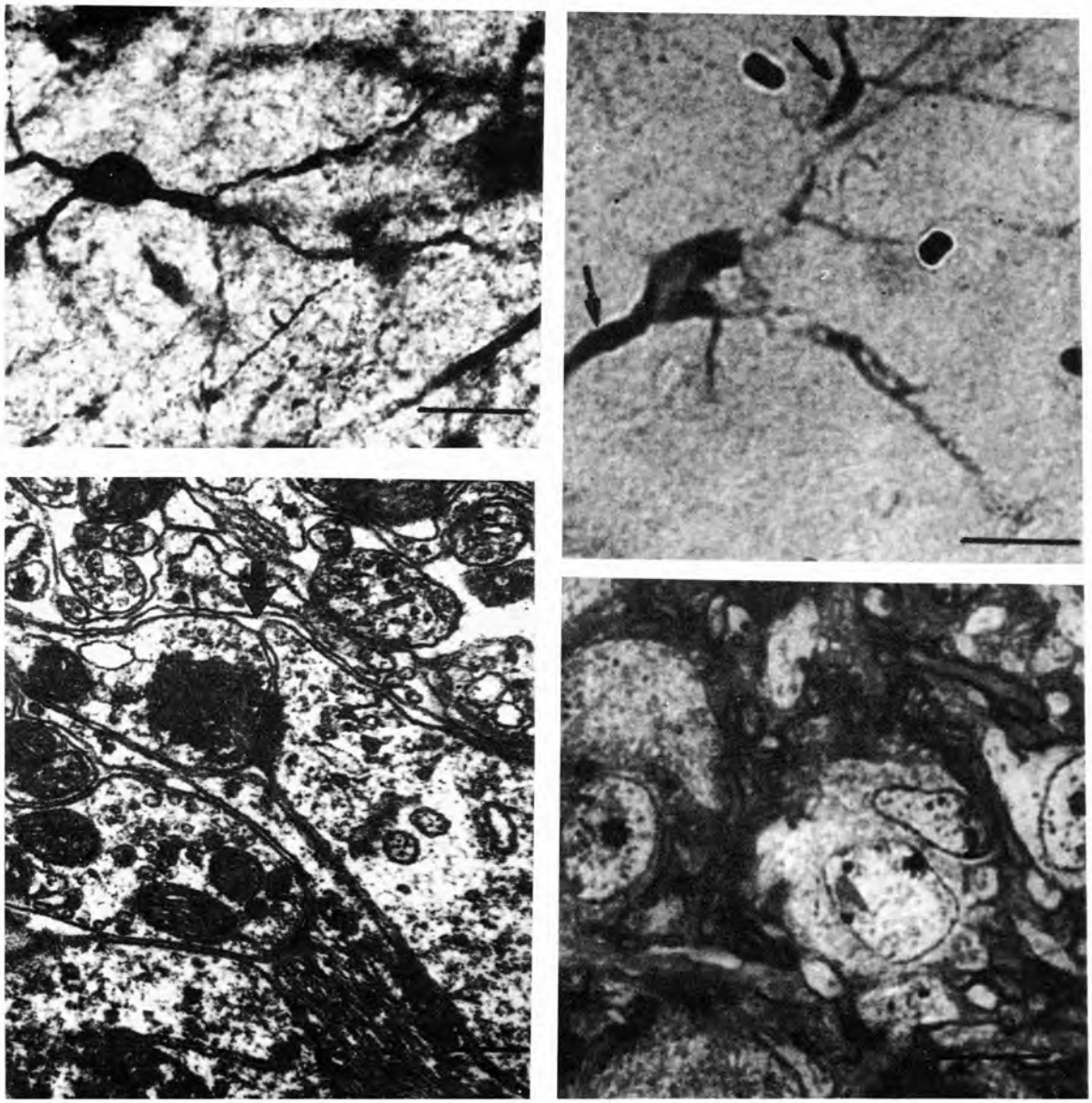
Another intriguing feature regarding transplantation in monkeys was the success obtained with fetal tissue preserved for 4 days in culture media as opposed to the high failure rate of fresh transplants. The presentation by Victorov and Lyjin in this symposium is of special relevance for our observation. This line of investigation need to be persued further, not only to elucidate the biological basis of this observation, but also for its relevance as and when fetal neural transplantation is adopted for clinical use. Needless to say many more animals needs to be studied prior to any definite conclusion.

In respect of the transplantation of dopaminergic tissue from substantia nigra and adrenal medulla in rats, the present study raises some important questions. Firstly while the transplanted cells acquire organotypic maturation, the morphological features are not always completely identical to the normal cells. The significance of frequent observation of close approximation of two neurons, the existence of gap junctions without any associated vesicles is still not clear. Yet these observations must be kept in mind. Furthermore the transplanted nigral enlarge cells do mature into neurons, glial cells and even myelinated fibres, however, the Golgi studies of the neurons show that neither the morphology of dendrites nor their arborizations are similar to the normal neurons of substantia nigra. The neuronal processes grow to a varying extent but seldom more than a millimeter or two. Most of the time these end up in the transplant itself. The synapses observed are between the neurons of the transplant. Thus, at best the neuronal circuitry is restricted locally. Once again the biological implications of this phenomenon have not yet been elucidated. There is no doubt that these neurons produce dopamine but the manner in which this locally produced dopamine helps to ameliorate the clinical symptoms is not clear.

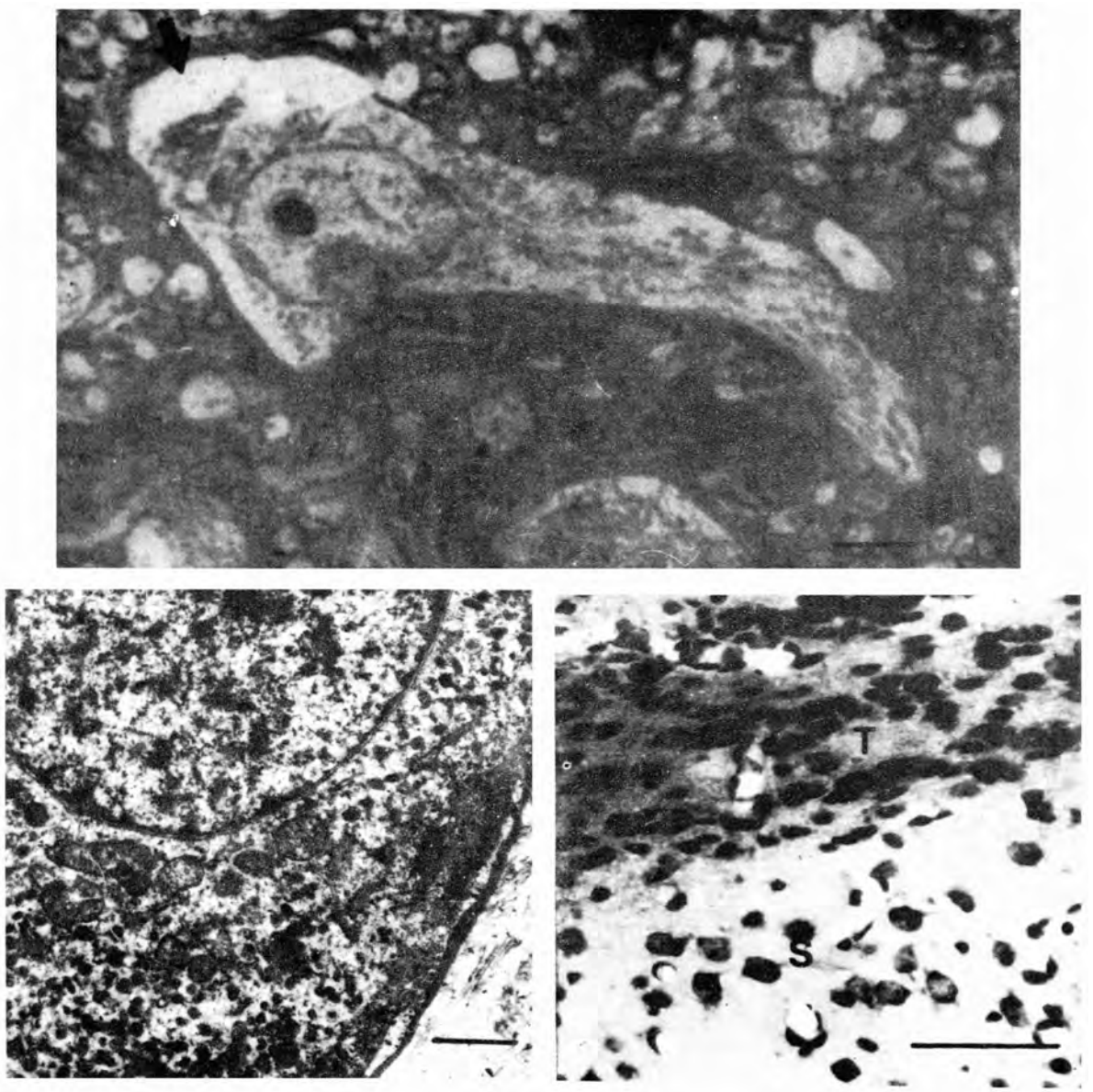
One of the important observations of the present study, so far not commented upon in the published literature, is in respect of progressively increasing number of "degnerating or ageing" neurons observed in animals surviving for more than six months. This does not appear to be an immunological rejection but a true degeneration. It is important to understand the underlying pathogenetic mechanism for this degeneration by further investigations. Needless to say this is of vital importance for its clinical implications.

With regards to the adrenal medullary transplants our observations confirm the earlier findings. However, it may be stated that unlike Olson (1970) and Olson et al. (1980), we did not observe any neuronal transformation of the aminergic cells of adrenal medulla even in the striatum. With increasing duration of transplant the density of aminergic fluorescence diminished. This may be indicative of fall out of the transplanted cells with passage of time.

A few words with regards to clinical implications of our observations in the light of published reports on adrenal medullary transplants in patients of Parkinson's disease would be in place. The immediate improvement in the clinical features of these patients reported by Madrazo et al. (1987) following transplantation in the caudate nucleus obviously cannot be due to growth, integration or development of even local circuitry. One of the possibility is local release of dopamine by the transplanted cells undergoing "lysis". There could be some other "neurotrophic" substance made available by the transplanted cells which may "stimulate" formation or release of dopamine by the host's intact cells. If this be so, one cannot explain the longterm good results claimed by the neurosurgeons atleast in some



Figures 1-4, 1, A Golgi-stained neuron from the nigral transplant in the striatum of adult rat. Bar = 40μ; **2**, A tyrosine hydroxylase positive dopaminergic nigral neuron in the ventricular transplant of 8 months duration. Thickening of the dendrites is seen as part of the ageing process (arrow). (Bar = 20μ); **3**, Electronmicrograph showing intrinsic synapse in the intrastriatal transplant grown from fetal substantia *nigra* (arrow) (Bar = 0.5μ); **4**, Photomicrograph of a semithin section of a transplant (introcular) to show myelinated fibres and double neurons. (Bar = 10μ) Toluidine blue stain.



Figures 5-7, 5, A neuron showing clear space in the cytoplasm after 6 month of transplantation (arrow). (Bar = 5μ) Semithin section stained with toluidine blue, **6,** Electron micrograph of a chromaffin cell in the medullary autograft showing degenerative changes of the aminergic granules & organells (Bar = 1μ); **7,** Photomicrograph of a 4 month old transplant (T) grown from fetal neocortex in the striatum (S) of rhesus monkey. (Bar = 50μ) Cresyl violet stain.

cases. It may thus be that development of neuronal circuitry is not a pre-requisite for the beneficial effect of the transplant.

It will be obvious from this presentation that while transplantation of the embryonic neural tissue in adult host of any species is a reality today and not science fiction, there are many remaining questions unanswered before we could safely recommend the procedure for treatment of human disease. It would not be wrong to state that from the rat to man is a long way, but the results in rats (and now in monkeys) are tentalizing enough to prompt intensive multidisciplinary investigations, not only morphological as presented here, but biochemical, immunological and behavioural with a sense of urgency. Even if a fraction of the success already achieved in animal models can be transplanted in human situation an immense amount of suffering can be mitigated.

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