Transplantation is one of the effective strategies in restoring cell and tissue function. Widely used in a variety of organ systems, transplantation approaches in the nervous system are still in their infancy. The complexity of the tissue and our limited knowledge about the mechanisms required for neuronal integration, have restricted therapeutic efforts to reconstitute local, well-characterised neurotransmitter deficits, such as restoring the nigro-striatal dopaminergic system in Parkinson's disease. Although transplantation of embryonic neurons has been carried out in Parkinson's patients, the technique has not yet reached the stage where it can be used as a therapeutic measure on regular basis. In spite of this, the possibility for neurons grafted into damaged brain to promote functional recovery, provides encouraging support to the idea that neural transplants might become therapeutically useful not only in case of Parkinson's disease, but also in other neurodegenerative disorders such as Alzheimer's and Huntington's disease.

Neurotoxicants have used with increasing frequency as investigative tools in neurobiology. Generally the neurotoxicants have been found to be potentially selective for a particular cell type or population. Their ability to destroy a selected cell population while sparing the surrounding cells render them useful for study of the functions of different brain regions. Some of the neurotoxicants are capable of
producing brain damage that in many respects resemble or mimic specific changes seen in neurodegenerative disorders. Therefore, the use of neurotoxicants is advantageous in studying the process of degeneration, repair and regeneration and also for studying mechanism of disease processes.

Trimethyliquinolin (TMT), belongs to the class of environmental toxins. TMT is a potent neurotoxicant and is capable of producing wide spread neuronal damage. Lesions induced by TMT have aroused attention as the damage produced by TMT is not dependent on any one neurotransmitter system and at behavioural levels TMT produces similar symptoms in rodents to those seen in the original reports on exposure to humans. The primary neuropathological effects of TMT occur in the limbic system, with the pyramidal neurons of the hippocampus being the most vulnerable, leading to a number of behavioral deficits including learning and memory, aggressive behaviour and hyperactivity.

Colchicine belongs to the class of investigative neurotoxins, as its administration has been found to selectively destroy the granule cells of the hippocampus. The granule cells of the hippocampus are involved in acquisition of information and learning and memory processes and thus their destruction have resulted in altered behavioral responses. Colchicine has been used to produce animal models of neurodegeneration (with behavioral deficits) and helped to study the role of granule cells and hippocampus in cognition and behavioral reactivity.

Intra-hippocampal transplantation of fetal neurons in such types of hippocampal damage could be useful in understanding the mechanism of transplantation induced restoration of functional deficits where selected neuronal populations are damaged.

Transplantation studies have previously been carried out in TMT exposed and colchicine lesioned rats but only partial recovery of function has been observed. In recent years several efforts have been made to increase the efficacy (survival and restorative potential) of the transplants in the damaged brain. Administration of neurotrophic factors have been found to play important roles in: (i) Promoting recovery of function after exposure to neurotoxicants and (ii) Promoting maintenance, functional competence and neurite out growth of developing neurons. Exogenous administration of neurotrophins especially NGF into the lesioned hippocampus have been studied to prevent the loss of cholinergic neurons and improve functional
deficits. Administration of NGF along with fetal transplants could be helpful in the maturation and survival of cholinergic rich grafts and hence increase their efficacy.

Studies in experimental animals exposed to neurotoxins have indicated that in cases of widespread damage in brain regions, the placement of several micrografts instead of a large single graft could help in better survival and effectiveness of the transplants. The concept of transplantation of small micrografts is that it causes minimal trauma and higher degree of reproducibility. Single site transplants are localized to a particular area and it is improbable that they can completely repopulate the damaged region, whereas distribution of small micrografts at multiple sites would lead to the sequential innervation of the lesioned area and provide better and improved restoration of the functional deficits caused by neurotoxicants.

In the studies presented in this dissertation an attempt has been made to evaluate behavioural, neurochemical and morphological outcome of TMT and colchicine induced neurotoxicity. This was followed by transplantation of specific fetal neuronal tissue (obtained from timed pregnant rats) into the hippocampus of the toxin-exposed rats. The objective was to understand the restorative potential of fetal neural transplants. Further, nerve growth factor infusions and multiple microtransplantation were used to enhance the restorative potential of the transplants. Studies were conducted one and six months post transplantation and/or NGF administration to evaluate the efficacy of the transplants on short and long term basis. The study was divided into two main parts which are described briefly under the following headings:

(1) Role of fetal neural transplants in trimethyltin exposed rats

Exposure to trimethyltin (7.5mg/Kg) was found to produce severe behavioural and biochemical alterations in rats. The damage to the pyramidal neurons (CA1, CA3/CA4 regions) of the hippocampus by trimethyltin was found to be associated with hypermotility, decreased learning and memory performances and aggressive behaviour in rats.

Neurochemical parameters studied, showed significant decrease in acetylcholinesterase activity and cholinergic muscarinic receptor binding in the hippocampus. Significant depletions in the levels of 5-HT and 5-HT receptor binding was also observed in the hippocampus of TMT exposed rats. Keeping in view the hippocampal degeneration and cholinergic deficits caused by TMT, fetal cholinergic
rich neurons were transplanted into the hippocampus of TMT exposed rats using stereotaxic co-ordinates. Transplantation of fetal cholinergic neurons at single sites into the hippocampus of TMT exposed rats were found to partially restore the learning and memory and locomotor deficit. The cholinergic deficits (ChAT and AChE activities and muscarinic receptor binding) were also partially restored following single site transplantation. The restorative potential of single site transplants was however, not found to provide long term benefits. Behavioural and neurochemical parameters show no or very little restoration following six months of transplantation.

Administration of NGF along with single site transplants was found to initially alleviate the restorative potential of the grafts. TMT exposed rats receiving both transplants and NGF showed better restoration of cholinergic deficits than those receiving transplants alone. This combined effect of NGF and fetal transplants was however, not seen in studies conducted after six months indicating that NGF administration was also not effective on long term basis.

The studies on multiple site microtransplantation showed that they not only provide better restoration of the functional deficits but the restoration persists on long term basis. Rats receiving multiple site microtransplantation, exhibited better restoration of locomotor, learning and memory function and cholinergic deficits as compared to the single site transplants both after one and six months post grafting indicating better survival and integration of the microtransplants in the host.

As transplants rich in cholinergic neurons provided restoration of the cholinergic deficits, serotonergic deficits were also found to be restored by serotonergic rich transplants in the hippocampus. Rats receiving intra hippocampal serotonergic rich transplants showed a significant restoration in TMT induced aggressive behaviour. 5-HT levels and receptor binding were not only restored but were overcompensated for, indicating serotonergic hyperinnervation following serotonergic rich transplantation.

Co-transplantation of both fetal cholinergic and serotonergic neurons in the hippocampus of TMT exposed rats showed a simultaneous recovery of both cholinergic and serotonergic deficits indicting that such type of co-transplantation approach could be useful; when more than one neurotransmitter system is affected.
Role of fetal neural transplants in colchicine lesioned rats

Colchicine (2.5μg) was directly administered into the hippocampus of rats using stereotaxic co-ordinates. Intra-hippocampal administration of colchicine was found to destroy the granule cell layer and mossy fibres in the hippocampus leading to learning and memory deficits and increased locomotor activity. Significant alterations in cholinergic markers viz. decrease in AChE and ChAT activity and muscarinic receptor binding were observed indicating the damage of cholinergic neurons by intra-dentate colchicine administration. Cholinergic denervation was also confirmed by the loss of ChAT immunoreactive cell bodies in the hippocampus and the septum.

Due to the cholinergic deficits caused by colchicine, cholinergic rich fetal neurons were transplanted into the hippocampus of colchicine lesioned rats. Intra-hippocampal cholinergic rich transplants at single sites were found to partially restore colchicine induced behavioural deficits after one month of transplantation but not after six months. AChE and ChAT activities were also initially restored, but not on long term basis by single site fetal transplants.

Infusion of NGF into the hippocampus also had some initial benefits on behaviour and cholinergic markers. NGF, when infused along with fetal transplants appeared to have some cumulative effect in the restoration of the functional deficits. The degree of restoration in the group receiving both fetal transplants and NGF was higher as compared to the groups receiving fetal transplants or NGF alone. These beneficial effects of NGF however, was not found to last on long term basis as no effects could be seen after six months.

Multiple site microtransplants not only showed a better degree of restoration of the behavioural and neurochemical deficits as compared to single site transplants, but the restoration also persisted and cholinergic parameters appeared to improve with time. The restoration of enzyme activities (AChE and ChAT) and muscarinic receptor binding in multiple site transplanted group was significantly higher than that exhibited by single site transplanted rats.

The restoration by fetal cholinergic neuronal grafting was confirmed by ChAT immunoreactivity in the hippocampus and septum. Well differentiated transplanted neurons could be seen after six months of transplantation which indeed would have helped in the restoration of colchicine induced functional deficits.
Results of electrophysiological studies also showed an increase in the firing rate in the transplanted animals indicating the presence of viable transplants in the hippocampus, which alleviate colchicine induced functional deficits.

Conclusions

The data from the studies conducted indicate that TMT causes irreversible, simultaneous cholinergic and serotonergic deficits in rats accompanied with severe behavioural alterations due to its destructive effects on the pyramidal neurons. Colchicine administration in the hippocampus damages the granule cells and mossy fibres also leading to specific behavioural and biochemical alterations, where cholinergic populations seem to be the most affected.

The study also shows that fetal transplants are capable of reversing the behavioural and biochemical abnormalities caused by neurotoxins, at least partially. NGF seems to have some beneficial effects on the survival of transplants or mediating functional recovery but this effect does not persist long after the withdrawal of the trophic factor. The results indicate that perhaps a continuous supply of NGF is required for its effects to last.

Finally, from the study, it is evident that small micrografts distributed over larger area help in the better restoration of functional deficits. The small micrografts could be leading to a sequential innervation of the lesioned area promoting the survival of the transplanted neurons and thus behavioural and biochemical functions.

Another finding of interest is that co-transplantation of different cell types could be helpful in providing more complete restoration when more than a single neuronal population is damaged by exposure to neurotoxicants.