Chapter 1
Review of literature
Neurodegenerative illnesses are characterized by gradually evolving, slow, progressive neuronal death unaccompanied by an intense tissue reaction or inflammatory response. As the regeneration of mammalian central nervous system (CNS) is extremely limited, such neuronal losses are permanent leading to various functional deficits. Rapid industrialization and environmental pollution have contributed in the augmentation of neurological disorders. Various industrial pollutants such as lead, tin, manganese etc and pesticides are known to have deleterious effect on the central nervous system, causing neuronal injury. The subtle onset of neurodegeneration leads to several diseases such as Parkinson's disease (PD), in which the dopaminergic neurons of the nigrostriatal pathway are damaged; Alzheimer's disease (AD), caused by the degeneration of basal forebrain cholinergic neurons and Huntington's disease etc.

In addition to such factors, conditions such as cardiac arrest, seizure activity and hypotension may lead to impaired cerebral blood flow (cerebral ischemia) causing irreversible brain damage and neurological deficits. Due to limitation in the spontaneous regeneration of the CNS the avenues in the treatment of neurological disease are themselves limiting.
In cases of Parkinson's disease only, current treatment is based on a dopamine replacement strategy using the dopamine precursor levodopa, but long term treatment with levodopa is complicated by involuntary movement (dyskinesia), neuropsychiatric side effects and fluctuations in motor functions (Olanow, 1996). Consequently, there has been search for alternative therapies that can reverse functional disabilities in patients with Parkinson's disease and other progressive neurological disorders that cannot be satisfactorily controlled with existing medications.

By analogy with drug induced effects, it was evident that dopamine producing cells implanted into the striatum of Parkinson's patients might be able to reverse some of the symptoms of a damaged or dysfunctioning dopamine system (Bjorklund, 1991). Currently, fetal neural transplantation appears to be a promising approach in the treatment of such neurological disorders (Kopyov et al. 1998).

The first studies on neural transplantation were carried out by W.G. Thompson in 1890, in which apparently viable cortical tissue of cat was implanted into the brain of dogs. Thompson concluded that the transplanted tissue could survive up to seven weeks after surgery. Del Conte (1907) made the first attempts to transplant embryonic tissues into the brain. This attempt was unsuccessful but Dunn (1917) successfully grafted neonatal CNS tissue into the brain. It was in 1940, LeGros Clark successfully grafted fetal neurons into the adult brain.

Since the first reports by Thompson (1890), sporadic studies over eight decades have characterized several different perspectives on neural transplantation. The initial fascination raised by the possibility of grafting brain tissue and ultimately, the reconstruction of damaged neural networks met with speculations and skepticism. However, experimental studies in the last twenty-five years have shown the reliability of fetal neural cells in restoring the functional deficits caused by neurodegeneration.

**Survival of Transplants in the Brain**

The earlier studies on fetal neural transplantation met with infrequent success, but Raisman (1969) provided the first electron microscopic demonstration of collateral sprouting in partially deafferented striatum. The regenerative capacity of nervous system
was reconsidered and several groups challenged the dictum regarding the absence of regeneration of CNS (Finger and Stein, 1982). Studies by Das and Altman (1971), Das et al (1980), Bjorklund and Stenevi (1971, 1977, 1979), Olson and Seiger (1972) and Stenevi et al (1976), demonstrated that the grafts could survive, extend axons in the host brain and establish appropriate patterns of reinnervation in areas from which the intrinsic host inervation has been removed by a variety of experimental lesions in rat. Das and Altman (1971), giving a comprehensive account of the transplantation technique studied the survival and growth of intracerebellar grafts in young rats. Olson and Malnfors (1970) and Bjorklund and Stenevi (1971) demonstrated the regeneration of axonal connections in the anterior chamber of the eye and brain respectively. From the initial studies conducted in the field of neural transplantation, research groups have outlined critical factors for survival of transplanted fetal neurons.

1. Transplanted tissues into the CNS are viable when obtained from immature (embryonic) donors (Stenevi 1976). The age of the donor tissue is crucial for their survival in the host (Seiger, 1985).

2. The age of the recipient affect the number of transplanted neurons that survive and extent of outgrowth of axons from transplanted neurons into the host brain (Gage et al 1983).

3. The site of transplantation in the host brain should be richly vascularized to provide support and rapid incorporation of newly grafted neurons into the host blood and cerebro spinal fluid circulation.

4. Transplanted neurons should be protected from immunological rejection by host (Widener and Brundin 1988). Graft failure generally occurs due to infection and damage during surgery. Hence transplantation should be performed under aseptic conditions.

Recently, Nikkhah et al (1995) showed that small grafts distributed at multiple sites survive better as compared to large grafts at single sites. Cells in small suspensions were found to form better connections with host neurons as well as with other transplanted neurons.
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Agrawal et al (1995), using fetal nigral cell suspension, have shown that transplants not only survive in the striatum of 6-hydroxydopamine (6-OHDA) lesioned rat striatum but assume physiological functions as well. Gopinath et al (1996) conducted morphological (electron microscopic) studies in 6-OHDA lesioned rats receiving nigral transplants and found that transplanted neurons survived at least upto 2 years after surgery.

Growth and Maturation of Transplanted Neurons in lesioned brain

Several microscopic and ultrastructural studies have been carried out to assess the growth and maturation of the transplanted neurons whereby they reinnervate the damaged brain region and provide functional recovery. The ability of different types of neurons to reinnervate and make synaptic contacts in the host depends on (a) the ability of specific neurons to survive in the denervated region of the host and (b) the capacity for each type of neurons to grow axons and form organotypic innervation patterns. Studies on the functional effects of neural transplantation undertaken in the 80s suggested that grafting of healthy neuronal tissue is a promising approach for amelioration of neurological dysfunction. Bjorklund and Stenevi (1979, 1984) and Dunnet et al (1983) showed that grafts of embryonic dopamine neurons in the neostriatum of 6-hydroxydopamine lesioned rats established neuronal circuitry and ameliorated amphetamine induced rotations. Bjorklund et al (1983), Cotman et al (1984), Schmidt et al (1983), Fine (1986), Fishman (1986) examined the anatomical, neurochemical and physiological relationship of the transplanted fetal neurons with the host tissue. The grafted neurons were found to survive, extend axons into the host and form synaptic connections with the host.

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hippocampus of chemically lesioned rats. Synaptic potentials in the pyramidal like neurons of the transplants indicated that normal afferent connection were made.

Clarke et al (1990) quantified cholinergic inputs into the hippocampus by choline acetyltransferase (ChAT) immunohistochemistry and observed that septal neurons after transplantation showed the best survival, reinnervation and organo-formation in denervated hippocampus as compared to neurons derived from other regions. Studies in global ischemia demonstrated substantial cognitive recoveries in rats with transplants of fetal CA1 region of hippocampus than with transplants from fetal CA3 region (Netto et al 1993, Hodges et al 1996). Histological studies by Mudrik and Baimbridge (1990), showed that grafts containing CA1 like pyramidal cells sent projections to host septal area, from which they also received innervation. Tonder et al (1989) showed that grafts containing normal CA1, CA2 and CA3 pyramidal cells showed normal levels of Glial Fibrillary Acidic Protein (GFAP) and received innervation from host septal area.

Katayama et al (1991) suggested that transplant survival and integration was more if transplantation of embryonic day 17-18 (E17-E18) neurons was done early after neuronal damage. Lindvall et al (1992), Takeda et al (1994), Stafkhina et al (1995) have shown that hippocampal cell suspension grafts, in contrast to solid grafts, survive and make better contacts with host neurons in the neocortex. Reinnervation of the hippocampal CA1 region and formation of synaptic contacts in CA3 and mossy fiber region by homotopic transplants have been observed (Tonder et al 1990, Field et al 1991). Farber et al (1988) and Shetty et al (1994) using horseradish peroxidase (HRP) and Bromodeoxy uridine (BRDU) labeled fetal neurons respectively, showed that the transplants survive and expand considerably from the initial injection site. The latter also confirmed by three dimensional graft reconstruction studies that absolute graft cell survival varied according to the location of the transplants. Pyapall et al (1994) showed that fetal hippocampal neurons mature and integrate in kainic acid lesioned hippocampus of rats. The results of the study conducted by Macklis (1993) in which neurodegeneration was produced by chromophore targeted laser photolysis, suggest that transplanted neocortical neurons migrate into regions of neurodegeneration to restore normal cytoarchitecture. Mudrik et al (1989) demonstrated that using cells from same/equivalent
regions have the ability to become well integrated with the host tissue. Several studies by Pohle (1987), Tandon et al (1988), Woodruff et al (1988) have shown that fetal homotopic transplants survive, integrate with the host and are capable of producing behavioural recoveries.

Studies on transplantation of fetal mesencephalic tissue on Parkinsonian non-human primates have also demonstrated usefulness of transplants in producing recovery. Bakay (1987) in his study on eight MPTP treated Rhesus monkeys showed behavioural recovery following transplantation and histochemically demonstrated catecholamine fluorescent cells in regions of the implant and a rich network of catecholaminergic fibers emerging from these cells. Bakay et al (1988) demonstrated in their anatomical studies, TH-IR cells and fibers within and adjacent to the injection tract used for stereotaxic implantation. Redmond et al (1986) and Sladek et al (1987) also showed tyrosine hydroxylase immunoreactive (TH-IR) neurons with and fibers ramifying extensively throughout the host striatum.

Bankiewicz et al (1990) carried out histological studies in Rehsus monkeys exhibiting bilateral signs of PD. After transplantation of fetal mesencephalon into the caudate, TH-IR cells were noted to survive and project neurites but the neurites did not extend beyond the graft. In a similar study by this group in 1988, grafting of fetal cerebellum into the caudate nuclei promoted sprouting of dopaminergic fibers from the ventral striatum of the host towards the implantation site with appropriate changes in rotational behaviour. The capability of fetal grafts to mature and exhibit axonal growth to distant targets has been demonstrated (Davies et al, 1994; Garcia et al, 1995). Studies have provided quantitative evidence that grafted fetal neurons located near the degenerated region are capable of establishing robust long distance projections.

**Mechanism of Transplant induced Restoration of Function**

Various biochemical and functional studies have been carried out in lesioned and transplanted animals and reports show that no single mechanism specifically accounts for graft induced functional recovery. Many different mechanisms apply in different model
systems, different mechanism could work in conjugation within the same system to produce restoration.

The foremost condition for underlying mechanism of graft induced functional recovery is to ascertain the exact pathway and circuitry damaged by toxin insult. Studies on dopaminergic degeneration by Alexander and Crutch (1990) and Delong (1990) demonstrate that a 6-ODHA lesions of ascending dopaminergic system affects one of the important component of the control of neuronal activity within the cortico-striatal loops but does not disrupt the integrity of the underlying neuronal organisation and topography. Such lesions of intrinsic and neostriatal neurons disrupts the nigro striatal pathway and will provide an essential disconnection of the cortico-striatal loop. Transplantation of fetal nigral tissue in such type of degeneration is found to be effective in providing behavioural recovery (Dunnet et al 1983, Dunnet et al 1987). The nigral grafts provide diffused striatal activation but cannot replace the damaged circuitry and thus cannot relay information to the striatum via the nigrostriatal pathway (Nieoullon et al 1997, Chiodo et al 1979, Grace and Bunney 1984 a,b). Striatal grafts on the other hand have a remarkable feature of connectivity (Wictorin, 1992). Studies have indicated that striatal grafts implanted into the denervated neostriatum receive extensive host innervations from all major afferents of the host striatum and give rise to reliable reinnervation to near and distant targets (Wictorin and Bjorklund 1989, Wictorin et al 1988,1989,1990,1991, Xu et al 1989, 1991). The chemical anatomy and synaptic connectivity within the graft is appropriate at the ultrastructural levels with host dopaminergic neurons making normal synaptic contacts with GABAergic neurons of the transplants (Clarke et al 1988, 1993). Studies on fetal intra hippocampal transplants to produce behavioural improvements and get integrated with the host hippocampus have been reported to have variable effects depending upon the extent of hippocampal damage, graft survival and integration.

Improvements have been observed following intra dentate colchicine lesioning followed by fetal hippocampal transplantation (Tandon et al 1988). Delayed but long lasting effects of hippocampal transplantation on anxiety responses were seen in rats with bilateral kainic acid lesions (Sprick 1991). Woodruff et al (1988) in their study on intra hippocampal transplantation in hippocampectomized and TMT exposed rats found that
transplants do integrate to some extent into the circuitry of the brain and could have provided a physical substrate for behavioural recovery. The results of immunocytochemistry provided evidence that the connections formed between transplants and host contained neurotransmitters appropriate to the type of the tissue comprising of the transplants. Woodruff et al (1988) suggested that if there is a loss of neuroplasticity, recovery of function may depend on creation of a certain amount of circuitry by the transplants and this circuit must be formed by tissue normally present in the host. Mudrik et al (1989) transplanted fetal hippocampal neurons in animals with CA1 cell loss after global ischemia and observed normal firing patterns.

The histological data showed that grafts contained CA1 pyramidal neurons and expressed proteins found in normal CA1 cells (Calbindin D and parvalbumin) and sent projection to host septal area from where they also received innervation. Electrophysiological studies by Tonder et al (1989) showed that grafts survived well in the hippocampus of ischämically lesioned rats, contained cells identical to normal CA1, CA3/CA4 pyramidal cells, showed normal levels of GFAP and received extensive innervation from host septum and the grafts sent projections to host CA1 and subiculum. Katyama et al (1991) suggested that survival and integration of transplants are more successful if conducted 4 weeks after ischemia, which may be due to release of trophic factors.

Streker et al (1987), Nilsson et al (1990) and Stromberg et al (1991) have demonstrated that grafted neurons are equipped with advanced functional regulatory systems. They found that transmitter release from grafted monaminergic neurons are impulse dependent and under autoregulatory control by autoreceptor mediated feedback regulation and neurotransmitter reuptake. Such type of control provided with an effective mechanism whereby neurotransmitter release from transplanted neurons can be maintained within physiological range. Autoregulated (synaptic and non synaptic) transmitter release may be both necessary and sufficient for grafted fetal neurons to mimic some aspects of the functions of host neurons. Agrawal et al (1995) in their studies on 6-ODHA lesioned and transplanted animals have shown 6-ODHA induced supersensitivity of dopamine neurotransmitter which is restored following nigral
transplantation. This observation is supported by electrophysiological results where it was found that firing patterns in transplanted rats were comparable and similar to those of control (unlesioned) rats after 8 weeks of transplantation. Similar electrophysiological studies have been shown for validation of functional host-graft connection in adult cortex, hippocampus and striatum (Segal 1987, Buzaki et al 1987, Bragin et al 1988, Lee and Ebner 1990).

Nilsson et al (1990), Buzaki et al (1987) and Vander wolf et al (1990) have demonstrated that intra hippocampal transplants of septal cholinergic neurons can be activated by behaviourally activating stimuli and transmitter release from the grafted cholinergic neurons. The graft activation can be modulated during ongoing behaviour and also those cholinergic rich transplants can reinstate normal behaviour dependent electrocortical activity in the hippocampus and cortex. Dunnet et al (1988) and Hodges et al (1990), in their studies on aged and ibotenic acid lesioned rats respectively, have compared the effect of cholinergic septal grafts on memory performance with the effects obtained by systemically administered cholinergic agonists. The effects obtained by agonist treatment did not match with those seen in grafted animals suggesting that transmitter release at synaptic sites may be a functionally advantageous feature of these type of grafts.

Studies on the enhancement and disruption of cholinergic transmission in the brain have shown that the density of muscarinic receptors is regulated by the amount of acetylcholine available at the synapse (Ben Bark and Dudai 1980, Ehlert et al 1980, Sutin et al 1986). Joyce et al (1989) in their study on regulation of muscarinic receptors in hippocampal degeneration and after transplantation showed that there is a coupling between presynaptic acetylcholinesterase and high affinity choline uptake (HACU). They have also demonstrated that fimbria fornix transections showed down regulation of acetylcholinesterase (AChE) activity and $^3$H Hemicholinium3 (HC3) binding in the hippocampus but reinnervation of the hippocampus by septal transplants significantly restored both these markers. The results suggested that muscarinic receptors are under the control of cholinergic neurotransmitter activity and that grafts of embryonic tissue are sources of cholinergic processes that make appropriate synaptic contacts providing
recovery of function. Studies on septo-hippocampal pathway degeneration showed that the decrease in HACU rates is due to (i) interruption of the septo hippocampal cholinergic neurons and (ii) loss of cholinergic terminals in the hippocampal formation (Tarricone et al 1993). Kaseda et al (1989), after grafting cholinergic rich neurons found that HACU levels were restored but the alterations in $^3$H Quinuclidinyl benzylate (QNB) binding was not restored upto 14 weeks post transplantation. However, studies by Tarricone et al (1993) showed that following 8 months of transplantation, the up regulation of muscarinic receptors in the hippocampus are restored to control levels. The restoration of muscarinic receptor binding following transplantation was postulated to be due to the release of acetylcholine from the grafted cholinergic neurons. Restoration of ChAT activity by intra-hippocampal transplants was significantly correlated with the performance on spatial reference memory, spatial navigation and spatial working memory. HACU concentrations which is correlated with spatial memory performance was also found to be significantly correlated with hippocampal ChAT activity, indicating neurotransmitter mediated recovery of function following intra-hippocampal transplantation (Dawson et al 1989, Tarricone et al 1989, 1993).

The extensive damage to the septo-hippocampal pathway not only induces a cholinergic denervation of the hippocampus but also deprives the hippocampus of the serotonergic and noradrenergic innervation arising from mesencephalic raphe and locus coerulesus respectively (Storm-Mathisen and Gulberg 1974, Gage and Bjorklund 1986). Co-grafts of cholinergic and serotonergic rich neurons in the hippocampus of lesioned rats demonstrated the restoration of ChAT activity HACU levels, serotonergic receptor binding and serotonin concentration same time. An overcompensation of the serotonin levels was observed following transplantation. The result indicated that co-grafting technique could be an interesting tool for investigating the reinnervation in cases of structure deprived of more that one neurochemical population.

Role of Neurotrophic factors

Recent studies have shown the influence of neurotrophic factors (NTF) & other growth factors in promoting recovery of function followings neuronal injury (Diener and
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Bregman 1994, Lindvall et al 1994, Kolb et al 1997, Liu and Holmes 1997). Neurotrophic factors, viz. nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and neurotrophins-3, 4/5 (NT-3, NT-4/5) are special proteins aimed at selected population of neurons in the central and peripheral nervous system. Their roles have been initially defined by the discovery and investigation of NGF, which acts to promote maintenance, functional competence and neurite outgrowth of certain sensory and sympathetic PNS neurons during development (Varon et al 1972, Levi Motalcini 1987). Further studies on NGFs and NTFs revealed that they are equally important for CNS neurons in adult as well as in developing animals, and could, therefore, be relevant to the pathophysiology and/or treatment of neurogenerative conditions in human patients (Appel 1981, Hefli 1983, Varon 1984, Connor and Dragunow 1998). The growth factors especially NGF has been shown to be a potent and selective stimulator of peripheral sympathetic and sensory neurons. The most pronounced effect of NGF is on growing and developing neurons (Eclancher et al 1985, Alderson et al 1990, Cavichiolli et al 1991, Alleva et al 1993). NGF has been shown to increase steady state levels of mRNA for ChAT presumably because of increased gene transcription and enhances the specific activity of ChAT and high affinity choline carrier proteins and thus increase acetylcholine release (Williams and Reylett 1990, Cavicchioli et al 1991, Lapchak and Hefli 1991). Data have indicated that administration of neurotrophins might counteract cell death following brain insult. In cell culture, BDNF ameliorates degeneration of mesencephalic dopaminergic neurons caused by 6-OHDA or MPP+ (Spina et al 1992) and of cerebral granule cells and hippocampal neurons caused by glutamate (Cheng and Mattson 1991, Lindholm et al 1993). Similarly NGF, BDNF and NT-3 protect cultured hippocampal, cortical and septal neurons against hypoglycemic insults (Cheng and Mattson 1991, Kokaia et al 1994). NGF has been shown to prevent degeneration of septal cholinergic neurons after fimbria fornix lesions (Hefli 1986) and of striatal neurons following excitotoxic lesion (Shumacher et al 1991). NT-3 secreting fibroblasts implanted close to locus coeruleus were shown to prevent degeneration of noradrenergic neurons after a 6-OHDA induced lesion (Arenas and Persson 1994). Intraventricular injections of NGF
or BDNF have been shown to ameliorate CA1 neuronal cell death following transient forebrain ischemia in rats (Beck et al 1994).

Studies have shown that the immunostainable content of NGF in the stomata of intact medial septum cholinergic neurons are rapidly depleted following intra-dentate colchicine administration, which is known to disrupt microtubule dependent retrograde axonal transport (Conor and Varon 1992). Axotomy of the septal cholinergic neurons, via fimbria fornix transections leads to disappearance of about two third of the cell bodies reflecting interruption of retrograde NGF supply (Sofroniew et al 1990). Continuous NGF administration into the lateral ventricle fully prevents the disappearance of medial septal cholinergic neurons when delivery starts at lesion time, and restores the number and size of demonstrable neurons when delivery is delayed even by several weeks (Hagg et al 1989). The apparent loss of septal cholinergic neurons is also prevented by intraventricular administration of BDNF (Knusel et al 1992).

Cholinergic neurons in the nucleus basalis magnocellularis (NBM) project to cerebral cortex with a more diffused axonal distribution. Cortical lesions reduce the immunostainable NGF content of NBM cholinergic neurons, which are largely prevented by NGF administration (Garofalo et al 1982, Haroutunian et al 1989, Maysinger et al 1992).

Basal forebrain cholinergic neurons play a major role in cognitive functions and damage to these neurons mimic cognitive losses in AD (Cummings et al 1992) and aging process (Brutus et al 1982, Berger-Sweeny et al 1994). Intraventricular NGF administration has been shown to induce cholinergic benefits with an alleviation of behavioral deficits in experimental animals (Will and Hefti 1985, Fisher et al 1987, 1991; Dekker et al 1992) and possibly in Alzheimer’s patients (Cadelli et al 1991). Cholinergic neurons in the striatum were found to respond to intraventricular or intraparenchymal NGF administration with increase in size, ChAT activity and low affinity NGF receptors (Gage et al 1989, Gall et al 1991).

Dopaminergic neurons of the nigrostriatal pathway are also responsive to NTFs. In animals with nigrostriatal transections, BDNF, NT-3 and NT4/5 were found to prevent neuronal loss and promote regeneration (Knusel et al 1992). Fibroblast Growth Factors
(FGF) have also been reported to provide trophic support to dopaminergic neurons in experimentally lesioned animals (Otto and Unsicker 1990, Date et al 1993).

Embryonic basal forebrain cholinergic neurons in vitro respond to the presence of NGF and BDNF with enhanced sprouting and expression of cholinergic phenotype (Alderson et al 1990). Junard et al (1990) and Barone et al (1991, 1992) have shown that the restorative effect of NGF does not persist and functional recovery was not observed after the withdrawal of NGF. Neurotrophins seem to prevent the immediate effects of target deprivation and permit the establishment of collaterals to other undamaged targets (Diener and Bregman 1994). NGF and other neurotrophins also seem to mediate recovery by inducing synaptic sprouting. NGF is known to stimulate terminal growth in uninjured basal forebrain cholinergic neurons promoting recovery after neurodegeneration (Chen et al 1995, Burgos et al 1995).

Neurotrophins could also act indirectly through enhanced synaptic activity, especially of cholinergic neurons, which in turn could stimulate dendritic changes in pyramidal neurons. BDNF has been shown to improve synaptic efficiency of cortical hippocampal circuits (Kang and Schuman, 1995). Experimental observations suggest that NGF and other neurotrophins are capable of stimulating plastic changes in neurons of developing and adult brain that have undergone degeneration or extensive devascularizing injury capable of supporting recovery from motor and cognitive deficits (Eclancher et al 1985, Pallage et al 1993, Kolb et al 1997). Due to the influence on developing neurons, exogenous administration of trophic factors may help in the growth and development of transplanted fetal neurons in experimentally lesioned animals.

Multiple Microtransplantation

Despite all efforts to improve graft survival and subsequently graft induced functional restoration, transplantation of fetal neural cells have met with limited success. With the standard techniques of implantation, transplanted cells are inevitably exposed to unspecific tissue reaction characterised by bleeding, necrosis and degeneration leading to decreased graft survival (Nikkhah et al 1994b). To counter such problems, Nikkhah et al (1993) developed a microtransplantation approach, involving the distribution of small
grafts over the degenerated brain region, for study of dopamine rich cell suspension transplants in a rat model of Parkinson’s disease. The distribution of fetal neurons was found to enhance ventral mesencephalon graft survival as compared to single site transplants, leading to improved striatal innervation and restoration of complex sensorimotor behaviour (Nikkhah et al 1993, 1994a).

One of the factors found to be important to produce enhanced graft survival was the high level of morphological integration and extensive THI-positive fibre outgrowth from the micrografts. Also the long term implantation trauma as assessed by GFAP expression was found to be minimised in the small micrografts thus making the multiple engrafts more viable (1994a,b). Studies have also demonstrated that the multiple transplants in the hippocampus are effective in producing enhanced behavioural and neurochemical recovery in TMT induced hippocampal degeneration (Roy et al 1997). Olson (1997) has suggested that the small micrografts of embryonic tissue could themselves become sequentially innervated, creating a channel and leading to the final target. Shetty and Turner (1997a,b) have also suggested that, as in case of hippocampal damage the location of transplants is critical, hence the placement of several micrografts along the degenerated layer may further improve reconstruction of the damaged circuitry and graft functioning in the hippocampal lesion models.

The concept of implanting small grafts with minimal trauma and high reproducibility seems a favourable choice for transplantation experiments. The modifications introduced in the microtransplantation technique could offer new ways to enhance morphological and functional efficacy of the transplants.

Clinical Studies Neural Transplantation

The first clinical trials with transplantation in Parkinson's disease were carried out by Backlund et al (1985) and Lindvall et al (1987). Adrenal medullary grafts were stereotaxically implanted into the caudate regions but only modest improvements could be observed. Madrazo et al (1987) reported the first successful adrenal medullary transplantation in Parkinson's patients showed good response. In their studies in 42 cases 1-3 months post transplantation, 60% of the patients showed good response, moderate
response in 20% and poor response in 20%. However, in other clinical studies only modest recovery was documented. The patients continued to show motor fluctuation and in most cases it was not possible to reduce antiparkinsonian drug. These preliminary studies met with limited success but fetal neural transplantation has been a rational consideration in the treatment of Parkinson's disease.

In studies by Lindvall et al (1989), two patients received ventral mesencephalon grafts from fetuses aged 7-9 weeks post conception unilaterally into the caudate and anterior putamen. A small but significant improvement in six months follow up in motor performance during the 'off' period was observed. Lindvall et al (1990, 1992) used a modified protocol for transplantation in which mesencephalic grafts were derived from 6-7 week old fetuses, stored for shorter time and implanted into the anterior and posterior putamen using a thin gauge needle. The patients experienced more prominent clinical benefits starting 6-12 weeks after transplantation over a three year observation period. There was a significant bilateral reduction in bradykinesia and rigidity. Peschanski et al (1994), using a similar protocol observed bilateral motor improvement and obtained comparable clinical and Positron Emission Tomography (PET) scan results. Freed et al (1992), implanted solid fetal nigral tissue and observed similar clinical benefits in patients 12-46 months post transplantation. Freeman et al (1995) observed clear and consistent improvements of clinical features and striatal fluorodopa uptake following fetal tissue transplantation in patients with advanced Parkinson's disease whose condition was not improved by drug manipulations. Studies by Spencer et al (1992) and Freeman et al (1995) showed that dopamine replacement therapy in Parkinson's disease provides dramatic clinical benefits and demonstrated that dopamine containing neurons subserve a modulatory function and under physiological conditions provide tonic stimulation of target receptors.

Bilateral implantation of ventral mesencephalon can induce substantial long term functional improvement in Parkinson's patients with severe dopamine depletion accompanied with increased uptake of fluorodopa in the striatum. Widener et al 1992 observed decrease in rigidity and akinesia and complete resolution of freezing episodes in a patient following intra-caudate, fetal nigral transplantation. In another patient there was
reduction in bradykinesia and rigidity and virtual disappearance of dyskinesia leading to greatly improved daily functions.

Kordower et al (1995) in their studies on a patient receiving bilateral fetal ventral mesencephalon grafts in the post commissural putamen found that the patient had sustained improvement in motor function and a progressive increase in fluorodopa uptake in putamen on PET scanning. On expiry of the patient 18 months after transplantation, tyrosine hydroxylase immocytochemistry in the brain revealed viability of the grafts. Each graft was integrated into the host striatum and contained clusters of dopaminergic neurons. Processes of these neurons had grown out of the grafts and provided extensive dopaminergic reinnervation to the striatum. Till date, all clinical studies on neural transplantation have been conducted in Parkinson patients only. The results of these studies have shown that restoration after transplantation in most cases have been moderate at the best. The improvement of grafting techniques (distribution of grafts at multiple sites) and increasing the survival rates of grafted neurons, could render fetal neural transplantation as a therapeutic choice for Parkinson's disease and other irreversible neurological disorders.

Current strategies in neural transplantation

Classical method of neurotrophic factor infusion employs intracerebral cannulation, which often evokes significant inflammatory responses that reduce survival of the grafts (Mayer et al 1993). In addition, a sustained release of neurotrophic factors to the target site is to be maintained to promote long term benefits. Two strategies have been developed which do not require intracerebral cannulation for delivery of neurotrophins.

1. Transplantation of polymer encapsulated cells
2. Transplantation of cells genetically modified to release growth factors.

Transplantation of polymer encapsulated cells

The concept of this technique is to encapsulate biocompatible, selectively permeable polymer membranes that can protect the transplant against immune rejection while allowing solute exchange between the graft and its environment (Galletti 1991). Hofman et al (1993) suggested that transplanted cells often grow beyond the implantation
site and form tumors. The encapsulation of the cell allows cell growth within the capsule space, while allowing neurotransmitter and trophic factors exchange between the transplants and host. Studies in fimbria fornix lesioned rats receiving intraventricular implantation of encapsulated cells loaded with NGF producing cells showed that the cells remained viable, confined to the capsule space and released sufficient NGF to prevent lesion induced loss of ChAT expression. Camarata et al (1992) grafted biodegradable polymer microspheres containing NGF to provide prolonged site specific delivery in a rat model of Parkinson’s disease. Immunohistochemical studies showed NGF release over 4-5 week period, promoting neurite outgrowth. Maysinger et al (1992) observed reduction in the loss of nucleus basalis magnocellularis cholinergic neurons produced by devascularizing cortical lesions following delivery of microencapsulated NGF in the forebrain of rats.

Transplantation of polymer encapsulated neurotransmitter secreting cells has been shown to allow inward diffusion of nutrients and outward diffusion of neurotransmitters, but prevents immunoglobulins or immune cells from reaching the transplant. Aebischer et al (1991) transplanted polyelectrolyte based microcapsules or thermoplastic-based macrocapsules loaded with PC-12 cells in an experimental Parkinson's model. Encapsulated PC-12 cells were able to reduce lesion induced rotational asymmetry in rats for at least 4 weeks.

Silver et al (1996) isolated suprachiasmatic nuclei from hamster fetuses within a semipermeable polymeric capsule there by preventing neural out growth but allowing diffusion of humoral signals. The capsules were transplanted in the third ventricle of suprachiasmatic nuclei ablated hamsters with altered circadian locomotor rhythms. Studies conducted 8 & 16 weeks post transplantation showed circadian activity rhythms can be sustained by means of diffusible signals from polymer encapsulated cells.

Techniques of transplantation of polymer encapsulated cells could be useful for delivering growth factors, proteins and other macromolecules intra-cerebrally over a prolonged time period. This technique allows transplantation of post mitotic cells across species. It also allows transplantation of transformed cell lines since the polymer capsule prevents the formation of tumors by physically sequestering the transplanted tissue.
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(Aebischer et al 1991). The growth factor containing polymer micro spheres can be used in work aimed at prolonging graft survival, treating experimental neurological disorders and augmenting peripheral nerve regeneration.

Transplantation of genetically modified cells

Research pertaining to gene transfer into the cells of the central nervous system is one of the fastest growing avenues of neuroscience. An important facet of application of gene transfer is gene therapy, which is based on introducing therapeutic genes into the cells of the CNS.

Gene therapy is conceivably applicable for genetic diseases in which gene defect has not yet been determined. Genes that encode for neuroprotective molecules can be introduced to prevent or delay destruction of affected cells. When extensive cell loss has occurred, other cells could be induced by gene transfer to produce key molecules (neurotransmitters, neurotrophic factors) of affected cell populations to act as denervated targets. The combination of gene transfer and intracerebral grafting can prove to be viable approach for treatment of certain CNS disorders like Parkinson's disease (Gage et al 1987, Takayama et al 1995, Freeman 1997, Kordower et al 1997). In degenerative encephalopathies such as Alzheimer's & Parkinosn's disease, developmental anomalies, ischemic and traumatic damage etc. the therapeutic gene might encode a neurotrophic molecule, a neuronal survival (anti-apoptotic) agent, a cytokine, a neurotransmitter or a receptor as the situation requires.

Chen et al (1991) studied the role of primary skin fibroblasts as donor cells for intracerebral grafting. Primary skin fibroblasts survive in the brain and remain in situ. A number of genes (NGF, Tyrosine hydroxylase, glutamic acid decarboxylase and choline acetyl transferase) can be successfully introduced through viral vectors and expressed in the primary fibroblasts. L-DOPA secreting fibroblasts were shown to reverse behavioural effects in rat model of Parkinson's disease upto 8 weeks post grafting. In the 6-hydroxy dopamine model, the gene that encodes tyrosine hydroxylase has been introduced into the lesioned area (using viral vector delivery systems) enabling a portion of striated cells to produce the enzyme (Kaplitt et al 1994, During et al 1994). The efficacy of this treatment was revealed by increased levels of L-DOPA and extracellular dopamine. There was also
a reduction in apomorphine induced rotations. Takayama et al (1995) employed the strategy for supplying basic fibroblast growth factor to grafted dopamine neurons in 6-OHDA lesioned rats. Primary skin fibroblasts were genetically modified to produce bFGF, mixed with fetal dopamine neurons and implanted into the striatum of 6-OHDA lesioned rats. Such cогrafting was observed to improve the survival, growth and functional efficacy of transplanted dopamine neurons.

Groves et al (1993) transplanted 0-2A progenitor cells genetically modified to express the bacterial beta-galactosidase gene, into the demyelinating lesions of spinal cord of adult rats. The bacterial b- galactosidase gene gives rise to b-galactosidase positive oligodendrocytes which remyelinate the demyelinated axons within the lesions. Transfer of the gene that encodes nerve growth factor has been proposed to prevent the loss of cholinergic neurons of the forebrain in Alzheimer's disease (Geschwind et al 1994, Federoff et al 1992).

Currently the majority of the work on gene therapy in being directed for the treatment of Parkinson's disease (Elsworth and Roth 1997 Mouradian and Chase 1997). Efforts are being made to transplant genetically engineered cells which induce dopamine synthesis, growth factors and promoters to provide functional recovery and to prevent levodopa associated motor complications (Elsworth and Roth 1997). Gene therapy can be a useful strategy for enhancing the clinical effectiveness of fetal neurons as also, intracerebral grafts of genetically modified cells, been found to survive within the brain, could be of utmost clinical significance for treatment of neurological disorders.

**Trimethyltin induced neurotoxicity**

Trimethyltin (TMT) and other organotin compounds are environmental pollutants and widely used as stabilizers for plastics, in fertilizer industry, as biocides for bacteria and fungus and as rodent repellents. TMT is a potent neurotoxicant and has been implicated in several episodes of human poisoning (Brown et al 1979, Dyer et al 1982 Kreyberg et al 1992). Neurobehavioural changes in humans after exposure to organotins have been observed. Case report of 6 exposed workers describes headache, deafness, impaired memory, disorientation, psychotic and other neuropsychiatric behaviour. The
two surviving workers with the highest urinary tin levels exhibited fixed neurological effects, which were not resolved more than 6 years after exposure. The remaining 3 workers returned to work but had memory loss which persisted for 6 months.

Human exposure to tin and organotins may occur by inhalation, ingestion or dermal contact. The exposure to general population occurs primarily by ingestion of food (WHO 1980). Estimates of daily dietary tin intake ranges from 1mg for diets consisting mainly of fresh meats, vegetables and cereals to 38 mg from canned foods (Schafer and Femert 1984, WHO 1980). Occupational exposure to tin may be substantial. Inhalation or dermal exposure to organotins used in fungicides or insecticides may occur during both manufacturing and application (NAS 1977, WHO 1980). Potentially high exposure to tin and its compounds occur in work places and industries producing or using organotin compounds. Individuals who eat acidic foods, may have potentially above average exposure to tin.

Trialkyltin have enhanced solubility in organic solvents, increased volatility and ability to penetrate biological tissues. The ability to attack central nervous system and inhibition of oxidative phosphorylation renders trimethyltin and other trialkyltin dangerous to handle and potentially hazardous to the environment.

In rodents TMT is capable of producing widespread damage to the central nervous system. The neuropathology of TMT is most evident in the limbic system including the hippocampus (Chang et al 1982, Chang and Dyer 1983 Dyer et al 1982). The pyramidal neurons of the CA3/CA4 regions of the hippocampus (fig. 1) are the most vulnerable to the toxic effects of TMT, leading to irreversible functional deficits (Dyer et al 1982, Earley et al 1992). Many of the characteristic behavioural symptoms at a single dose of TMT of 6 mg/kg or higher are evident after 7 days while hippocampal damage becomes maximal within 21 days of administration. With doses over 10mg/Kg mortality occurs within 48 hours after injection (Hasan et al 1984).

Tin concentrations in rat brain after TMT exposure is linearly related to dosage (Cook et al 1984). The slow increase in brain levels may be related to high affinity of TMT for rat hemoglobin (Brown et al 1979, Aldridge et al 1981). Haemoglobin serves as a reservoir, slowly and continuously releasing TMT into the plasma from where it enters
the brain. The widespread hippocampal damage caused by TMT leads to behavioural changes in rodents including aggressive behaviour, spontaneous seizures, hyperactivity and learning and memory impairment (Brown et al 1979, Early et al 1992).

TMT induced decrease in GABA concentration might be suggestive of alterations in convulsive thresholds, kindling and electrophysiological changes (Hasan et al 1984, Hanin et al 1984, Doctor et al 1982). Earley et al (1992) have also shown depletion in 5-HT level in the hippocampus following TMT administration suggesting damage of 5-HT projection from raphe nuclei. Earley et al (1989) have shown severe reduction in $^3$H QNB binding in the septo-hippocampal region indicating damage to cholinergic neurons following TMT exposure to rats. Spatial memory functions, which depend upon muscarinic receptor density (Gardner et al 1984), have also been found to be impaired, suggesting cholinergic denervation following TMT exposure.

Synapsin I is a neuron specific phosphoprotein associated with synaptic vesicles, present on all nerve terminals and ontogeny of which coincides with synapse formation (DeCamilli et al 1983a,b; Lohman et al 1978). Miller and O'Callaghan (1984) found significant decrease in synapsin I concentration following TMT administration, suggesting a reduction in the synaptic density within hippocampus. The results indicate that TMT interferes with the events related to synaptogenesis.

Bordie et al (1990), have suggested that another likely cause of pyramidal cell loss by TMT could be due to elevated extracellular levels of glutamate, an amino acid which is known to be cytotoxic. Glutamate causes lesions experimentally when high affinity uptake of this amino acid is blocked (McBean and Roberts 1985), a situation which might contribute to TMT induced toxicity as TMT itself blocks glutamate (Naalsund and Fonnum 1986). Morphometric studies on trimethyltin induced neuronal damage indicate that TMT exposure may disrupt brain structures important for linking neocortex with sub cortex via structures in hippocampal region (Kutscher 1992). The widespread damage caused by TMT possibly leads to complex behavioural alteration and changes in multi neurotransmitter systems. Such type of neurodegeneration could be helpful in understanding the mechanism of recovery of function following transplantation of co (multiple) grafts, specific for different neurotransmitter systems.
Fig. 1: Diagramatic representation of the hippocampus of rat showing the pyramidal neurons of the *Cronus Ammonus* (CA) and granule cells of the dentate gyrus with associated mossy fibres and collaterals.
Colchicine and its neurotoxicity

Colchicine ($C_{22}H_{25}NO_6$, molecular weight 399.4), is an alkaloid and is derived from the plant *Colchicum autumnale*. The main clinical use of colchicine has been in the treatment of gout (Wallace and Ertell 1978). Colchicine is a known microtubule inhibitor and blocks microtubule assembly by binding to tubulin. Goldschmidt and Steward (1982) have reported that direct administration of colchicine into the hippocampus results in preferential destruction of the dentate granule cells and mossy fibers (fig. 1). Several other reports have replicated this observation of specific site of colchicine action (Lothman et al 1982, Johansen et al 1986, Frush et al 1986, Walsh et al 1986). The mechanism by which colchicine produces the destruction of dentate granule cells is believed to be related to its effect on tubulin, however, tubulin binding characteristics of some drugs may not correlate with the ability to cause damage to the dentate granule cells (Dashieff and Ramirez, 1985). Hence, the mechanism of colchicine induced damage is unclear. The reason why inhibition of microtubule function should be preferentially toxic to dentate granule cells in poorly understood. It has been suggested that colchicine may be preferentially taken up by some cell types and not others and that different isomers of tubulin which exist in the brain bind colchicine with different affinities (Mundy and Tilson 1990). Other possibilities have also been suggested which include the accumulation of toxic products after microtubule dysfunction, which are handled differently by different cells or blockade of retrograde transport of a critical neurotrophic factor (Maudy and Tilson 1990).

Apart from its activity as an inhibitor of microtubule function, colchicine can bind to cell and nuclear membranes, inhibit enzymes, nucleoside uptake (Wallace and Ertell, 1978) and can also disturb calcium homeostasis (Hindelang-Gertner et al 1976). The selectivity of colchicine induced neurotoxicity has also been studied using radioimmunoassays of hippocampal dynorphin and in some cases metenkephalin (Hong et al 1979, 1984). Significant depletions of dynorphin have been observed 1 week after colchicine administration, which persisted up to 12 weeks after dosing. Hippocampal met-enkephalin measured 12 weeks after lesioning was also found to be decreased but only by 20% as compared to the controls. These data have suggested that colchicine has
preferential effects on granule cells and mossy fibers since dynorphin is highly localized in these cells while met-enkephalin has a more generalized distribution in the hippocampus (Bloom 1993, Hong and Schmid, 1981). Similarly, levels of neurotransmitters (Serotonin, 5-HIAA, GABA, Glutamate etc.) which are not localized in the granule cells were not found to be altered following intradentate administration of colchicine.

Histological and immunocytochemical analysis have revealed extensive loss of granule cells throughout the superior and inferior blade of the dorsal and ventral hippocampus following intradentate colchicine (Walsh et al 1986). Pyramidal cell of CA1, CA2, CA3 and CA4 region have been found to be essentially spared following colchicine administration (Goldsmidt and Steward 1980, 1982, Sutherland et al 1983). Reduction in hippocampal size has been observed which seemed to be largely related to the loss of granule cell processes in the molecular layer of the dentate gyrus and CA3/4 region (Walsh et al 1986). Morphological studies (Tilson and Peterson, 1987) have revealed that colchicine produced approximately 60% decrease in granule cells in the dorsal hippocampus. The length of the pyramidal cells was decreased only by 10%. The width of the granule cells in dorsal hippocampus was found to be decreased by 50%-60% while width of the pyramidal cells was unaffected. Colchicine also produces approximately 60% decrease in volume of mossy fibers in the dorsal hippocampus. Other morphological measures show slight (10%) shrinking in brain and enlargement in lateral ventricles following colchicine administration.

The dentate granule cells of the hippocampus are active during the acquisition of new information, and respond quickly to changes in environment (Segal 1973, Deadwyler et al 1979). Dentate gyrus has also been shown to have an important role in short term and or working memory (Zornetzer et al 1974, Collinier et. al. 1982). Therefore, intradentate administration of colchicine has been reported to interfere with radial arm maze performance, impair retention of a step through passive task and facilitate acquisition of a two way shuttle box response (Jarrad et al 1984, Walsh et al 1986, Tilson et al 1987).
Colchicine induced damage of dentate granule cells has been found to significantly elevate motor activity compared to controls (Walsh et al 1984, Tilson and Peterson 1984, Tilson and Peterson 1987). Influence on nucleus accumbens might contribute to the expression of hyperactivity induced by various types of hippocampal lesions (Reinstein et al 1982, Hannigan et al 1984). Hippocampus has been demonstrated to provide a major excitatory input into the nucleus accumbens. Furthermore, the dopaminergic afferents from ventral tegmental area projecting to the accumbens inhibit this excitatory hippocampal input. Based on the sequential processing of information in the hippocampus, it would be expected that the disruption of the hippocampal cascade at any point from dentate gyrus to subiculum might modify the influence of hippocampus on the nucleus accumbens and alter the motor activity (Walsh et al 1986). The damage to the dentate granule cells induces hyperactivity following colchicine administration.

Colchicine has been suggested to induce reactive sprouting of the septo hippocampal pathway (Cotman and Nadler, 1978: Stanfield and Cowan 1982). The disruption of cholinergic pathway leads to alternations in cholinergic (muscarinic) receptor binding. The function of muscarinic receptors are known to be modulated by cholinergic inputs in the hippocampus and hippocampal degeneration by colchicine has been found to decrease the receptor number (Tandon et al 1989). Intra-dentate colchicine has also been shown to increase agonist induced inositol phosphate turnover in brain slices, suggesting its effect on the second messenger system (Tandon et al 1989, Barone et al 1992).

Colchicine has been found to reduce the number of ChAT positive neurons in the medial septum and AChE positive neurons in the hippocampus (Gilbert and Peterson 1991). A blockade of axoplasmic transport rather than direct cytotoxicity of the cell bodies within the septum may mediate colchicine disruption of septal cholinergic neurons. Dipatre et al (1990) reported that intradentate colchicine blocks retrograde transport of NGF from hippocampus to medial septum. Colchicine appears to cause degeneration of cholinergic neurons by blocking axonal transport and reducing the availability of critical NGF like substance. It is also possible that colchicine interferes with the uptake, synthesis or transport of acetylcholine in septal neurons (Gilbert and
The alterations in the cholinergic properties by colchicine could be due to the disruption in the transport of ChAT from endoplasmic reticulum, where it is synthesized, to the terminal region, where it is stored (McGeer et al 1987). It may be possible that ChAT is being synthesized but is unable to reach the normal sites of storage, in the terminal regions. High affinity choline uptake is also decreased, and may be due to the fact that colchicine exerts a direct toxic effect on the uptake process. It may be disrupting a portion of the cytoskeletal network responsible for anchoring the transport system and rendering it incapable of maintaining its normal spatial orientation within the energy dependent kinetic properties (Emerich and Walsh 1990).

Shigematsu and McGeer (1992) have demonstrated the production of amyloid precursor proteins (APP) by neurons after colchicine administration and its accumulation in perikarya and proximal axons in major dendrites. In Alzheimer's disease, brain APP is concentrated in degenerating neurites and neuropil threads as well as in senile plaques (Cole et al 1991, McGeer et al 1992). Overproduction or accumulation of APP may play a role in amyloid plaque and neurofibrillary tangle formation. Since intra ventricular colchicine administration causes APP accumulation in neurons and neurites it may be useful in providing an experimental model for such aspects of Alzheimer's disease pathogenesis.

Transplantation of fetal neural cells in colchicine lesioned hippocampus could be helpful in understanding the mechanism of functional restoration in neurodegenerative conditions where selected neuronal populations are damaged.