Chapter-6
General discussion
The nervous system is a unique target for toxic agents in several ways. First, the adult neuron does not divide and therefore, replacement of lost cells is not possible. Second, there is a barrier to passage of many blood-borne substances unless they are non-polar or are actively transported. Third, since the normal function of the nervous system requires the action of a complex integrated network, damage to even a small portion of the nervous system can result in marked effects on function. This situation is unlike that in many other target tissues such as kidney or liver, where specialized cell-to-cell networks are less diverse in structure and function. Finally, neurons are dependent on glucose as a constant source of energy and cell bodies and processes in some brain areas exist at borderline levels of oxygenation.

If high energy demands are placed on the system or if delivery of oxygen to the neurons is reduced by any mechanism, selective cell death may result. The structural complexity of the nervous system is reflected in the large number of different responses to toxic agents. Each portion of the peripheral nervous system involves several relay areas in the CNS. When damage to motor, sensory or autonomic nerves is combined with damage to the integrative system within the CNS, the complexity of
responses to toxic agents is obvious.

The broad spectrum manifestation of lead toxicity have been recognised for centuries. Recent years have seen considerable advancement in our ability to identify and characterize the neurological consequences of lead poisoning, including changes in cognition and other behavioural functions (Bellinger and Stiles, 1993; Needleman, 1993; Otto and Fox, 1993). However, the cellular basis for the neurobehavioural deficits associated with low level lead exposure has not been identified. Two alternative pathogenic events related to lead toxicity are possible: (1) that lead attacks multiple targets in the developing brain, each becoming vulnerable during a specific period of development, (2) that lead damages one particular function which is critical to many developmental events. Cookman et al (1987) account for the prolonged period of vulnerability of the developing rat brain by proposing that lead impairs the early synaptic elaboration of neurons and its subsequent remodelling during later brain development. In support of this idea, central synaptic deficits (Avervill and Needleman, 1980; McCauley et al, 1982) and impairment of the conversion of neural cell adhesion molecule from its embryonic to adult form (Cookman et al, 1987) have been reported in animals exposed to lead.

The present study suggests that an additional mechanism underlies neurobehavioural deficits induced by low levels of lead i.e. the impairment of the polyamine biosynthetic pathway. This view is supported by evidence that polyamines play a crucial role in the regulation of important biochemical events during critical stages of brain development (Bell and Slotkin, 1986). As a consequence of lead toxicity, polyamine turnover is altered leading to disruption of neuronal differentiation, proliferation and migration, which causes subtle changes in defined neuronal pathways in the hippocampus and cerebellum, finally culminating into learning and memory and motor deficits.

This study provides evidence for fetal neural transplant mediated amelioration of the behavioural and cognitive functions in rats with lead induced deficits and lends
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credence to the thesis that neural grafting is a viable approach in not only restoring function but also understanding the basic mechanism(s) of toxin induced deficits. The ability of implanted grafts of neural tissue to promote functional recovery in brain damaged recipient has raised the question of how such implants exert their functional effects. The available data do not permit exact specification of the mechanism(s) whereby the grafts promote behavioural recovery rather they allow several interpretations.

Dunnett and Bjorklund, 1987; Bjorklund et al, 1987; Dunnett, 1987 and Woodruff et al, 1988 have proposed various potential mechanisms whereby fetal grafts might produce recovery of behavioural function in a damaged brain. These include:

1) Non-specific, diffuse release of neurotransmitters into the host neuropil. This mechanism would account for the recovery reported to follow grafts of adrenal medulla into the brain in both animal models of Parkinsonism (Freed, 1983) and in humans suffering from the disease (Madrazo et al, 1987),

2) The ability of some types of grafts to act as "bridges" across which or through which several host axons can reconnect to deafferented targets. Such bridges might account for recovery observed in some of the experiments in which septal tissue was placed in the cavity created by an aspiration lesion of the fimbria-fornix (Low et al, 1982),

3) Reinnervation by axons from the graft neurons in the area of host brain denervated by the lesion, but without reciprocal connections from the host brain to the transplant. The transplant neurons would then provide physiological levels of specific transmitters to the host brain, but the release of transmitters would not be regulated by the host brain. Behavioural recovery associated with dopaminergic fetal substantia nigra cells to the denervated striatum (Bjorklund et al, 1980; Dunnett et al, 1983 and Freed, 1983) or with grafts of fetal cells from the cholinergic nucleus basalis magnocellularis (NBM) to neocortex in rats in which the NBM has been destroyed (Arendash et, 1985; Dunnett, 1987) provide examples of this mechanism of transplant action.

It seems unlikely that any of these three mechanisms participate in production of the recovery of function found in the present study. The first and the third
Mechanisms have been linked with behavioural deficits produced by destruction of the neurotransmitter system and subsequent specific replacement of this neurotransmitter by transplanted cells which produce it. The transplants made in the present study are not associated with production of specific neurotransmitter, the loss of which is known to produce the precise behavioural deficits observed in lesioned rats. It also seems unlikely that transplants made in the present study are providing bridges for specific regeneration.

The two mechanisms discussed by Dunnett and Bjorklund (1987) provide better explanation for the results of this study. As noted by Dunnett and Bjorklund (1987), these mechanisms are associated with very different levels of integration of the transplant into the circuitry of the host brain. One explanation involves integration of the transplant into the functional circuitry of the host brain. The transplant then "replaces" to some degree the circuitry lost when the host brain is damaged. The transplant made in the present study, especially of cholinergic rich intra-hippocampal transplants provided some degree of repair of the cholinergic denervation as suggested by Bielarczyk et al. (1996) although analysis at the level of electron microscope would be necessary to determine whether or not synaptic connections were formed between the transplant and the host brains. Moreover, using horse radish peroxidase as a labelling agent, indicates that transplants especially made in the cerebellum develop extensive connections with the host brain. These anatomical observations provide some evidence that the transplants do integrate to some extent into the circuitry of the brain and, therefore, could provide a physical substrate to produce behavioural recovery (Woodruff et al., 1988).

The final mechanism of transplant related recovery relies on the positive influence on the damaged host brain of neurotrophic factors presumed to be released by the transplanted tissue and has been invoked to explain the recovery of behavioural function observed following transplantation of fetal cortex into medial frontal lesion cavities (Dunnett et al., 1987; Kessler et al., 1986; Stein and Mufson, 1987). As pointed out by Woodruff and Baisden (1988) there is little direct evidence that transplanted
issue produces neurotrophic substances that promote repair processes in damaged brain, which might lead to subsequent behavioural recovery. However, Springer et al (1988) and Silver et al (1996) demonstrated that neurotrophic influence of the grafts is important for functional recovery. Springer et al (1988) demonstrated that grafts of male mouse submaxillary gland placed into the lateral ventricles of female rats given transections of fimbria-fornix reduce the amount of lesion induced atrophy of cholinergic neurons within the diagonal band/medial septal nuclei (DB/MS). This effect was not produced by grafts of sublingual gland. The submaxillary gland of the mouse is rich in nerve growth factor (NGF), while the sublingual gland is not. Presumably diffusion of NGF from the grafted submaxillary gland to the cholinergic cells of DB/MS was responsible for the reduced atrophy observed by Springer et al (1988). Additionally, Silver et al (1997) enclosed transplants within capsules that prevented integration of the transplant with the host brain, but allowed diffuse release of neurotrophic factors across the capsule. When these capsules were transplanted into rats with complete ablation of the suprachiasmatic nucleus, a complete restoration of the circadian cycle was observed, thus lending support to the hypothesis that neurotrophic influence by the transplant plays a crucial role in the behavioural recovery, associated with the transplant. By inference, then, the potential presence of neurotrophic factors released by the transplants used in the present study cannot be disregarded for explanation of the observed recovery in behaviour.