

# Graft/Host Relationships in the Developing and Regenerating CNS of Mammals

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**ABSTRACT:** A new light was shed on the utility of neural grafts when it was recognized that donor tissues and cells offer more than a source of immature progenitors potentially capable of cell replacement: First, they have the inherent capacity to produce multiple trophic and tropic factors promoting cell survival and tissue plasticity often characteristic of the immature central nervous system (CNS). Second, by their interaction with the host microenvironment via cell/cell and cell/ECM interactions, these grafts are capable of re-establishing homeostasis, which can be, for example, reflected in rescue and protection of host elements from harmful influences. This second capacity of donor cells relies, in part, also on a “dormant” but still present regenerative capacity of mature or even aged CNS and on the possibility of its mobilization in the damaged nervous system by neural grafts. For this to occur efficiently after transplantation, a bi-directional dialogue between donor and host cells must gradually be established, in which both “partners” transmit signals (cell/cell contact, molecular messengers), “listen to” and “understand” each other and are able to react by modifying their own plasticity- and development-related programs. Thus, for the best possible recovery of functionality in the injured adult and aged nervous system, neurotransplantation must always try to find optimal conditions for all three of the mentioned qualities of neural grafts, especially for the protection and/or reactivation of neural circuitry embedded in non-neurogenic CNS areas. Once fully understood, this newly recognized aspect of neurotransplantation (and topic of this review) might, someday, even allow the recovery of systems that would otherwise be doomed, such as cognition- and experience-related circuitry.

**KEYWORDS:** CNS; regeneration; neuroprotection; rescue; graft; neurotransplantation; neural stem cell

## GRAFTING AS A TOOL FOR STUDYING CNS DEVELOPMENT AND REGENERATION

Experimental neurotransplantation was first attempted more than one hundred years ago and used by neuroanatomists and embryologists as a tool for addressing basic questions in early ontogeny. While grafting into the mammalian nervous sys-

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tem (NS) remained for a long time unsuccessful, transplantation in other species, such as fish, amphibians, reptiles, and birds, was relatively easy and could be done in the very early stages of embryonic development—during the incubation of the egg. These experiments helped to resolve some fundamental problems of developmental biology and led to essential discoveries such as neuronal induction, cephalization, and body polarization. It became evident that the ontogeny of individual organs depends also on the development of surrounding tissue, and that close communication between individual cells and structures is needed and regulated according to a genetically defined developmental program in time and space.

In contrast to lower vertebrates and birds, the mammalian class is characterized by substantially diminished plasticity in the adult NS. This is mainly due to the highly specific and complex neural connectivity, characterized by precisely controlled communication between billions of cell types with defined tasks and characteristics. Especially in higher mammals such as primates, many of these connections are of a cognitive nature and are pruned and specified only postnatally, obeying learning- and experience-dependent input and escaping early development-based repair mechanisms. It has also become apparent that the number of precursor cells and, importantly, their potential to differentiate into various types of neural cells, decreases progressively throughout the development of an individual. The regression of germinal and neurogenic zones is probably one of the main reasons for the progressive loss of the nervous system's restorative capacity.<sup>1</sup> Other inhibitory factors are continuously being discovered in the molecular composition of the adult CNS environment<sup>2</sup> and in the formation of the blood-brain barrier, which prevents the entrance not only of many noxious substances, but also of endogenous and systemically applied therapeutic substances into the CNS from the vascular compartment.

Thus, it seems to be almost impossible that the cytoarchitectonic patterns established during the developmental and learning phases of the CNS by genetically coded spatio-temporal morphogenetic gradients, can ever be re-established when damaged. However, encouraging evidence exists for anatomical and functional modification and adaptation of connections during normal life as well as in the injured adult.

The ancient concept that Arneemann formulated in 1787 rejected, for decades, any regenerative possibility in the postnatal NS of mammals, both peripheral and central.<sup>3</sup> Only in 1901, did Bielchowski and Cajal observe that a peripheral nerve can regenerate when it stays in contact with its cell body in the CNS.<sup>4</sup> With the aim to “transfer regenerative propriety of the peripheral nervous system into the central nervous system,” Ramon y Cajal transplanted peripheral elements (peripheral nerves and dorsal root ganglia) into the brain.<sup>5</sup> From these experiments, he postulated that the determination of axonal regeneration depends more on the microenvironment of the peripheral axon than on the cell body situated within the CNS.

Today, a new concept of the interaction of intrinsic and extrinsic developmental factors is dawning and opening new avenues. It is of prime interest for modern neuroscience research to unravel the molecular and functional principles underlying the development and regeneration of CNS cytoarchitecture and the character and roles of the ontogenic components. To what extent these principles are determined by genetic and epigenetic (other neurons, neuroglial cells, chemical gradients, learning, disease, and other factors) means, and how they mold the structure and function of neurons and glial cells, still remains largely unknown.

Thus, current neurotransplantation research not only offers hope for human medicine, but has also become an important tool for gaining new insights into neural plasticity, that is, into dynamic mechanisms at all levels of the neuroaxis, such as axonal reorganization and synaptogenetic reactivity. In a broader biological sense, all of these activities are responsible for the structural and functional establishment and agility of the mammalian NS. Especially in the brain, the cytoarchitecture is continuously sculpted in immediate response to experience and learning processes, based on external input and the interaction of the individual with the environment. This, however, causes questions about determinism and indeterminism, or nature versus nurture, to gain renewed importance in neuroscience, and begs for new experimental approaches. Such questions can be investigated in two classic ways: either by observing cellular interrelations in normal development or by observing patterns of variation and/or invariance which may arise in abnormal circumstances as a result of particular mutations or chemical or mechanical insults. In this second approach, neurotransplantation has joined in as a strategy to explore CNS development and plasticity in non-traditional ways. It soon became one of the modern strategies of choice in both basic and applied neuroscience and today helps answer questions concerning cell proliferation, differentiation, programmed cell death, and target-oriented migration in the CNS, as well as the formation of neural circuitry, CNS immune response, and many other issues of critical interest.

### TIMID BEGINNINGS

The fact that in some animals, and during early development, regeneration of the CNS does occur soon tempted researchers to engage in neurotransplantation experiments. The first attempts occurred as early as 1890, followed by others at the beginning of the 20th century. For example, Thompson transferred allografts (transfer of tissue between individuals within one species) and xenografts (between individuals belonging to different species) into the cerebral cortex of a dog,<sup>6</sup> and Saltykow performed allografting between adult rabbits.<sup>7</sup> Since, at that time, there existed little knowledge about the immune response and its causes, these pioneer experiments with grafts into the adult CNS within or between species did not lead to long-term survival of the transplanted material. The repeated failure led only to skepticism about mammalian neurotransplantation and was taken as a confirmation of the plastic rigidity of the mammalian CNS.

The modern concept of neurotransplantation in mammals did not appear until 1917, when E.H. Dunn transferred embryonic neural tissue into the rat CNS, thereby formulating two fundamental principles: (1) the graft has to be composed of immature tissue, and (2) it must get trophic support from the recipient.<sup>8</sup> This original approach was thus to take advantage of the highly plastic characteristics of dividing cells residing in the germinative regions of the developing CNS. The vivacity and adaptability of the immature neural tissue contrasted sharply with the regenerative rigidity of the adult mammalian CNS, and the possibility that immature donor tissue might deliver new "building matter" or missing substances into the impaired host CNS soon sparked new hopes. Nevertheless, despite all the promises, this innovative

“avant-garde” concept of neurotransplantation introduced by Dunn was abandoned for many decades to come.

A new wave of neurotransplantation finally started to take off in the late 1970s, when Perlow and collaborators demonstrated in rodents that non-differentiated dopaminergic (DA) neurons could compensate for a motor deficit induced by the unilateral damage of the substantia nigra (SN).<sup>9</sup> From that time on, experiments employing grafting into mammals multiplied and diversified tremendously, rising from a purely empirical phase into a stage firmly rooted in the rational bases of anatomy, physiology, and molecular biology. The quickly accumulating knowledge prompted the development of various animal models recapitulating certain neurological syndromes in man and allowed theoretical and some practical investigations envisaging the application of neurotransplantation in clinical therapy.

### A RENAISSANCE OF TRANSPLANTATION INTO THE CNS

The appeal of clinical relevance of neural grafts became particularly strong during the late 1980s, when Dunn’s original approach was again remembered, and researchers started to experiment with neural grafts dissected from different parts of the fetal or early postnatal CNS. This approach took advantage of the highly plastic characteristics of dividing cells residing in the germinative regions of the developing CNS and, simultaneously, of the relatively higher immunological tolerance of the CNS (for a long time, it was believed that the CNS is an “immunologically privileged” organ in the body, but this opinion was later substantially revised (see, for example, Xiao and Link<sup>10</sup>). The so-called “primary grafts” were transferred, in solid form or as cell suspensions, into intact pre- and postnatal brains and/or into the CNS of animals serving as models of various neurological disorders in humans. Particularly in the treatment of Parkinson’s and Huntington’s diseases, this approach eventually resulted in impressive anatomical and biochemical repair, paralleled by significant amelioration of the behavioral symptoms.<sup>11–15</sup>

Although primary neural grafts do occupy a firm place in the history of neurotransplantation, the limitation of material accessibility, concerns about its purity and viability, as well as political and ethical issues led many researchers to look for alternatives. These appeared in the early ‘90s in form of the freshly discovered neural stem cells (NSCs), demonstrating great plasticity and multipotency (see, for example, Refs. 16 and 17). The possibility of maintaining NSCs in culture, and expanding and manipulating them quickly and efficiently, stirred great interest in the biomedical research community as representing a potentially rich source of grafting material to replace fetal tissues.

Soon, the applications of stem cells in transplantation became as diversified as their sources and characteristics, which can be divided conceptually into various groups according specific parameters. While NSCs are isolated directly from the CNS (fetal germinative zones or adult neurogenic niches), other cells with stem cell properties can readily be obtained from various non-neural organs and/or in the form of pluripotent embryonic stem cells from very early embryos prior to germ-layer formation (blastocysts).<sup>18</sup> The source of NSCs is, however, of crucial importance and defines their biology, their ability to survive, migrate, and incorporate into the host

CNS, as well as their potential to differentiate into neural cells. These and other properties of NSCs may be further modified experimentally with the aim of enforcing the cells' capability to serve as vectors for the delivery of defined substances, to enhance their migration, or to guide their differentiation predominantly into one specific cell type.<sup>19</sup>

Depending on the donor material, neural grafts (both tissue and NSCs) have been proposed to help morphological and functional recovery of the recipient CNS by adopting one or more of the following strategies: (1) replacing affected cell populations (or structural components like myelin) and their connections, which would correlate with functional recovery; (2) delivering missing neuroactive molecules, such as enzymes and neurotransmitters, by targeted genetic engineering of the grafted cells, as occurs, for example, in most of the current therapeutic approaches for Parkinson's disease; (3) creation of highly growth-permissive tissue "bridges" for host axonal regeneration and target-oriented guidance of growing axons; and (4) provision of neurotrophic and other regeneration-promoting substances through natural and endogenous secretion by the transferred cells supporting the survival and growth of graft and host neural structures (this last strategy is discussed in more detail below).

The application of these principles in neurotransplantation has provided us with a wealth of information about normal CNS development and the characteristics of a disorder-dependent variability of the host environment and its impact on the behavior of the grafted tissues and cells. However, it also constantly highlights our persistent lack of understanding of the mechanisms controlling stem cell differentiation under the host's microenvironmental influence. Especially in the adult and aged CNS, the prevailing non-neurogenic and growth-inhibitory milieu has a strong negative impact on that process<sup>20-22</sup> and substantially reduces the success rate of neurotransplantation.

To unravel at least some of the mysteries pertaining to the problems alluded to in the last paragraph, we need to realize that in neurotransplantation, it is not only the graft that is exposed to and that reacts to environmental changes nor is it only the recipient that secretes signaling molecules influencing the behavior of donor cells. After grafting, an intimate cellular and molecular relationship is established between graft and host in which both try to reach an "ideal" homeostatic equilibrium. In this fusion of two worlds of quite diverse characteristics, only a detailed knowledge of the laws that govern the resulting reciprocal interactions will allow us to take advantage of them for clinical benefits.

### GRAFT-INDUCED CNS PLASTICITY

Today, it seems more and more obvious (and we were among the first proponents of this idea back in the early '90s<sup>23</sup>) that the ability to replace damaged or missing cell populations or to deliver missing enzymes and structural molecules is by far not the only beneficial feature of grafted fetal tissue and NSCs that deserves all the credit. In the light of new findings regarding postnatal CNS plasticity<sup>24</sup> and intercellular graft/host communication,<sup>25</sup> a much broader image begins to emerge of the roles and consequences of this complex cellular/molecular dialogue between essentially two

different worlds coming together. It forces us to go back and to try to understand regeneration of the grafted CNS in light of mechanisms and principles governing CNS development in order to fully apprehend the full spectrum of events that occurs during the intricate dialogue between graft and host cells.

In the early '90s, together with the late Hendrik Van der Loos, we worked on repairing a mechanically damaged somatosensory cortex by transplanting fetal tissue into juvenile mice. After a series of grafting experiments, we observed that, instead of replacing the damaged and missing cells in the unilaterally created cortical cavity in the primary somatosensory barrel field area, primary neural tissue isolated from embryonic day 14 (ED14) mouse neopallium could induce repair from within the juvenile host in a manner not seen spontaneously in the absence of transplantation.<sup>23</sup> The reconstituted cavity (in 70–80% of cases) appeared to become “filled” by relatively well-organized cortical tissue of *host* origin that even presented cellular arrangements reminiscent of barrels, which even displayed, at least partially, electrical activity (unpublished observations). The broader significance and applicability of this graft-induced but host-dependent cortical reconstruction was later confirmed in a xenotransplantation paradigm in which mouse tissue was transplanted into the lesioned CNS of kittens.<sup>26</sup>

In an attempt to ascertain a mechanism underlying this graft-evoked regeneration, the possibility of *de novo* neurogenesis was investigated in animals pulse-labeled with tritiated thymidine (<sup>3</sup>H]T) or bromo-deoxyuridine (BrdU), both nucleotide analogues labeling dividing cells during the S phase of proliferation. Our hopes that we could reactivate the formation of neurons in the host were, however, not met. The vast majority of [<sup>3</sup>H]T- and BrdU-labeled host cells turned out to be of glial and endothelial origin (although no significant gliosis or glial scar were ever found). The formation of new host neurons, on the other hand, was quite rare, and therefore insufficient to account for the substantial tissue recovery. Thus, alternative mechanisms needed to be explored that could have led to the observed plasticity. Such mechanisms may include remodeling of surrounding tissue by existing postmitotic cells, activation and differentiation of dormant progenitor cells present in the postmitotic CNS, and prevention of necrosis and secondary host cell death in the vicinity of the cavity.

At that time, on the basis of the observation of graft-induced host plasticity in damaged mouse and kitten neocortex, we predicted that this “regenerative phenomenon” is likely to depend on a favorable cellular and molecular environment that had to be created in a specific and reciprocal communication between graft and host elements, since neither non-grafted cavities nor cavities receiving non-neural fetal tissue presented any signs of recovery. Certain cellular elements within the fairly heterogeneous fetal tissue grafts seemed to modify the brain environment such that intrinsic, albeit latent, regenerative responses by the host were being uncovered, triggered, and amplified. The long-range effects of grafted tissue in some of these experiments suggested at least a partial role for secreted and diffusible substances mediating the necessary graft/host interaction. This assumption was corroborated by others investigating the production of growth factors by grafts and the responsiveness of the adult CNS to them.<sup>27,28</sup> Our thoughts led us to speculate that a source of such trophic and neuroprotective factors might well be the non-differentiated stem cells residing in the germinal parts of our neopallial grafts (which always included parts of the fetal ventricular zone).

## COOPERATION OF HOST AND DONOR STEM CELLS IN THE DEVELOPMENT OF GRAFTED FETAL CNS

In both the developing and the adult nervous system the behavior of cell precursors is regulated by environmental factors encountered either in the germinative zones or in the stem cell niches. Thus, the ultimate NSC behavior *in vivo* is the final cellular response to converging signals coming from various neighboring cell types such as endothelial and glial cells, or from the local extracellular matrix (ECM).<sup>29,30</sup> This continuing metamorphosis is reflected in both genetic and epigenetic components of ontogeny.

What happens, however, when such continuity of the intact CNS development is affected by transplantation—the coming together of two worlds of quite different cellular and biochemical properties? Today, we know that the fate of *both* interacting entities can begin to change and to be redefined depending on the character and intensity of mutual signals and the presence of cells capable of receiving and reacting to them. Thus, in a graft, represented by primary fetal tissue or by certain types of stem cells (SCs), the molecular principles underlying its response to the environment are governed by the developmental stage and the area from which its material was derived, and by the way it was processed prior to transplantation. Although this mutual signaling and responding is still far from being completely understood, it seems that the underlying “language” is of a universal character and can be studied even after xenografting.

To address some of the issues discussed in the preceding paragraph, we recently inspected the behavior of human NSCs grafted *in utero* into the ventricles of a mid-gestation non-human primate brain.<sup>31</sup> We observed that the donor cells not only could survive the xenotransplantation, but they also became integrated into the organogenetic program of the host. The injected cells entered the subventricular zone, interdigitated with the endogenous NSCs, and both together subdivided into different cell components of the developing brain. This “teamwork” of donor and host NSCs was reflected in a prevalent morphogenetic scenario. Thus, many of the NSCs soon left the subventricular zone, migrated along the processes of radial glia into the growing brain parenchyma, and started to differentiate into various types of neural cells, while contributing to the spatio-temporal morphogenetic gradient of the fetal monkey brain. Other subpopulations of donor and host NSCs remained undifferentiated and formed quiescent pools of multipotent cells, possibly for later use during ontogeny or maintenance of CNS homeostasis.

The presence of dormant undifferentiated cells within the adult CNS has by now been firmly established, and we believe that these cells may be used in later life for self-repair and literal cell turnover or to exert a protective effect upon imperiled juxtaposed neurons by providing trophic support.<sup>27,28</sup> The idea that this “chaperone” population can be augmented by transplantation and used effectively for the rescue of impaired brain populations<sup>25</sup> is effectively demonstrated in the experiments described in the next chapter.

## NSCs RESCUE DYSFUNCTIONAL NEURONS

The slow disintegration of neural function associated with aging and many neurodegenerative diseases provides an ideal opportunity to test the putative NSC capacity

to rescue dysfunctional cells in an environment *not* confounded by excessive necrosis, excitotoxicity, anoxia, or trauma.

One well-characterized and easily-identifiable neuronal cell type with stereotypical projections that is typically compromised in the aged brain—the dopaminergic (DA) neuron—was used for the following study.<sup>25</sup> To accelerate the process of aging and to simulate more of the pathological processes known to accompany neurodegenerative diseases such as Parkinson's disease, aged mice were exposed to systemic injections of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a toxin selectively affecting DA neurons. The animal model was carefully designed with the aim of not killing the mesencephalic DA neurons, but only to cripple them chronically in both cerebral hemispheres. Unilateral implantation of murine NSCs above the right ventral tegmental area (VTA) occurred 1 and 4 weeks post MPTP. In contrast to young or intact aged brains, the progressive immigration of the donor cells into both hemispheres was very extensive in the aged, MPTP-treated hosts. While in sham-transplanted animals (receiving MPTP treatment but no NSC graft) no recovery ever happened, the neural transplants resulted in a gradual reconstitution of the damaged mesostriatal system; first uni- and then bilateral, the recovery became apparent through the re-expression of two of its key markers, tyrosine hydroxylase (TH) and the membrane-bound DA transporter (DAT). Interestingly, the steady reactivation gradient of TH and DAT expression in the DA pathway was associated with corresponding D-amphetamine-evoked rotation of the animals during the first week after grafting. This rotation was a sign of an initial post-transplantation imbalance in dopamine levels between the two brain hemispheres and a consequence of the progressive recovery gradient in the DA system. Although the restoration of DA function was graft-dependent, it was not predominantly due to differentiation of the donor NSCs into DA neurons. While there was a sporadic and spontaneous conversion of NSCs to DA cells contributing to nigral reconstitution in the depleted mesencephalic areas, the vast majority of the recovered mesencephalic neurons were actually "*rescued*" *host cells*. Thus, in this example, a damaged host CNS did benefit from transplanted NSCs not because of their capacity to replace cells, but mainly because of a channel of communication with host cells leading to significant sparing and rescue of damaged but still persisting host cytoarchitecture.

This often ignored new possibility of saving damaged host circuitry and increasing the regenerative capacity in an adult injured CNS by a plasticity-promoting dialogue between graft and host has been, by now, corroborated in several other studies. Thus, it was demonstrated that NSCs seeded upon a synthetic biodegradable scaffold and grafted into the hemi-sectioned adult rat spinal cord, induced a significant improvement in animal movement by reduction of necrosis in the surrounding parenchyma and prevention of extensive secondary cell loss, inflammation, and formation of a glial scar. Here again, as tract tracing and GAP-43 immunoreactivity showed, the host tissue displayed regenerated neurites *not* derived from donor NSCs, but of recipient origin.<sup>32</sup> A more recent study in the lesioned spinal cord showed convincingly how such host neurite sprouting can be influenced by variation of the spectrum of donor cell–released trophic factors which can, for example, selectively stimulate the regeneration of either motor or sensory fibers.<sup>28</sup> Finally, a substantial reconstitution of the brain parenchyma and structural connectivity was reported when NSCs were transferred into regions of extensive brain degeneration caused by hypoxia, particularly when donor cells were transiently supported by biodegradable

scaffolds.<sup>33</sup> Also, in this case, the injured brain interacted reciprocally with the exogenous NSCs, which resulted in partial tissue reconstitution and host neurite sprouting.

The interaction between neural graft and host CNS is very complex and is likely to involve not only trophic and growth factors, but also cell-recognition molecules and the ECM.<sup>29,30,34</sup> The latter two, in particular, have so far hardly been explored in the context of neurotransplantation. To explore their involvement in the engagement of NSCs in regeneration, in the following study, we therefore elected to examine as a prototypical neural adhesion molecule, one of the earliest and best characterized of that class of molecules, the integral membrane glycoprotein L1 (Ourednik *et al.*, submitted for publication). L1, a member of the immunoglobulin superfamily, has been reported to be associated with a number of actions in the nervous system pertaining to important developmental and regenerative processes, including neurite outgrowth and fasciculation, myelination, synaptic plasticity, axonal regeneration, and cellular migration.<sup>35</sup> During these processes, L1 has been shown to be involved in homophilic and heterophilic signal transduction events leading to changes in steady-state levels of intracellular messengers involving, for example, the mitogen-activated protein kinase (MAPK) cascade and regulating gene transcription.<sup>36,37</sup> Thus, the major aim of our study was to gain insight into the contributions of L1 (as a prototypical representative of other adhesion molecules such as CAMS, integrins, cadherins, and others) in helping the bi-directional communication of graft and host elements.

Using the same MPTP-based lesioning paradigm mentioned above and various permutations of L1 overexpression in grafted NSCs and/or host astrocytes (the astrocyte having been identified as a cell type pivotal for defining the stem cell niche), we indeed found beneficial effects of this cell-recognition molecule on donor cell migration and survival, which resulted in faster and more robust recovery of the crippled host DA neurons (Ourednik *et al.*, submitted for publication). Intriguingly, this was obvious even in animals receiving time-delayed (4 weeks post MPTP) grafts. Thus, as originally postulated, in the hierarchy of molecular interactions between SCs and the adult CNS, adhesion/recognition molecules may play a pivotal—perhaps enabling—role in allowing SCs to help restore a homeostatic milieu, and a controlled manipulation of molecules like L1 could, someday, become an additional and important tool in the design of more efficient stem cell-based therapies.

### CNS PROTECTION VIA “STEM CELL VACCINATION”

In the preceding sections, we have adduced ample evidence supporting our hypothesis that grafted and residential SCs do collaborate in developmental and regenerative processes of the mammalian CNS and can play decisive roles in the re-establishment of the homeostatic milieu. However, we can speculate even further that by seeding the nervous system with SCs prior to the occurrence of damage we might actually prevent the often devastating sequelae of many hereditary diseases and CNS trauma. We tried to investigate the usefulness of such “preventive” grafting by using spontaneous cerebellar mouse mutants in the following set of experiments.

A number of naturally occurring mouse mutants exist that emulate various aspects of neurodegenerative diseases. Many of these mutants are characterized by the

degeneration of Purkinje cells (PCs), an important but quite vulnerable neuronal cell type within the brain. Three mouse mutants that we have begun to use in pilot studies are, in increasing severity and virulence, the *nervous* (*nr*) mouse, the *Purkinje cell degeneration* (*pcd*) mouse, and the *Lurcher* (*Lc*) mouse. Ostensibly, the only similarity these three mutants have in common is their loss of PCs (and secondarily granule cell neurons) at various times throughout their lives, usually beginning at early postnatal time points; the etiology for PC death seems to vary from mutant to mutant, although further investigation in this regard is ongoing. In all three mutants, however, grafted NSCs, depending on the time of their transfer, appeared to exert an impressive protective influence on the vulnerable PC population. While a small number of NSCs differentiated into PC-like cells, the overall appearance of PC reconstitution derived from the rescue of *host* PCs. This strong potential by NSCs to preserve neuronal populations in the mutant cerebellum seemed most prominent if NSCs were implanted before or at the onset of PC degeneration—that is, on postnatal days 0–10 (P0–10)—rather than during more advanced stages of PC loss (postnatal day 14 to 12 months). This positive effect of the grafts was reflected in both an impressively improved cerebellar cytoarchitecture and in the correction of motor behavior in the host animals.<sup>38</sup>

We have to bear in mind that an important component of many such neurodegenerative disorders—including Parkinson's disease and the PC death in our mouse mutants—is the occurrence of oxidative stress. The latter can be induced in experimental animal models by chemicals like 3-nitropropionic acid (3-NP), a drug that evokes progressive degeneration of striatal neurons and leads to behavioral changes similar to those accompanying Huntington's disease in humans.<sup>39</sup> By using this approach, we are presently testing whether it might actually be the buffering and homeostasis-maintaining capacity of grafted NSCs to diminish the impact of oxidative stress on host cells that allows their preventive usage *prior* to the occurrence of the insult in the CNS. Although preliminary, the first sets of data are very encouraging and indicate that preventive grafting can indeed result in substantial rescue of neurons from oxidative stress by the mobilization of protective antioxidant mechanisms (for more details see the paper by Madhavan *et al.* in this volume).

## SUMMARY

The research summarized in this review provides us with rather strong evidence that grafted NSCs can be a rich source of cells as well as factors helping the injured CNS to cope with or recover from an unfavorable environment and protecting its structural integrity and functionality. A better understanding of the regulatory signaling mechanisms and of the molecules involved should lead not only to a better understanding of brain development, but also to a better control of NSC behavior in neurotransplantation, which might help to open new therapeutic avenues. The discovery of neurogenesis and persisting endogenous NSCs even in the adult CNS, and the evidence that these dormant progenitors can be stimulated to neurogenesis by an injury and can integrate functionally into existing adult neural networks, have rendered the scientific community more receptive and positively inclined to accept and further evaluate an increased role of the host in its repair. In consequence, this trend allowed our pioneer studies from the '90s to gain significance and to be revisited.

The effectiveness of neural transplantation depends on, among other factors, the type of NSCs used, as well as on their survival and ability to successfully infiltrate damaged brain regions. Unfortunately, both latter processes are drastically reduced in adult or aged CNS and in the presence of chronic insults. The reasons for this include a lack of neurotrophic factors, the presence of myelin-associated inhibitory molecules, and age- or lesion-dependent accumulation of particular variants of the ECM (e.g., chondroitine sulphate proteoglycans) interfering with the signaling between NSC cells and their environment.<sup>1,2,20,29,30</sup> It seems, therefore, that in order to augment regeneration and plasticity in the adult and aged CNS, we may need a combinatorial approach, which would include cell replacement, trophic support, protection from oxidative stress, and the neutralization of the growth-inhibitory and tissue-plasticity-preventing components with “lubricating” molecules like L1. Such a global approach cannot be overestimated and needs to be our highest priority in achieving the best possible in graft-aided CNS regeneration.

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