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# The Role of Cell Therapy in the Treatment of Epilepsy: Lessons from Animal Models and Clinical Trials

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## 1. Introduction

The notion of replacing brain cells affected by a particular disease or unable to synthesize a molecule crucial for adequate brain function has been around for many years. The first experiments entailed the transplantation of adult tissue into an adult or young animal; they were met with limited success (Björklund & Stenevi, 1984). Later, scientists started working with fetal tissue, because this tissue fares better when dissected, axons are shorter, cells are still dividing, and growth factors are present in the tissue. These studies were more successful and led to clinical trials using human fetal tissue, and autologous transplants of adrenal chromaffin cells, especially for Parkinson's disease (Freed, 1991). Transplants were first used in this neurodegenerative disorder because it was assumed that the main problem was the lack of dopamine in the striatum, and this neurotransmitter could be readily provided by chromaffin cells from the adrenal medulla or by mesencephalic dopaminergic cells. Initial preclinical studies had shown, using the 6-OHDA model developed by Ungerstedt (Ungerstedt 1968; Ungerstedt & Arbuthnott, 1970), that intrastriatal grafts of mesencephalic cells could reduce the number of ipsilateral or contralateral rotations induced by apomorphine or amphetamine, suggesting that the dopaminergic tone had been restored in the lesioned striatum. These studies led to others using fimbria fornix lesions (e.g., Dunnett et al., 1982), diabetes insipidus model (e.g., Gash & Sladek, 1980), Huntington's disease models (e.g., Norman et al., 1989), and epilepsy models (e.g., Castillo et al., 2006). In addition to the experiments showing functional effects of the transplants, there were others looking for the mechanisms underlying the effects of the transplants. A series of studies showed that transplanted cells could establish reciprocal anatomical connections with the host (Wictorin, 1992; Murata et al., 1990). A couple of studies using electrophysiology, and markers or cell activation showed that these connections were functional (Xu et al., 1991; Rutherford et al., 1987; Labandeira-Garcia & Guerra, 1994). Neuroscientists involved in this field concluded that neural transplants could affect the host brain by releasing growth factors, and neurotransmitters, and by establishing functional connections with the host brain, thus reestablishing lost circuits.

However, the source of tissue for transplantation was still an issue to be considered. Fetal tissue was not readily available in all countries, and its use implicated ethical and legal

issues not easily resolved. In addition, fetal tissue included all kinds of cells, and in spite of the immuno-privileged status of the brain, it could lead to an inflammatory response in the host. Autologous transplants of chromaffin cells (Freed et al., 1990) and xeno-transplants (Fink et al. 2000) had been used as an alternative source of tissue for transplantation. Then, with the advent of new molecular biology techniques, cell lines with particular properties and characteristics were developed. GABAergic cell lines proved easier to develop than dopaminergic cell lines, and given the fact that epilepsy has been considered as a disorder in which there is an imbalance between excitation and inhibition, these cell lines started to be tested in epilepsy animal models. More recently, once it was demonstrated that Cajal's notion that in the adult nervous system neurons no longer divide was incorrect, and that there are neurogenic pools in the adult brain (Nottebohm, 2002), the potential use of these immature cells for transplantation became a possibility. Furthermore, a myriad of studies started looking for factors to differentiate these immature nerve cells into the desired type of nerve cell, dopaminergic, cholinergic, GABAergic, among others (Vazin & Freed, 2010; Mejía-Toiber et al., in press). Cell lines could be developed from these adult but immature cells, thus solving the problem of having a source of tissue for transplantation. Moreover, now we know that cells, such as fibroblasts can be reprogrammed into neuronal cells (e.g., Takahashi & Yamanaka, 2006), thus, in theory the host could eventually replace its own damaged cells. In this brief overview of the field of neural transplantation, we have seen how the sources of tissue for transplantation have expanded, the techniques have been refined, and new possibilities have emerged to try and restore function in the nervous system. Still, questions remain, how is it that the new cells can modify the function of the host nervous system, is it the transplant that changes the host brain and takes some of its functions, or is it the host brain itself that is modified as a result of the presence of the alien cells in its midst. Is it necessary to transplant new cells into the brain, or do we have to learn how to activate the neurogenesis that is already taking place in the adult brain. How can cells be differentiated without posing a risk for the host, which plasmids are safe to us, and which are not. In the following sections we will focus on the advances of neural transplants in animal models of epilepsy, and in the clinical setting in Parkinson's disease.

## **2. Preclinical studies using cell transplants of fetal tissue and neural stem cells in animal models of epilepsy**

Epilepsy is a neurological disorder with a prevalence of about 1% (Morimoto, Fahnestock et al. 2004) characterized by seizures that occur in a spontaneous and recurrent manner. Seizures are classified as generalized and can manifest themselves as uncontrolled muscular activity or as a sudden interruption of physical and mental activity (absence seizures); or as focal seizures, that are classified according to their manifestation, i.e., if they involve psychic or sensory phenomena, motor or autonomic components, loss of awareness, or evolving to a bilateral, convulsive seizure (Berg et al., 2010). Epileptic seizures are the result of abnormal, excessive and synchronic discharges of groups of neurons that occur as a result of changes in synaptic function and intrinsic properties of neurons that upset the balance between inhibitory and excitatory neurotransmission, favoring the latter (Cossart et al., 2001). In the recent document published by the International League Against Epilepsy (ILAE) Commission on Classification and

Terminology (Berg et al., 2010), the underlying type of causes of epilepsy are considered to be genetic, structural-metabolic or unknown.

Epilepsy is one of the neurological disorders that could benefit from transplantation of cells, since some types of epilepsy are focal, and in the case of generalized seizures they can be suppressed by local injections of GABAergic agents into particular areas of the brain such as the substantia nigra, or piriform cortex (Gale, 1992). Indeed, Kokaia et al. (1994) observed a reduction in generalized seizures in rats previously kindled in the amygdala when GABA-releasing polymer matrices were implanted bilaterally and dorsal to the substantia nigra. The effect lasted until GABA release from the polymer matrices was reduced. Thus, local delivery of an inhibitory neurotransmitter could reduce the neuronal excitability in brain regions from which seizures originate or in brain regions from which they propagate to the rest of the brain.

Initially, studies using transplants in epilepsy models used fetal tissue with some success. Barry et al., (1987) (Table 1), evaluated the effect of fetal noradrenergic transplants in the hippocampus using the kindling model of epilepsy. This model consists in the use of repeated subthreshold electrical stimulations of specific regions of the Central Nervous System (CNS). Afterwards, the animal presents focal epileptiform seizures that then generalize to the rest of the brain. The work of Barry et al. (1989) involved first, the administration of 6-hydroxydopamine (6-OHDA) icv, and then the transplant of fetal noradrenergic cells into the hippocampus. The authors of the study found that animals required a greater number of stimulations to induce the various phases of kindling, in comparison with rats that did not receive the transplants. Also the delay in kindling was related to the density of innervation of the transplant in the host hippocampus. They obtained similar results when transplanting cells of the locus coeruleus into the amygdala and piriform cortex (1989) (see Table 1). A study using noradrenergic cells obtained from locus coeruleus (LC) from E13-E14 rats showed that these cells could reinstate noradrenergic transmission in the noradrenaline-depleted hippocampus (Bengzon et al., 1991). In this particular study, they measured noradrenaline-release using microdialysis after electrical stimulation of the hippocampus, and observed that noradrenaline release from intrinsic and grafted LC neurons occurred concurrently with their seizure suppressing action; thus LC grafts in the hippocampus appeared to be functionally integrated into the host brain, at least during generalized kindled seizures (Bengzon et al., 1991).

Fetal GABAergic cells have also been used with varying degrees of success in other epilepsy models. Fine et al. (1990) used the pilocarpine model in rats with ibotenic acid striatal lesions. Fetal GABAergic cells from the ganglionic eminence (E16) were transplanted into the substantia nigra pars reticulata (SNr), and suppressed the motor seizures; however, similar effects were observed after sciatic nerve transplants, suggesting a non-specific effect. Almost a decade later, Löscher et al., (1998) implanted fetal striatal GABAergic neurons into the substantia nigra in fully kindled animals, and evaluated the threshold for focal discharges (ADT), afterdischarge duration and severity, and duration of seizures during ADT. They found a significant increase in ADT and marked reduction in seizure severity compared with pre-transplantation values; however, the seizure-suppressing effect of GABAergic grafts was not permanent but slowly disappeared over the weeks after transplantation (Löscher et al., 1998).

Another decade later, better results were obtained by Rao et al., (2007) and Hattiangady et al., (2008) when these groups evaluated the effects of transplants of hippocampal cells

(E19), and lateral ganglionic eminence (E15) into the hippocampus in a model of spontaneous recurrent motor seizures (SRMS), respectively. Rao et al., (2007) pretreated the cells with brain derived neurotrophic factor, and neurotrophin-3, plus a caspase inhibitor, or with fibroblast growth factor (FGF-2). They observed that hippocampal cells not treated with trophic factors failed to reduce the SMRS, in contrast to those treated with the trophic factors and the caspase inhibitor; greater survival was observed with FGF-2. Hattiangady et al., (2008) pretreated precursor cells with FGF-2 and a caspase inhibitor, and found an important reduction in SRMS induced by kainic acid. In addition they found that 69% of surviving transplanted neurons differentiated into GABAergic cells suggesting that the reduction in seizures is related with increased inhibitory control in the hippocampus.

Stem cells with their properties of self-renewal and multipotentiality (Wang et al., 2007) represent another source for transplantation into the nervous system. Pluripotent stem cells (PSC) can generate cells from all three embryonic germ layers, mesoderm, endoderm and ectoderm. Two types of mammalian PSC have been identified, the embryonic stem (ES) cells derived from the inner cell mass of the blastocyst, and embryonic germ (EG) cells obtained from post-implantation embryos (Yu & Thomson, 2008). Recent studies have identified different means of obtaining desired lineages from these human ES, for example neural stem cells (NSC) (Carpenter et al., 2001; Reubinoff et al., 2001; Zhang et al., 2001), further differentiated into midbrain dopaminergic neurons (e.g. Perrier et al., 2004), neural crest lineages (Lee et al., 2010), and neural progenitor cells from human EG cells (Pan et al., 2005). Various groups have also described differentiation of mouse stem cells into neural precursors, (e.g. Westmoreland et al., 2001; Kim et al., 2002; Barberi et al., 2003; Lang et al., 2004), and found positive functional effects after transplantation (Rodriguez-Gomez et al., 2007).

In terms of their potential use in epilepsy models, it has been shown that NSC derived from the medial ganglionic eminence and transplanted in the hippocampus of adult rats showing spontaneous recurrent motor seizures, reduced motor seizures, but did not restore cognitive impairments (Waldau et al., 2010). The NSC differentiated mostly into astrocytes, neurons, and oligodendrocyte progenitors. About 50% of the cells expressed GDNF and 10% expressed GABA. NSC grafts restored GDNF expression in hippocampal astrocytes. The authors of the study suggest that the addition of new GABA neurons plus GDNF positive cells may underlie the therapeutic effects they observed.

A different group has developed adenosine-releasing ES cells, by disruption of both alleles of adenosine kinase (*Adk*) which phosphorylates adenosine in eukaryotes and catalyzes the reaction: Adenosine + ATP = ADP + AMP. The cells were encapsulated in semipermeable polymer membranes and implanted into the lateral ventricles of kindled rats (Güttinger et al., 2005). The authors observed transient protection from convulsive seizures and a profound reduction of afterdischarge activity in EEG recordings. However, they suggest that long-term seizure suppression was precluded by limited viability of the encapsulated cells (Güttinger et al., 2005). In a more recent study with male Sprague-Dawley rats (Li et al, 2007) the same group derived NSC from *Adk*<sup>-/-</sup> or *Adk*<sup>+/+</sup> ES cells. They implanted them into the hippocampus before kindling, and compared *Adk*<sup>-/-</sup> cells to adenosine releasing baby hamster kidney cells *Adk*<sup>-/-</sup> (BHK-AK2), and to wild type NSC. They observed that wild type NSC delayed the development of seizures; BHK-AK2 exerted a moderate protection, whereas *Adk*<sup>-/-</sup>-NSC retarded epileptogenesis during



kindling development and prevented the occurrence of generalized seizures. The effect was sustained for 3 weeks; 26 days after grafting, histological analysis revealed dense cellular transplants in the vicinity of the injection tract, with 56% expressing mature neuronal markers (NeuN).

NSC engineered to express enhanced green fluorescent protein under the control of the tau promoter were transplanted one month after pilocarpine treatment in the hippocampus of Wistar male rats (Rüschenschmidt et al., 2005). Electrophysiological recordings 13-34 days after transplantation showed that the NSC developed characteristic intrinsic and synaptic neuronal properties, and received excitatory and inhibitory synaptic inputs; there was no evidence of tumor formation. Jing et al. (2009) described the transplantation into the hippocampus one-week after kainic acid-induced status epilepticus (SE) of NSC obtained from the subventricular zone of green fluorescent protein-expressing Sprague Dawley rats. In this study an infusion of erythropoietin was delivered by means of an osmotic pump into the right lateral ventricle. Erythropoietin (EPO) is a glycoprotein produced primarily in the kidney but also produced locally in neural tissues, according to the authors it has shown neuroprotective effects in nervous system disorders including during the process of SE. The results of this study indicated that NSC transplants were able to prevent the development of SRMS through the suppression of aberrant mossy fiber sprouting and by increasing the number of inhibitory neurons. EPO infusion increased survival but not differentiation or migration of NSC; in terms of effects on EEG, no differences were observed between NSC + EPO, and NSC + vehicle groups.

Human NSC have also been used in models of SE. Chu et al (2004) obtained human NSC from the ventricular zone of embryonic brain (15-weeks gestation); one of the clones obtained was infected with a retroviral vector encoding  $\beta$ -galactosidase ( $\beta$ -gal) and puromycin-resistant genes. These cells were injected intravenously in a tail vein of male Sprague-Dawley rats, 24-h after pilocarpine-induced SE; the control group received only vehicle. Six weeks later histological analysis or electrophysiological recordings of hippocampal slices took place. In the transplanted group the authors observed a reduction of convulsive seizures;  $\beta$ -gal+ cells were found in the hippocampal area (CA1, subiculum, hilus of the dentate gyrus, CA3), amygdala, and piriform cortex; in kidney, lungs, and spleen, without evidence of tumor formation. In rats without SE, no cells were found in the brain. Cells were mostly GABA+ and parvalbumin+; field excitatory postsynaptic potentials in CA1 induced by stimulation of Schaffer collaterals were smaller in transplanted animals. The authors concluded that intravenously transplanted human NSC suppress spontaneous recurrent seizures. Using the same model Costa-Ferro et al. (2010) evaluated the therapeutic potential of bone marrow mononuclear cells (BMC) obtained from transgenic mice. BMC cells have been reported to stimulate endogenous glial or neural stem cells, reduce neuronal apoptosis and neurodegeneration, modulate inflammatory responses, release trophic factors and cytokines involved in tissue repair and regeneration, and promote self-repair mechanisms in the brain (Costa-Ferro et al., 2010). Ninety-min after pilocarpine-induced SE the animals received BMC or saline only via the tail-vein. By 15 days after transplantation, none of the BMC transplanted animals showed SRMS, while all saline-treated animals did; 120 days later only 25% of transplanted animals showed SRMS with a lower frequency and duration than control

animals. Transplants attenuated cell loss in the hippocampus and BMC were found distributed in the brain of pilocarpine-treated recipient animals. In addition, long-term potentiation was preserved in the transplanted animals compared to pilocarpine-treated non-transplanted animals. The authors suggested that BMC could prevent the development of chronic seizures, reduce neuronal loss, and influence the reorganization of the hippocampal network.

Maisano et al., (2009) have also used ES-derived NSC in animal models of epilepsy, and have transplanted the YC5 mouse ES line into the CA3 region of the hippocampus one week after kainic acid or pilocarpine administration. The NSC expressed immature stem cell markers shortly after transplantation; with longer survival time (4-8 weeks) cells in the kainic acid model some cells expressed an early marker of granule neurons (Prox1). Eight weeks after transplantation many grafted neurons received synaptophysin-positive synaptic terminals, suggesting their integration into the host hippocampus. The same group used human NSC and observed similar results after transplantation into the kainate-lesioned brain (In: Maisano, et al., 2009). The cells migrated to the upper blade of the dentate gyrus only in mice that had experienced seizures, and expressed neuroblast markers (doublecortin, PSA-NCAM). Mouse but not human NSC formed teratomas when transplanted into the mouse hippocampus. More recently, the same group has made electrophysiological recordings one month after transplantation into the dentate gyrus in kainate-treated mice. Their results showed that the cells have normal electrophysiological properties, receive synaptic inputs, and have the ability to fire spontaneous potentials. In their 2009 paper, Maisano et al. suggest the use of protocols to enrich for GABAergic precursors, based on the expression of glutamic-acid decarboxylase-67 (GAD67); they suggest the use of conditioned media, gene transfection of transcriptional factors, or sequential treatment with growth factors and molecules to guide progenitor cells toward GABAergic lineages.

In a recent critical review (Shetty & Hattiangady, 2007) the authors state that there is no evidence in support of using stem cells in the treatment of temporal lobe epilepsy yet, although they acknowledge that the field is still in its initial stage of development. This conclusion is still valid at present. Among the strategies that Shetty and Hattiangady (2007) suggest for further advance in the field, two of them are particularly relevant for theme of the present review. First, is the need for rigorous analyses of the efficacy of grafts of ES cells or NSC placed into the hippocampus after the onset of chronic epilepsy for suppressing seizures as well as learning and memory deficits. In particular, the question of the duration of the suppressive effects is particularly relevant, because as can be gathered from the evidence presented above, the grafts of either fetal tissue or NSC have been evaluated for relatively short periods. These analyses should include evidence for the long-term survival of the grafts, and the differentiation of the cells in these grafts into functional principal hippocampal neurons, or GABA-ergic interneurons. Second, is the need for evaluating combination therapy, i.e., NSC or ES cells transplants and delivery of anticonvulsant compounds into the hippocampus during chronic epilepsy. As these authors suggest, this strategy might result in significant seizure suppressing effects. Finally, it should be emphasized that studies using fetal tissue and NSC transplants must include adequate control groups so that the effects of the grafts can be unequivocally attributed to the particular cell type or compound being tested.

Cell type	Model	Site of transplant	Behavioral effects Histological findings	Reference
Noradrenergic (locus coeruleus, E13-14)	NA-depleted rats; kindling 6-11 months after transplant.	Hippocampus	Delayed onset and retarded progression of kindling; correlated with neuron survival	Barry et al., 1987
Noradrenergic (locus coeruleus, E13-14)	NA-depleted rats; kindling 2-3 months after transplant.	Amygdala-pririform cortex	Delayed onset and retarded progression of kindling.	Barry et al., 1989
Noradrenergic (locus coeruleus, E13-14)	NA-depleted rats; kindling 3 months after transplant, then midrodialysis.	Hippocampus	Retarded progression of kindling; restored basal and seizure-induced extracellular noradrenaline levels in hippocampus, cells immunopositive for tyrosine hydroxylase.	Bengzon et al., 1991
GABAergic (ganglionic eminence, E16)	Ibotenic acid lesioned animals; pilocarpine administration 1 month after transplant.	Substantia nigra reticulata	Reduced occurrence of motor limbic seizures, and intensity score; grafts localized in the substantia nigra.	Fine et al., 1990
GABAergic (ganglionic eminence E14)	Transplanted after fully kindled.	Substantia nigra reticulata	Increased afterdischarge threshold, decreased severity; short-lasting effects.	Löscher et al., 1998
GABAergic (lateral ganglionic eminence, E15)	KA-induced SE; transplanted 4 days after SE ; evaluated 9-12 months after SE.	Hippocampus	Reduced number of SRMS; differentiated into GABAergic cells.	Hattiangady et al., 2008

Table 1. Studies using transplants of fetal tissue and neural stem cells into the brain in animal epilepsy models.



Cell type	Model	Site of transplant	Behavioral effects Histological findings	Reference
Hipocampal cells (E19)	KA-induced SE; transplanted after showing spontaneous motor seizures; evaluated 2 monts after grafting.	Hippocampus	Reduced number of SRMS; greater survival of neurons and GABA interneurons in hippocampi if pretreated with neurotrophic factors and caspase inhibitor.	Rao et al., 2007
Neural stem cells (medial ganglionic eminence, E14)	KA-induced SE; transplanted after showing spontaneous motor seizures; evaluated 3 months after grafting. Evaluation of seizures and learning and memory.	Hippocampus	Reduced frequency and duration of SRMS; no improvement in cognitive function; differentiation into neurons (some GABA+), astrocytes and oligodendrocyte progenitors, restored GDNF in hippocampus.	Waldau et al., 2010
Adenosine-releasing ES cells encapsulated in polymer membranes	Kindling model, evaluated 3 days after grafting until 7 days.	Lateral ventricle	Transient protection from convulsive seizures and reduction of afterdischarge activity in EEG recordings.	Güttinger et al., 2005
NSC from <i>Adk</i> <sup>-/-</sup> or <i>Adk</i> <sup>+/+</sup>	Kindling model, grafted before kindling; evaluated for 3 weeks.	Hippocampus	NSC from <i>Adk</i> <sup>-/-</sup> retarded epileptogenesis, prevented occurrence of generalized seizures. Dense cellular transplants, some cells NeuN+.	Li et al., 2007

Table 1. Studies using transplants of fetal tissue and neural stem cells into the brain in animal epilepsy models. (continuation)

Cell type	Model	Site of transplant	Behavioral effects Histological findings	Reference
NSC	Pilocarpine treatment, transplanted one month later, evaluated 13-34 days after transplantation.	Hippocampus	NSC showed intrinsic and synaptic neuronal properties, receive excitatory and inhibitory inputs.	Rüschenschmidt et al., 2005
NSC	KA-induced SE, transplanted one week later, EEG recorded 3 weeks after transplantation.	Hippocampus, with or without EPO (icv)	Reduced frequency of abnormal spikes; attenuates aberrant mossy fiber sprouting; differentiated into GFAP+ astrocytes; EPO enhanced survival.	Jing et al., 2009
hNSC	Pilocarpine-induced SE, 24h later cells injected; 6 weeks later, histology or electrophysiological recordings of hippocampal slices.	Intravenous administration, tail vein	Reduction in convulsive seizures; smaller fEPSP in CA1; labelled cells found in hippocampus and other brain areas, no tumor formation.	Chu et al., 2004
BMC	Pilocarpine-induced SE, 90 min later cells injected; evaluated 15 and 120 days later.	Intravenous administration, tail vein	Short-term suppressed SRMS; long-term only 25% animals with BMC showed SRMS of lower frequency and duration than controls; attenuated cell loss in the hippocampus; long-term potentiation preserved.	Costa-Ferro et al., 2010

Table 1. Studies using transplants of fetal tissue and neural stem cells into the brain in animal epilepsy models.(continuation)

Cell type	Model	Site of transplant	Behavioral effects Histological findings	Reference
NSC and hNSC	KA or pilocarpine administration, transplanted one week later.	Hippocampus CA3	Expression of immature cell makers, and of granule cells after longer survival times. Inputs of synaptophysin+ synaptic terminals. Mouse NSC formed teratomas. hNSC migrated to dentate gyrus. Neuronal electrophysiological characteristics.	Maisano et al., 2009

Table 1. Studies using transplants of fetal tissue and neural stem cells into the brain in animal epilepsy models. (continuation)

*Adk*: adenosine kinase, enzyme that phosphorylates adenosine in eukaryotes; BMC: bone marrow mononuclear cells; CA: *Cornu Ammonis*; EEG: electroencephalogram; EPO: erythropoietin; ES: embryonic stem cells; fEPSP: field excitatory postsynaptic potentials; GABA: gamma-aminobutyric acid; GDNF: glial-derived neurotrophic factor; GFAP: glial fibrillary acidic protein; hNSC: human neural stem cells; icv: intracerebroventricular administration; KA: kainic acid; NA: noradrenaline; NeuN: a mature neuronal marker; NSC: neural stem cells; SE: status epilepticus; SRMS: spontaneous recurrent motor seizures.

3. Preclinical studies using cell transplants of cell lines in animal models of epilepsy

The use of fetal tissue for transplantation involves ethical and technical issues that can be circumvented by using cell lines. The advances in molecular biology techniques provided the means to immortalize and genetically modify cells to produce specific growth factors, and enzymes. One example of a cell line with intrinsic GABAergic properties is the clone M213-20, derived from the ganglionic eminence of E14-15 Sprague-Dawley rats. This cell line was obtained by immortalizing neuroblasts using the temperature sensitive allele (A58) of the SV40 large T antigen, and was found to have some GABAergic characteristics (Giordano et al., 1993; 1996). This cell line was further modified by transfection with the hGAD<sub>67</sub> cDNA by means of a plasmid based on the Epstein-Barr virus. From the clones obtained, one was selected, the M213-20 CL-4 that synthesizes and releases significantly more GABA than the parent cell line (Conejero-Goldberg et al., 2000), reuptakes GABA, responds to glutamate, presents calcium transients, and releases the neurotransmitter in a calcium-dependent manner (Mejia-Toiber et al., 2010). This cell line when transplanted into the inferior colliculus increases the latency for audiogenic seizures (Ross, et al., 2002); it increases the latency for tonic-clonic seizures induced by kainic acid, and decreases their severity when transplanted into the SNr (Castillo et al., 2006) (Table 2). In a more recent

Cell type	Model	Site of transplant	Behavioral effects Histological findings	Reference
M213-2O CL-4	Audiogenic seizures.	Inferior colliculus	Increases latency to audiogenic seizures.	Ross et al., 2002
M213-2O CL-4	KA-induced SE; transplanted 8 weeks before.	Substantia nigra reticulata	Increases latency for tonic-clonic seizures, reduces their intensity.	Castillo et al., 2006
M213-2O CL-4	KA-induced SRMS; evaluated at 4 and 12 weeks after transplantation.	Substantia nigra reticulata	Reduces the percentage of animals showing SRMS; longer survival times for transplanted rats, GABA content increased in area of graft.	Castillo et al., 2008
M213-2O CL-4	Kindling model; evaluated for 8 weeks.	Substantia nigra reticulata	No improvement; strong tissue reaction in kindled animals.	Nolte et al., 2008
M213-2O CL-4	Genetic model of absence seizures (GAERS)	Substantia nigra reticulata	No reduction in seizures.	Castillo et al., 2010
Cortical cell line CN1.4 with GAD65cDNA	Transplanted, then kindled in amygdala	Piriform cortex	Increases in pre-kindling partial seizure threshold, and increased latency to the first generalized seizure during kindling. GAD65 long-term expression.	Gernert et al., 2002
Cortical cell line CN1.4 with GAD65cDNA	Pilocarpine-induced SE, then transplanted, evaluated 3 days and 7-8 days after surgery	Substantia nigra reticulata	Fewer SRMS, integration into the host, effect diminished if transgene is suppressed.	Thompson & Suchomelova, 2004
Cortical cell line CN1.4 with GAD65cDNA	Transplanted, then hippocampal stimulation; transplanted, then kindling in entorhinal cortex	Dentate gyrus	Increased after discharge threshold; longer latency to first generalized seizure.	Thompson, 2005
GDNF cells encapsulated in semi-permeable membrane	Kindling, then implanted, then rekindled 4 weeks later.	Ventral hippocampus	Lower levels of GDNF reduced duration of afterdischarges, and seizure severity.	Kanter-Schlifke et al., 2009

GAD: glutamate decarboxylase, the synthetic enzyme for the neurotransmitter GABA; GAERS: Genetic Absence Epilepsy Rat from Strasbourg; GDNF: glial derived neurotrophic factor; KA: kainic acid; SRMS: spontaneous recurrent motor seizures.

Table 2. Studies using cell lines in animal models of epilepsy.

study, it was found that intranigral transplants of this GABAergic cell line decrease the percentage of rats showing spontaneous seizures induced by kainic acid, at 4 and 12 weeks after transplantation into the SNr (Castillo et al., 2008) (Table 2). Beneficial effects are not observed in all animal models using nigral transplants of cell line M213-2O CL-4. In Wistar rats in a model of kindling a strong immune reaction was observed (Nolte et al., 2008). This strong immune reaction was only observed in kindled animals transplanted with cell line M213-CL-4. In another study, transplants of this cell line did not reduce absence seizures in the genetic epilepsy model of the Strasbourg rats (GAERS) (Castillo et al., 2010).

A different cell line, CN 1.4, contains GAD<sub>65</sub> cDNA the other enzyme involved in the synthesis of GABA. The vector used to generate this cell line, produces two gene products, a tetracycline-responsive transactivator (tTA) and neomycin phosphotransferase (Thompson et al., 2000), thus, when doxycycline is present tTA cannot bind to the promoter, and cannot direct transcription (Thompson 2005). Transplants of this cell line into the SNr reduce susceptibility to crisis in the model of kindling and reduce the number of epileptiform spikes in lithium pilocarpine-induced seizures (Gernert et al., 2002; Thompson & Suchomelova, 2004) (Table 1). In the entorhinal cortex-kindling model Thompson (2005) observed increased GABA levels in the hippocampus 3 and 10 days after transplantation of this cell line. They found that behavioral seizures and afterdischarges were reduced only in the animals with transplants of the GABA cells. GABA levels *in vivo* and *in vitro* decreased when the cells were treated with doxycycline, and so did the behavioral effects.

In a recent study a genetically modified cell line that synthesizes and releases the glial derived neurotrophic factor (GDNF) was encapsulated in a semipermeable membrane and transplanted into the hippocampus. Those animals transplanted with capsules that released a moderate amount of GDNF showed a decrease in the severity of the seizures and in the duration of the afterdischarge (Kanter-Schlifke, et al., 2009).

At present, there are no other cell lines that have been tested in animal models of epilepsy. These studies taken together, have demonstrated that increasing levels of the inhibitory transmitter GABA into the SNr, hippocampus, amygdala, piriform cortex, or inferior colliculus, does reduce seizures in a variety of animal models. The challenge is to take this knowledge and transform it into a therapeutic strategy that may be successful in the clinical setting.

#### 4. Clinical studies using cell transplants: lessons from Parkinson's disease

Preclinical proof-of-concept studies are necessary to show that transplanted cells can survive, differentiate, integrate, and exert functional effects in the animal model of choice. They are also necessary to find the shortcomings and undesirable side effects of these procedures. However, animal models can only go so far in providing information about the use of neural transplants in the clinical setting. These experimental models have evident shortcomings because they cannot reproduce the particular environment of the diseased brain, the progression of the illness, or the survival time of the host, among others. In this sense, only clinical studies can definitively answer the question of whether or not transplants can reduce the symptoms or slow the progression of the disease, and for how long they may give the patient an overall better quality of life.

The clinical studies using neural and adrenal transplants in Parkinson's disease (PD), can provide with a wealth of information regarding the potential of this procedure, its shortcomings, and the factors that still need to be resolved. PD is a progressive neurological



disorder characterized by tremor, rigidity, and slowness of movements, associated with cell loss in the substantia nigra, pars compacta, and other brain structures (Tolosa et al., 2006). Substantia nigra pars compacta cells (SNc) produce and release dopamine (DA) in the caudate nucleus and putamen, a neurotransmitter involved in motor behavior. In addition to loss of DA cells in the nigra, other histopathological signs include the presence of dystrophic neurites, and Lewy body inclusions in the surviving DA cells. Lewy bodies are  $\alpha$ -synuclein and ubiquitin-containing intracytoplasmic inclusions (Kövari et al., 2009). PD was the first neurological disorder in which neural transplants were used. The source of tissue for transplantation initially were adrenal medulla chromaffin cells, since they produce and release substantial quantities of catecholamines (Drucker-Colin & Verdugo-Diaz, 2004). In 1990, Freed et al., wrote a thorough review about the status of intracerebral adrenal medulla grafts, they reviewed the results of about ten of the early studies of adrenal grafts for the treatment of Parkinson's disease. Two primary methods of autotransplantation were used then, the first involved transplantation of adrenal medulla into the striatum by stereotaxic injection; the other involved transplantation of the adrenal tissue to cavities in the wall of the lateral ventricle. The conclusions of this review indicated that intrastriatal adrenal medulla grafts caused only a transient improvement, whereas intraventricular grafts appeared to have a more lasting effect. The degree of improvement, though, differed among studies, and ranged from substantial to modest. The major improvements had to do with increases in duration of "on" times, that is the time when the medication is having an effect, and the patient can move. In Parkinson's disease there are fluctuations in the response to the medication, these periods are known as "on", and "off". Great variability was observed from patient to patient, the majority improved only slightly and a few had greater effects. The evidence also suggested that the creation of a cavity on the wall of the ventricle could enhance the trophic effects of the grafts. In this review, the authors advocated the use of quantitative objective measures of motor function, in addition to rating scales only; the inclusion of control trials involving comparisons between patients receiving grafts and controls, non-treated groups, or groups receiving other treatments. They concluded that when adrenal medulla grafts survive the functional effects could be the result of release of dopamine, and possibly other substances. When they do not survive, the functional effects must be attributed to the host brain reaction to injury or to the release of chemical substances with trophic effects outlasting their production, or the production of substances by surviving non-chromaffin cells. At that time, postmortem studies in human patients showed lack of tyrosine hydroxylase (TH) positive cells (tyrosine hydroxylase is the rate-limiting enzyme in the synthesis of dopamine and norepinephrine) but there was not enough data to correlate the clinical outcome to the presence or absence of TH positive cells. In a later review of the field, Freed (1993) addressed the results of fetal mesencephalic transplants in human patients with Parkinson's disease. These studies reported clinical improvement over the course of several months after transplantation. Early changes were, according to the author, qualitatively similar to those observed after adrenal grafts. Positron emission tomography (PET) studies indicated increased 6-[ $^{18}\text{F}$ ]-fluoro-L-dopa (FD) uptake in the region of the graft. FD is a marker that allows monitoring of the uptake and decarboxylation of FD to fluorodopamine (FDA), and the subsequent storage of FDA in synaptic vesicles, thus giving an indication of pre-synaptic DA function (Au et al., 2005). At that time, no controlled studies on mesencephalic grafts had taken place. A decade later, Roitberg et al. (2000) reviewed the clinical studies using neural grafting for Parkinson's and

Huntington's disease. At that time more than 150 procedures for fetal transplantation in Parkinson's disease had been reported over the world. The range of clinical benefits was variable, some patients showed improvement in terms of reductions in "off" periods, reduction of medication, increase in FD uptake in the grafted areas, improvement in activities of daily living scores, while others showed no improvement, and some patients developed dyskinesia contralateral to the implanted side. Some degree of clinical improvement had been observed even 60 months after transplantation. In terms of anatomical characteristics, the authors indicated that TH positive neurons were identified in the graft of a patient that died 18 months postoperatively from an event unrelated to the surgery. Electron microscopic studies indicated synapses between transplanted TH cells and host cells in the putamen.

Several technical improvements were made in the decade between 1990 and 2000 that could account for better outcome of grafting procedures. Among them were a more favorable donor age, considered to be when dopaminergic cells first appear in the subventricular zone just prior to neuritic process extension. Grafts of fetal tissue older than 9 weeks gestational age did not show significant clinical effects or survival of TH positive cells. Another issue was time between obtaining the tissue and grafting, studies showing clinical benefits used tissue within 24-48 h of tissue acquisition (Roitberg et al., 2000). An advantage of solid versus suspension grafts was suggested, and the number of transplanted cells was also important. Apparently the greater the number of replacement cells, the better the clinical effect. Another factor was the location of the transplant, whether caudate nucleus or putamen, Roitberg et al., (2000) suggested that the putamen was a better site for implantation, and that greater improvement could be achieved by bilateral transplants. Importantly, transplants of TH+ cells had been shown to extend neuritic processes up to 5-7 mm from the grafted site, although complete reversal of symptoms had not been achieved in spite of this innervation pattern. The need for immunosuppression had not been firmly established.

In the first decade of this century, the results of two double-blind controlled trials were published. The first in 2001 by Freed et al., reported on the results of operations on 40 patients that began in 1995 and ended in 1998, and included a sham group, that later had the option of receiving the graft. The results of the study were rather sobering, motor aspects improved 18% in the transplanted group as a whole, and 34%, if only patients 60 years of age or younger were considered. Rigidity and bradykinesia were reduced in the younger groups, but no improvement on tremor was observed. FD indicated an increase in radionuclide uptake in the putamen of the transplanted group, without changes in the sham group. One 66-year-old patient died of causes unrelated to the surgical procedure seven months after transplantation, and the postmortem analysis of the brain indicated the presence of TH+ cells with outgrowth of 2-3 mm, without Lewy bodies. Postmortem analysis of the brain of another patient (a 68-year-old man who died three years after the transplantation procedure, also from causes unrelated to the surgery) showed surviving dopamine cells in the tissue, with outgrowth that extended the full width of the putamen. PET-FD had shown 100% improvement in uptake over baseline measures. On a three-year follow-up, there was a 38% improvement in the younger group of patients and 14% of older patients, five patients developed dystonia and dyskinesia. The authors concluded that their results were similar to those of open studies. In 2003 the results of another double-blind controlled study were reported (Olanow et al., 2003) with similar results. Comparisons

between placebo and transplanted groups were not significant even though striatal FD uptake was significantly increased in the transplanted groups, and survival of dopamine neurons was observed at postmortem examination of five patients who died during the study from causes unrelated to the transplant. Number of surviving TH+ cells ranged from 30,000-120,000 per side depending if the patient had received a one-donor or a four-donor graft. Activated microglia and immune reactivity was observed in the transplanted group in and around the region of the graft. In addition 56% of transplanted patients developed dyskinesia. The authors concluded that fetal nigral transplantation was not recommended at that time. Goetz et al. (2005) reached a similar conclusion after an evidence-based medical review update on the various treatments for PD from 2001-2004. On the basis of their analyses, the authors concluded that fetal mesencephalic grafts were non-efficacious meaning that evidence showed that the intervention did not have a positive side effect on studied outcomes.

In 2005 Piccini et al., addressed some of the issues pertaining to the clinical outcome after neural transplantation in PD. On the basis of their results with nine patients from a full series of fourteen grafted PD patients, they suggested that clinical outcome is best when more than 100,000 DA neurons are grafted and at least 1/3 to 1/2 of the putamen is reinnervated. This degree of innervation corresponds to 50% of normal FD uptake, although as indicated in the previous studies it does not always match with the level of clinical improvement. This study contributed importantly to the discussion of the mechanisms that underlie the development of dyskinesia, they found no association between graft-induced dyskinesia and DA release under basal or methamphetamine administration. They also concluded that grafts in the putamen do not protect intrinsic DA neurons in the nigra from degenerating, that a better outcome can be found in patients in whom the degeneration of the intrinsic DA system was confined to areas reached by the graft, and that immunosuppression could be stopped without compromising the survival of the grafts or the clinical improvement. From these studies it became clear that the response to the grafts varies between individuals, that factors such as tissue handling and storage, immunosuppressive treatment, and patient selection needs to be improved, thus this type of therapy requires that it be tailored individually to each patient (Winkler et al., 2008).

In a follow-up to the study published by Freed et al. in 2001, Ma et al. (2009) reported the results obtained two and four years after transplantation. Interestingly the motor scores of the transplanted patients improved 25% in the 4-year evaluation in the group as a whole. In addition, differences found previously between younger and older patients, and men and women did not persist over time. Increased FD uptake was found in both hemispheres, and it was correlated to clinical outcome. They also observed that better outcome was associated with the degree of FD uptake present preoperatively in the ventrostrual putamen. In the same region they detected progressive loss over the 4-year period, suggesting ongoing neurodegeneration.

Recently, various studies have addressed the issue of the presence of Lewy bodies, and activated microglia in the grafts of patients that received transplants a decade or more before (Li et al., 2008; Kordower et al., 2008a, 2008b). In these studies it was suggested that the presence of Lewy bodies could indicate that the microenvironment of the host brain may affect DA neurons, or that somehow the pathological process can spread to the grafted cells, although the presence of Lewy bodies may not affect the functional effects of the grafts. Importantly, these studies also found survival of TH+ cells in the transplants.

In contrast, Mendez et al. (2008) after evaluating the histopathological characteristics of 3-4, and 9-14 year-old transplants did not find Lewy bodies in the grafts. In this study, they reported that serotonin cells were present, and they suggested that the presence of this type of cell could be involved in the development of dyskinesia. Cooper et al., (2009) evaluated the histological evidence of the studies dealing with post-mortem cases of patients with neural transplants, and concluded that the presence of Lewy bodies could be related to the type of graft, solid tissue versus cell suspension, the former having resulted in greater microglial activation indicating an increased host inflammatory response that could have contributed to the development of PD-like pathology, and to accelerated aging in some transplants in PD patients. In the view of Cooper et al. (2009) the vast majority of transplanted neurons in the studies reviewed, remained healthy and continued to provide substantial clinical benefits for over a decade. They suggest that future studies should look into the use of pluripotent stem (iPS) cells as a source for donor cells. This technology could improve transplant quality and content, by providing sorted pure DA populations. A similar proposal has been expressed in other reviews on the future of cell and gene therapies for PD (Isacson & Kordower, 2008; Wang et al., 2007; Li et al., 2007; Winkler et al., 2005).

In conclusion, the review of the studies of neural transplants in PD shows that this therapy has to be tailored to each patient, that issues such as the transplantation procedure, i.e., solid tissue grafts versus cell suspension grafts, location of the grafts, tissue handling and storage, age of donor, immunosuppression therapy, and patient selection in terms of age, gender, and degree of degeneration as evaluated by PET-FD uptake have to be taken into consideration. They have also shown that fetal grafts can survive for extended periods, and can improve the clinical status of some of the patients for a considerable length of time. However, it does not appear to be a therapeutic alternative suited for the majority of PD patients. The experience obtained in this regard, needs to be taken into consideration before proceeding to the use of cell transplants in epilepsy. Animal studies using models of epilepsy have provided valuable information about the functional effects of transplants however, a drawback of these studies is the duration of the effects; therefore alternatives such as the use of gene therapy are now being considered (Löscher et al., 2008).

## 5. Closing remarks

Advances in molecular and cell biology have now made it possible to immortalize neural cells, including stem cells, to replace lost cells or replenish the supply of selected molecules (Mejia-Toiber, in press) and have thus provided a new source for neural transplantation, one that could possibly be tailored to each patient. Although unquestionably promising and exciting, there are still some issues to be solved before these cells can be used routinely in the clinic. Several authors have voiced concerns similar to those expressed for transplants using fetal tissue (e.g., Allan et al., 2010; Kim et al., 2004; Li et al., 2008; Lindvall et al., 2004; Muller et al., 2006; Snyder et al., 2004; Storch et al., 2004). We have classified these concerns in two categories. The first one includes the negative effects of transplantation of stem cells into the brain. These include the immune rejection of the transplant, and the effects of prolonged immunosuppression. Other negative effects are the potential for tumor formation after differentiated ES are transplanted resulting either from residual proliferating ES cells or precursors, or by the epigenetic changes resulting from their manipulation and the



presence of retroviral vectors. In the case of cell transplantation in PD there is also the concern for graft-induced dyskinesia (Allan et al., 2010).

The second category of concerns includes the need to tailor cell therapy to the pathology of interest. It must be considered if it would be preferable to transplant a homogeneous population, for example, neural cells, versus transplanting a mixed population including cells of glial lineage in order to favor their integration into the host's nervous system to promote functional recovery. Stem cells can provide the host environment with trophic and neuroprotective support to promote the recovery of endogenous cells, to mobilize host progenitors, and to favor inherent neurogenetic programs within the host, in addition to replacing host cells (Snyder et al., 2004). Furthermore, the reciprocal stem cell-host interaction needs to be taken into consideration, because the host environment may affect stem cell behavior by exposing these cells to factors related to the brain pathology of the host that may confer an invasive phenotype (Muller et al., 2006). Indeed, NSC and BMC have been shown to migrate to localized and also widespread lesions after transplantation (e.g., Costa-Ferre, 2010; Chu et al., 2004); at least three processes seem to influence this migratory behavior: inflammation, reactive astrogliosis, and angiogenesis (Muller et al., 2006).

Undoubtedly, all these developments from adrenal to fetal grafts, to the generation of NSC with specific characteristics have increased our knowledge about the neurologic disorders themselves, about the potential for regeneration and restoration of function that the brain has, about the effects of neurotrophic factors, and about brain development. New technical alternatives have been found, the use of stem cells, the possibility of induced PSC, and gene therapy. One should bear in mind that restoring lost functions by replacing damaged cells is not a trivial process if one considers the intricate relationships that exist among nerve cells, and that complex neural circuits underlie sensory, motor, and cognitive functions. After more than two decades of basic and clinical work in neural transplantation, it has become evident that in order to ensure long-term effects neural transplants need to do more than just supply the molecule of interest. However, the positive results obtained in the past together with the recent technical advances indicate that in the future we can expect that therapeutic alternatives will be available in the clinical setting to improve the quality of life of human patients.

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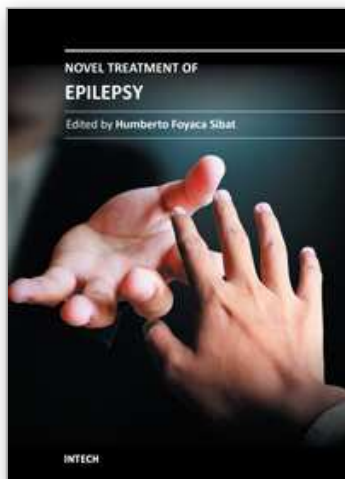


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### **Novel Treatment of Epilepsy**

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Epilepsy continues to be a major health problem throughout the planet, affecting millions of people, mainly in developing countries where parasitic zoonoses are more common and cysticercosis, as a leading cause, is endemic. There is epidemiological evidence for an increasing prevalence of epilepsy throughout the world, and evidence of increasing morbidity and mortality in many countries as a consequence of higher incidence of infectious diseases, head injury and stroke. We decided to edit this book because we identified another way to approach this problem, covering aspects of the treatment of epilepsy based on the most recent technological results “in vitro” from developed countries, and the basic treatment of epilepsy at the primary care level in rural areas of South Africa. Therefore, apart from the classic issues that cannot be missing in any book about epilepsy, we introduced novel aspects related with epilepsy and neurocysticercosis, as a leading cause of epilepsy in developing countries. Many experts from the field of epilepsy worked hard on this publication to provide valuable updated information about the treatment of epilepsy and other related problems.

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