Parkinson’s Disease:  
Part III. Surgical and Emerging Therapies

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For the past 50 years, enthusiasm has remained high that parkinsonism is a treatable disorder, whether by surgical or medical means. The development of varied surgical approaches over the years has proved only partially successful in palliating the common symptoms and motor complications of Parkinson’s disease, yet these efforts helped to highlight the basal ganglia as the primary region of disease pathology. Surgery targeting the basal ganglia remains highly successful in reducing motor deficits in patients. Deep brain stimulation targets the same regions using high-frequency electrical impulses to control tremor. Newer emerging treatments, such as gene therapy and restorative cell approaches, seek to correct dopamine deficits and to restore diseased neurons.  

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This article reviews the development of surgical treatments for PD and explains how their results helped to illuminate the functions and interactions of the various anatomical structures involved in PD.

Early Surgical Treatments
Alleviated Only Some Parkinson’s Symptoms

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The history of surgical treatments for movement disorders, in which a great many targets were initially chosen, serves to reinforce the fact that Parkinson’s disease is not an isolated problem of the substantia nigra, as is often assumed, but rather PD involves the gradual and progressive impairment of a neural network composed of the basal ganglia, motor cortex, brainstem, and cerebellum.

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Various operations have been developed historically for the treatment of Parkinson's disease. In the 1950s, Cooper noted that "operations on the cortex and pyramidal tract never relieve rigidity, incapacitation, or various other symptoms of parkinsonism. ... [Performed] solely to relieve tremor, they succeed in this respect only at the expense of motor power." Conversely, operations targeting the basal ganglia are capable of alleviating most of the cardinal features of parkinsonism. Currently, only operations on the basal ganglia are performed for the treatment of PD, although new approaches, such as deep brain stimulation or cell transplants, may eventually target multiple components of the motor system.
Basal ganglia dysfunction in Parkinson’s disease and treatment targets. In PD, dopamine depletion in the striatum leads to disinhibition of D2-receptor-bearing neurons in the indirect pathway, producing increased inhibition of the globus pallidus pars externa (GPe) and disinhibition of the subthalamic nucleus (STN). The resulting overactivity in STN neurons leads to excessive excitation of the globus pallidus pars interna (GPI)/substantia nigra pars reticulata (SNr) and overinhibition of the thalamocortical and brainstem motor centers.

Various surgical and emerging therapies target different structures in this network. Cell transplants and deep brain stimulation may target other structures in addition to those shown in this figure. (SNC, substantia nigra pars compacta; VL, ventrolateral thalamic nucleus.)

The father of basal ganglia surgery is widely considered to be Dr. Russell Meyers, an American neurosurgeon, who in the 1940s began to develop a number of surgical approaches to parkinsonism. These included resection of the head of the caudate nucleus, resection of the caudate with interruption of the anterior limb of the internal capsule, resection of the caudate with the anterior one-third of the globus pallidus, and putamen, and resection of the pallidofugal fibers emerging from the medial globus pallidus.

Only the last approach is still in use today, as it was found that resection of the caudate does not alleviate either tremor or rigidity.

Drs. Ernst Spiegel and Henry Wyss, at Temple University in Philadelphia, introduced their “stereoecephalotome” in 1947, and then in 1964, they reported six cases of lesioning of the pallido-fugal fibers using stereotactic electrolytic lesions. This operation, when done on the ansa lenticularis (“ansotomy”), was able to reduce contralateral tremor and rigidity without causing paralysis.

At the same time, Dr. Irving Cooper, at St. Barnabas Hospital in New York, pioneered chemi-pallidectomy, which he found to produce good results in many patients. The operations by Cooper were done freehand, without a standard stereotactic frame. Feneon and Guiot similarly used freehand techniques to approach the pallidal region or ansa lenticularis.

As new drugs such as levodopa were introduced, these highly invasive procedures could no longer be justified and were essentially abandoned for three decades. Beginning around 1990, a renaissance of pallidotomy was initiated by Finnish neurosurgeon Dr. Lauri Lahtinen and coworkers.
Pallidotomy Involves Selective Ablation of the GPI

Pallidotomy was the first successful surgical approach for PD, starting with the pallidofugal section by Meyers. As outlined in Part II of this series, a loss of nigrostriatal dopaminergic activity in PD produces reduced net inhibition of the globus pallidus pars interna (GPI) through its various inputs. This in turn causes excessive pallidothalamie inhibitory output to the ventral thalamus and impaired activation of the premotor and motor cortex via the thalamocortical pathways.

Pallidotomy not only improves bradykinesia but also can decrease tremor and rigidity and alleviate dopamine-induced dyskinesias.

After successful pallidotomy, PET imaging studies have shown increased activation of the supplementary motor cortex, lateral premotor cortex, and dorsolateral prefrontal cortex.

It should be noted that dampening the inhibitory pallidothalamic output not only improves akinesia but, paradoxically, also helps with the seemingly opposite phenomenon of dyskinesia, suggesting that complex intersecting motor pathways are involved. Further studies in human patients are needed to clarify the functional organization of the GPI and its physiologic influence on other anatomic structures.

Given the somatotopic organization of the globus pallidus, putamen, internal capsule, and other structures that lie in close proxim-
The thalamus is a relay and signal-processing center, subserving both sensory and motor functions. Appropriate to its position at the center of the brain, its name comes from the Greek word for "vault" or "inner sanctum." The right thalamic hemisphere (shown here) is linked to the left thalamus by the intrathalamic adhesion.

The relevant portion of the thalamus in PD is the ventrolateral (VL) region. This is further subdivided into the Voa/Vop (anterior) and Vim (posterior) regions. [LD, lateral dorsal; LP, lateral posterior; VA, ventroanterior; VP, ventroposterior.]

The Swedish neurosurgeon Dr. Lars Leksell pioneered anterodorsal GPI pallidotomy starting in 1952, but he later altered the target to the posteroventral GPI (i.e., close to the origination of the ansa lenticularis) after noting better results. Within the posteroventral GPI, anteromedial lesions appear to provide greater improvement in off-medication rigidity and on-medication dyskinesia, central lesions allow greater improvement in akinesia and postural instability/gait, and posterolateral lesions allow greater tremor relief.

Thus, the precise site to be targeted may depend in part on the symptoms found in a particular patient. In recent years, most GPI lesioning has been in the posterolateral GPI. These pallidal lesions can improve akinesia, motor fluctuations (reduction of "off" periods), tremor, and dyskinesia/dystonia, but are not very helpful with postural symptoms.

Complications of pallidotomy arise in part from the close proximity of the GPi to the internal capsule and optic radiations, which are critically important in motor control and vision. Improvements in stereotactic guidance make these surgeries quite safe, but possible complications still include contralateral (to the surgical lesion) motor deficits, visual field cuts, worsening of dysarthria/dysphagia, and cognitive impairment.

Another major limitation of pallidotomy is that, in general, lesioning has been done on one side of the brain only, to correct the side of the body most severely affected (i.e., contralateral to the lesion). When performed bilaterally, these operations are frequently staged rather than simultaneous in order to avoid complications in speech, swallowing, and cognitive function, which is also true with bilateral thalamic lesioning.

Thalamotomy was the next important basal ganglia surgery to be adopted, after autopsies showed that some surgeries that had targeted the globus pallidus had in fact impinged on the thalamus. As it turns out, freehand chemopallidectomy operations by Cooper and others were often incorrectly targeted, which is not surprising in the absence of stereotactic guidance; some of the best results for tremor relief but worst results for akinesia relief in fact were found on autopsies to be thalamic lesions.

Cooper was keenly aware that the anterior choroidal artery feeds both the GPi and lateral thalamus, and he used ligation of this artery to introduce deliberate GPI infarcts before inventing his various chemopallidectomy procedures. The original observation of beneficial effects on tremor following anterior choroidal artery ligation was actually incidental, seen in a parkinsonian patient in whom the artery was ligated to prevent hemorrhage during another procedure. Cooper detailed this case report in the journal Science in 1953.

Based on the classical model of basal ganglia organization, lesioning of the thalamus would be expected to exacerbate symptoms of akinesia and rigidity. In fact, Laitinen observed that pallidotomy lessens akinesia, while thalamotomy appears to worsen it. Both operations can significantly decrease tremor and rigidity, but only pallidotomy improves akinesia.

Thalamic lesions have differing effects depending on their location. Lesioning in areas that receive basal ganglia input (Voa, Vop) may alleviate rigidity and dyskinesias, whereas lesioning in regions with cerebellar inputs (Vim) are more effective against tremor. Thalamotomy usually targets the Vim for relief of tremor, whereas more anterior targeting (Voa/Vop) may be used to alleviate dyskinesias.
Common complications of thalamic surgery include weakness or hemiplegia, aphasia, dysarthria, ataxia, and worsening of bradykinesia. As with pallidotomy, bilateral surgeries have a much higher complication rate.

In view of the relative strengths and weaknesses of the various ablative surgeries, isolated tremor is most effectively treated by ablation of the Vim nucleus of the thalamus, whereas akinesia is most effectively treated with pallidotomy. Depending on the chief complaints of a given patient, it may be possible to choose a surgical approach rationally.

**“Brain Pacemakers” Stimulate Basal Ganglia Structures**

The most promising modern surgeries are not ablative at all but instead involve deep brain stimulation (DBS), or the implantation of high-frequency stimulators to basal ganglia structures. These devices are commonly termed “brain pacemakers.”

Implantable biostimulation was originally discovered in the context of real-time electrophysiologic recording (microweighting), which was performed to help pinpoint the site of lesioning electrodes (e.g., in the Vim nucleus of the thalamus) in ablative procedures. During electrode localization, it was found incidentally that high-frequency stimulation could reduce tremor.

DBS of the thalamus (Vim) was first introduced by the French neurosurgeon Dr. Alim Benabid in the 1980s and appears to be as effective as lesioning in the treatment of tremor. DBS also has been used in the Gpi in lieu of pallidotomy or as part of a staged operation in patients with a unilateral palilidotomy who develop symptoms on the other side.

Another important surgical target for DBS is the subthalamic nucleus (STN), which was considered a suitable target after primate studies showed that it decreased parkinsonian symptoms after unilateral ablation, a procedure not done in many patients due to the serious complication of hemiballismus. Now that DBS is commonplace, the STN is actually the preferred site for bilateral DBS.

One major question with DBS is that despite the excellent clinical results, its precise mechanisms remain to be elucidated. Thalamic DBS for tremor has an optimal frequency of 100 to 350 Hz, and slower frequencies (<100 Hz) actually can potentiate tremor. The effects of DBS on tremor are immediate and dramatic, and therefore they do not appear to depend on gene expression.

It is still unclear if the beneficial effects of DBS in the thalamus are due to activation of certain Vim neurons or inhibition of efferent neurons through connections with other structures. In the STN, high-frequency DBS has the same effect as lesioning, and its effects can be assumed to be due to depolarization block and/or disruption of the network connections mediated by the STN. The effects could be partially mediated through STN connections with the Gpi, Gpe, cortex, brainstem, or other regions.

For DBS, anatomic targeting using preoperative MRI and assignment of stereotactic coordinates is used first to approximate the electrode position. Because substantial error may occur when targeting solely with stereotactic coordinates, microweighting of electrical potentials in different brain regions is routinely used for real-time electrophysiologic confirmation. Each basal ganglia region has a characteristic pattern of neuronal firing, and burst firing of neurons can be visually monitored on an oscilloscope and with audio as the various brain layers are penetrated.

So-called macrostimulation of the electrode targeting site can be useful in defining the functional result, such as demonstrating an intraoperative reduction of tremor on Vim macrostimulation. The

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In deep brain stimulation, an electrical stimulator implanted under the skin of the chest sends high-frequency electrical impulses through electrodes implanted in the globus pallidus or STN. The electrical stimulation of the GPi effectively mimics the effects of pallidotomy. Electrodes may be introduced unilaterally or bilaterally. The DBS electrode contains contacts (white rectangles), which following implantation in the globus pallidus, are aligned to provide optimal therapeutic benefit.

The main advantages of microelectrode neuronal recording, as opposed to macro- or micro-stimulation, is that it is not as dependent on patient cooperation, and it is able to precisely map somatotopy, or the anatomic structure-function. The main limitation is the slower speed at which information is acquired and the high cost.

Small variations in DBS electrode targeting are very important. For example, DBS of the GPi region currently favored for pallidotomy (posteroventral GPi) may reduce rigidity and dyskinesias but exacerbate symptoms such as akinesia and gait disturbance. A slightly more dorsoventral target in the GPi may alleviate more symptoms (e.g., gait, akinesia, rigidity) but may induce abnormal involuntary movements. These kinds of intraoperative observations have added to our knowledge of the somatotopic organization of the GPi.

DBS of the GPi has been demonstrated to help with most late-stage symptoms of PD, including tremor, rigidity, akinesia, and dyskinesias. Results of a multicenter GPi-DBS study with a double-blind crossover design were reported in the New England Journal of Medicine in 2001. The United Parkinson's Disability Rating Scale (UPDRS) score was found to be improved 37% when the stimulator was functioning as compared to when it was not. Moreover, dyskinesias were improved, “on” time was improved, and both patient and physician impressions of disability improved.

Studies by Benabid's group in Grenoble have reported significant benefits with DBS targets in the ventroposterolateral GPi or STN, both unilaterally and bilaterally. Following FDA approval of DBS devices for use in the STN in January 2002, a study done in Philadelphia reported 47% improvement in the UPDRS motor score at 12 months with DBS stimulation. Improvements were seen in dyskinesias, motor fluctuations, and "on/off" duration.

Connectivity of the STN is quite complex, and DBS may act in part through STN connections with the brainstem, especially the pedunculopontine nucleus. It has been proposed that the interconnectivity of the STN is what prevents the emergence of dyskinesias following STN ablation or stimulation; the fact that there are projections to
the GPe as well as the GPI may partially dampen the loosening of inhibitory output from GPI to the thalamus in DBS. It is clear that network connections are important, and there appear to be multiple processing loops for motor outputs.

In general, the GPI and STN studies have shown similar results, and it may be that the effects of DBS in these regions are not immensely different. Both GPI and STN stimulation are able to improve tremor, rigidity, akinesia, and dyskinesia. Other considerations, such as complication rates and cost, may prove to be deciding factors in choosing the site of DBS implantation.

At this time, bilateral STN targeting with DBS is generally the option of choice, given its significant effects on akinesia and rigidity, which are the most dopamine-dependent symptoms. Bilateral STN stimulation is particularly effective against tremor and dopamine-induced dyskinesia, a common result of long-term L-dopa therapy.

When compared to ablative procedures, DBS holds many advantages. Although pallidotomy is also effective against tremor and L-dopa-induced dyskinesia, it is less effective for rigidity and akinesia. Likewise, thalamotomy procedures are effective for tremor but far less so for other symptoms. The main drawbacks of DBS include the use of an indwelling device that may be prone to malfunction, infection, or breakage, and the fact that the subcutaneously implanted batteries must be replaced periodically.

The frequency of electrical stimulation used in DBS varies with the target site chosen. It remains to be seen if combinations of electrodes in different brain regions, perhaps operating at different frequencies, could correct some of the system-level problems in the basal ganglia and perhaps even retard the progress of neurodegeneration. For example, if electrical stimulation could be targeted to both the thalamus and STN or GPI, then integrative circuits might be affected. Other targets such as the GPe or cortex also could be explored in the future. It remains to be seen if surgical and technical considerations will make this “re-wiring” of the brain unrealistic, and how it might be combined with other emerging treatment strategies.

For patients who cannot tolerate DBS (typically a very long procedure) for other health reasons, surgical options such as stereotactic radiosurgery ablation may be possible. This relatively noninvasive procedure has afforded acceptable results in thalamotomy for relief of tremor but disappointing results in the GPI for relief of rigidity, tremor, and akinesia. In fact, half of patients given radiosurgical pallidotomy in one series got worse, and only one third showed any improvement.

In patients in whom open surgery presents an unacceptable surgical risk, it may be possible to do a radiosurgical Vim thalamotomy, but the instances are very limited. One obvious drawback of radiosurgery is that electrophysiologic testing cannot be done at the time of surgery to confirm location, and the radiation may also produce complications.

Gene Therapy Has Targeted Dopamine Synthesis and Neurotrophic Factors

Gene transfer approaches to treat PD have existed for over a decade. Preliminary work in animal models strongly suggests that high levels of gene expression can be obtained in the substantia nigra pars compacta (SNc), striatum, STN, and other brain areas using techniques of viral-mediated gene transfer (e.g., using lentivirus or adenovirus vectors). However, these approaches are unproven in humans.

In some ways, PD is a better target than Alzheimer’s disease for gene therapy, because the pathology is less diffuse in early stages of the disease, and if initiated in time, gene transfer could offer substan-
Gene transfer may be performed using two basic procedures: In 'ex vivo' gene therapy, the relevant genes are introduced to cells which are then cultured and implanted into the brain. In 'in vivo' gene therapy, the desired genes are transferred to a vector, such as a viral capsid or analogous lipid-protein coat, which then is implanted into relevant regions of the brain. The use of native brain cells may be an advantage or a disadvantage, depending on the extent of pathology and the potential for regeneration of existing structures versus the need to build new cellular architecture. Because stem cells used in ex vivo approaches appear to have some ability to migrate to areas of injury, targeting presents less of an issue and stereotactic guidance may not be necessary.

In most cases, though, diagnosis occurs only after the death of more than 50% of the SNc dopaminergic neurons, by which time the effects may be widespread and irreversible. Clearly, replacing or introducing additional copies of relevant genes through gene therapy may not be a viable approach unless the recipient cells are still viable in sufficient numbers.

Most published gene therapy experiments in animal models of PD have focused on replacement of dopamine biosynthetic enzymes or the addition of neurotrophic factors to the nigrostriatal system for the protection and restoration of dopaminergic neurons. Initial studies into the replacement of dopamine focused on transfer of the gene encoding the l-dopa-synthesizing enzyme tyrosine hydroxylase (TH). Experiments in animal PD models, using either implantation of cells engineered ex vivo to produce l-dopa or in vivo techniques involving direct injection of TH-expressing vectors into the striatum, showed only partial improvement in a behavioral measure in rats.

To improve levels of dopamine production in the transduced striatum, researchers found it necessary to combine TH with the cofactor tetrahydrobiopterin (BH4), either by exogenous administration or by coexpression of GTP-cyclohydrolase-1 (GCH1), a rate-limiting enzyme in BH4 synthesis. Other accessory enzymes, such as amino acid decarboxylase (AADC), also have been delivered with TH, but the incremental effect is negligible.

Despite sustained gene expression, the functional effects of TH gene delivery in rat and primate PD models have been disappointing, and it is unlikely that gene therapy aimed at replacing dopamine levels alone will be sufficient. The poor efficacy is likely due in part to the continued progression of disease, which robs the striatum of dopaminergic receptors.

A second approach has focused on the transfer of genes expressing neurotrophic or growth factors, which are secreted proteins developmentally expressed in the brain. These factors, which are active at nanomolar to picomolar levels, include neurotrophins, acidic and basic fibroblast growth factor (FGF), brain-derived neurotrophic factor (BDNF), and others which may exhibit neuroprotective and neurorestorative activity.

BDNF is neurotrophic for dopaminergic neurons and is normally...
Imaging Methods Offer the Possibility of Earlier Diagnosis of PD

Improved genetic screening technologies to identify persons at high risk of PD, in conjunction with new methods of noninvasive brain scanning, may help to make the diagnosis of PD earlier, which would improve the chances of successful gene transfer therapy. Currently, PET and SPECT are two techniques that provide information on the integrity of the dopaminergic system, but they are not generally available outside special clinical trials.

PET with $^{18}$F-fluorodopa is especially useful in the assessment of presynaptic dopaminergic neurons in vivo. An alternative is SPECT with presynaptic dopamine transporter ligands, typically cocaine structural analogues known as tropanes. Post-synaptic density can be tested with other compounds, such as PET with $^{11}$C-raclopride or SPECT with $^{123}$I-iodobenzamide (IBZM).

Idiopathic PD demonstrates a loss of presynaptic dopaminergic neurons projecting from the SNc to the striatum, and imaging permits longitudinal assessment of dopaminergic function in the striatogniral system. Also, these imaging techniques allow for a quantitative measure of cell viability in striatal cell grafts, useful for measuring outcomes in clinical trials. These imaging techniques have yet to find a wider clinical use, however, mainly because there are inadequate preliminary screening criteria for asymptomatic people who go on to develop PD.

expressed in the human nigrostriatal system, although levels are significantly decreased in the substantia nigra of patients with PD. In rats, results with BDNF have been uneven. BDNF-transfected fibroblasts or astrocytes implanted in the rat striatum resulted in behavioral improvement but with variable results on cell viability. Putative neuroprotective effects of BDNF appear to depend in part on administration prior to insults of dopaminergic neurons, which is not a clinically relevant paradigm.

Glia-derived neurotrophic factor (GDNF), a member of the transforming growth factor-$\beta$ superfamily, has particularly potent effects on dopaminergic nigrostriatal neurons, although it is virtually undetectable in the normal adult human brain. Yet, GDNF has shown efficacy in animal models of PD.

In one trial, GDNF was delivered in vivo via a lentivirus vector to the striatum and substantia nigra of rats. Following injection of 6-OHDA, animals showed protection against dopaminergic neuron loss and improvements in behavioral outcomes compared to control rats.

In another study, Dr. Jeffrey Kordower showed reversal of motor deficits as well as protection against nigrostriatal degeneration following neurosurgical delivery of GDNF-encoding vectors to the striatum and substantia nigra in an MPTP monkey model of PD.

However, until the majority of genes involved in idiopathic PD are known, many experts believe that clinical gene transfer is premature. Approaches aimed at reconstituting the dopaminergic biosynthetic pathway originally showed promis-
Viral gene transfer vectors now can be engineered to contain promoter elements that allow gene expression to be regulated by orally administered drugs, thus offering the possibility to tightly regulate expression of genes such as GDNF that otherwise could prove to be toxic in high amounts. The problem remains that, as with standard drugs, most current gene therapy strategies tend to attack the symptoms rather than the genetic defects themselves.

The technology to cheaply sequence the entire human genome on a large-scale basis will probably exist within the next decade, and eventually it may be possible for a patient with a significant genetic risk for PD to decide with a physician which genes will be administered to ward off the disease (e.g., “preemptive” administration of neurotrophic factors).

At present, however, knowledge of the genetic and biochemical pathways in PD is still piecemeal, despite the recent identification of a number of susceptibility genes. Unlike genes that actually may be causative in PD, many genes probably fall into a category of “downstream” genes that are perturbed as a secondary phenomenon as neurons respond to insults of one type or another.

Based on the importance of dopaminergic, GABAergic, and glutamatergic signaling in the basal ganglia, the genes that control the biosynthesis of neurotransmitters or their receptors have received attention in examining the pathogenesis of PD. Although gene transfer aimed at basal ganglia neurotransmitters and their receptors or transporters may not prove to have a therapeutic application, studies in animal models can clarify the physiologic roles of the different biochemical pathways.

One such pathway is the conversion of glutamate to GABA, a single-step enzymatic process catalyzed by glutamate decarboxylase (GAD). Because glutamate and GABA have essentially opposite effects in the basal ganglia, a relative shift in expression of the genes involved in this pathway could have major effects on neurotransmission.

Scientists at the Parkinson’s Disease Society Brain Bank in London have shown that in PD, GAD gene expression is decreased by as much as 50% in the GPi, which ordinarily inhibits the STN via direct GABAergic projections. The relative lack of GAD enzyme may account in part for dysregulation of the GPi/STN circuit in the basal ganglia in PD, and so recent gene transfer strategies could target GAD gene transfer to the GPi or directly to the STN to offset the deficiency of gene expression.

Another target for gene modulation is the GPi, where excess GABA could be converted to glutamate through upregulation of the gene for glutamate synthetase (though glutamate-mediated excitotoxicity is a limiting factor). In the thalamus, genes for glutamate or GABA receptors may be altered to test the effects of a relative imbalance.

In view of the fact that other surgical procedures already offer excellent results, the effects of gene transfer will need to be substantial in order to justify the risk of any such procedure. Much work remains to be done in understanding the complex interactions among genetic factors implicated in PD before gene therapy can live up to the promise offered by new vectors and targeting techniques.

Cell Therapy Is a Paradigm for Regenerative Medicine

Cell therapy for PD holds great promise but has not yet yielded consistent results. For many reasons, PD lends itself to a strategy of augmenting dopaminergic neurotransmission through simple cellu-
lar replacement. Major unresolved technical issues include the source of cells (e.g., fetal neural stem cells, fetal mesencephalic tissue, embryonic stem cells, other sources) and how the cells should be prepared (e.g., growth factor co-administration, genetic manipulation to secrete growth factors) and regulated in situ.

The proximal cause of PD is thought to be loss of neurons in the nigrostriatal pathway, and this loss is already severe in most cases by the time the disease is diagnosed. It is appealing to consider the possibility of replacing lost cells with new neurons, which would secrete neurotransmitters as well as integrate functionally as neurons.

For much of the past century, the prevailing thinking has been that once lost, neurons could never be regained. In the last decade, a major shift in thinking took place with the discovery that neurogenesis appears to occur to a limited extent in the brain as a natural phenomenon, and stem cells of various derivations can be treated to produce functional, post-mitotic neurons.

Despite some basic knowledge about the genes and proteins that are implicated in this process of cellular differentiation, many details remain unknown. At this time, the key issues for therapeutic cellular delivery are twofold: (1) obtaining the ideal, physiologic

**Sources of neural stem cells.** An activated human oocyte may be derived from any patient, using somatic cell nuclear transfer to a generic enucleated oocyte. The developing blastocyst consists of inner cell mass (ICM), blastocoeel, and trophoblast. The ICM is harvested and blastocyst-derived stem (BDS) cells are generated on a feeder cell layer or bioscaffolding. These cells are expanded in culture and treated with protein differentiation factors and gene vectors as needed to obtain lineage-specific stem cells for ex vivo cell/gene therapy and neurosurgical implantation in the brain. Other possible sources of stem cells include fetal brain neural stem cells or adult mesenchymal stem cells, but these will not be genetically identical to the host. In cases where an underlying genetic defect is strongly implicated in PD, it may be preferable for a blastocyst or blastocyst-derived stem cells to be generated from donor sources.
Dopaminergic cells in the substantia nigra decline in number with advancing age. In PD, the rate of cell death is accelerated or begins earlier than in normal life until the presymptomatic phase is passed and the parkinsonian range is attained (roughly two-thirds cell death).

cells for neuronal replacement in sufficient quantities; and (2) putting the cells in the proper place and regulating their development.

Fetal mesencephalic tissue transplantation for PD has had a long history that began in the 1980s, when it was shown that these cells could be harvested and maintained in culture and then functionally integrated into the substantia nigra or striatum in parkinsonian animals. The idea with fetal cell transplants has been to replicate the dopaminergic environment that would be present if cell loss had not occurred, without introducing nonphysiologic and detrimental influences at the same time.

Apart from fetal mesencephalic cells, a few other cell derivations have been used clinically in a limited number of cases (e.g., adrenal medulla cells, porcine xenotransplants), but these other cell derivations were not successful.

Fetal mesencephalic cell transplants to the substantia nigra or striatum are able to survive and do in fact produce dopamine in physiologically relevant amounts. Some patients who received cellular transplants had marked improvement in their symptoms of akinesia, tremor, and gait instability for years following surgery. In particular, gait instability responded remarkably well to cell transplants in certain patients, despite the fact that L-dopa therapy has not shown the same effects.

However, as Dr. Stanley Fahn has discussed following his recent double-blind, placebo-controlled surgical trial, results have been uneven, and some patients have suffered uncontrollable dyskinesias as a result of striatal cell transplants. These dyskinesias can be surgically corrected by ablative procedures, but this complication underscores the importance of developing better cells with built-in regulatory mechanisms.

Perhaps the most promising cell lines for eventual use in transplantation are human embryonic stem cells, which unlike fetal mesencephalic cells can be grown and expanded in culture to provide material for many such procedures. In addition, primordial neural stem cells can be harvested and expanded from specific regions of the fetal or adult brain for transplantation, but this source of material is more difficult to obtain in quantities. Other potential sources of stem cells for clinical use in the brain include mesenchymal stem cells or bone marrow-derived stem cells.
At present, human embryonic stem cell lines are not being actively developed in the United States, because of federal guidelines which prevent scientists from deriving new such cell lines. Ironically, fetal nigral cell transplants using material from aborted fetuses are legal and fetal neural stem cells are also open to investigation, but federal funding for developing new human embryonic stem cell lines from human blastocysts, which could replace the current dependence on fetal tissue, is currently prohibited. Despite the legal and political issues, it is expected that human embryonic or blastocyst-derived stem cells will be widely available in the future and also could be engineered with additional genes for trophic factors, allowing for ex vivo gene therapy (i.e., delivery of genes using a cellular vector) in addition to functional reconstitution of nigrostriatal or other neurons. Because these cells in theory could be engineered to contain gene-regulatory mechanisms, it may be possible to turn on or off the production of dopamine using an inducible/repressible gene promoter, such as the tetracycline-responsive element or rapamycin element.

Using the techniques of somatic cell nuclear transfer, it is anticipated that embryonic stem cells will be custom-derived using an individual patient’s own cell nuclei, thus avoiding potential problems of antigenicity. Based on results of recent animal experiments, stem cells transplanted simultaneously into different regions of the basal ganglia (e.g., substantia nigra and striatum) will probably give optimal results, especially given the importance of the neural network or system approach.

It should be added that important questions of uncontrolled or dysfunctional cell growth will need to be carefully addressed in future clinical trials.

The limited efficacy of both surgical and drug-based treatments in use today is due partly to the fact that Parkinson’s disease has run a large part of its course by the time symptoms become apparent. At clinical presentation, up to 70% or more of the ~500,000 tyrosine hydroxylase-positive cells in the substantia nigra pars compacta (SNc) may be dead, and nondopaminergic systems are also affected, including regions outside the basal ganglia such as the cerebral cortex. The pathology spreads beyond the SNc as the entire motor system is dysregulated. The window for effective therapy may be limited.

As our genetic screening techniques and noninvasive diagnostic tools improve, the major challenge will be to predict the death of neurons before clinical symptoms appear, so that interventions may be tried to prevent continuous loss of cells in the SNc. Once the inevitable aging-related damage has begun, though, future treatments will aim to prevent additional damage through the introduction of appropriate counter-regulatory genes and cellular replacement strategies.

Some treatments to look for in the near future will be new neurosurgical depot drug approaches which will effect continuous dopaminergic stimulation, as well as gene and cell-based approaches that cater to an individual’s underlying risk factors and extent of damage at diagnosis.

As we discover how gene expression changes in different brain regions over the course of disease progression, it may become possible to select anatomic targets depending on the stage at which a patient presents. As more is learned about oscillatory networks in the basal ganglia, new biostimulator approaches may be designed that target different brain regions or several regions simultaneously.

Future genetic discoveries and refinements of gene and cell-based treatments are bound to contribute to our knowledge of PD, and the sanguine predictions of Dr. Cooper and others will finally be realized.