Two types of dopamine neurons, called A9 and A10, can be extracted from fetal brain, he explained. The A10 cells develop into the limbic region of the striatum, including the accumbens.

The A9 cells develop into the motor region of the striatum, including the caudate and putamen, he added. This regional development is partly influenced by the way the fetal brain is organized, with the motor cortex sending axons to the motor striatum but not the limbic striatum.

For right now, that's a long-term goal, and it's probably not going to be immediately realized because of the challenges that still lie ahead of us, said Samuel Pfaff, PhD, who studies the embryonic development of motor neurons at the Salk Institute in La Jolla, CA.

Nonetheless, he said, If we can understand the tricks embryos use to create motor neurons, that might have a direct application in converting stem cells to motor neurons for treatment. Research by his group and others has shown that the notochord provides key triggers for motor neuron development, in the form of the secreted glycoprotein sonic hedgehog (Shh).

For amyotrophic lateral sclerosis (ALS), the almost complete absence of other treatments makes optimism about stem cell therapy understandable. But replacement of motor neurons also poses one of the biggest challenges for stem cell therapy. Not only must cells be transplanted and survive, but they also must grow out and form functional synapses at precise targets. Even the most optimistic proponents recognize the enormous challenges in developing that kind of therapy.

For Parkinson disease, the challenges are even larger. Motor neurons will need to be wired up correctly to become functional, and we're going to have to understand that process in more detail.

The future holds even larger challenges, though. Motor neurons extend an axon and form a synaptic connection with muscle, Dr. Pfaff said. We're going to have to find ways to engineer that process, or to allow it to take place. Motor neurons will need to be wired up correctly to become functional, and we're going to have to understand that process in more detail.

The therapeutic prospects of neuronal stem cells for neurologic disorders were discussed by three researchers at a daylong conference during the AAN Annual Meeting in San Diego. This is the second in a series of reports from that conference.

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CHALLENGES FOR PARKINSON DISEASE

Parkinson disease presents another challenge for stem cell therapy. Years of experiments and early clinical trials of fetal dopamine neuron transplantation have provided a prototype for stem cell therapy in PD, according to Ole Isacson, MD, PhD, Associate Professor of Neuroscience at Harvard Medical School. But the difficulties with this prototype may offer sobering lessons for the application of stem cells.

Among the most important lessons is that the exact phenotype of the transplanted cell matters a great deal. Complications from fetal transplants for PD have been partly related to the amount of non-neuronal tissue in the graft, Dr. Isacson said. This is less likely to be a problem with stem cell transplants, which are derived from purer cell cultures. However, obtaining exactly the right kind of dopamine neuron may pose greater challenges, he added.

Figure. Dr. Ole Isacson said that years of experiments and early clinical trials of fetal dopamine neuron transplantation have provided a prototype for stem cell therapy in PD.

Figure. Dr. Samuel Pfaff: We now have a good idea how to trigger motor neurons from embryonic stem cells. At the lab bench, we can convert embryonic stem cells into motor neurons. There's no debate about that. But it requires a delicate balance; depending on the concentration, Shh can induce glial cells, motor neurons, or interneurons.

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caudate, and limbic cortex. That kind of dopamine neuron differs in function from the A9 cells that die in PD, Dr. Isacson said.

Dr. Isacson has shown that forty to fifty genes are expressed at significantly different levels in A9 and A10 cells, and some of these seem to account for the difference in vulnerability of the cells. Not all dopaminergic cells are the same, he said.

But experiments in rats show how important A9 cells are. While A10 cells grow away from the motor region of the striatum, A9 cells grow into it. Committed fetal dopamine neurons know their targets in the adult brain, he said. If you just transplant A10, you probably wouldn't get proper reinervation.

That means that if embryonic stem cells are to replace fetal cells in PD, they must be induced to expand and differentiate, he explained, and not just into dopaminergic cells, but specifically A9 cells. Currently several protocols attempt this, but trying to simulate normal development in a dish is difficult, he said.

It's a complicated set of sequences, Dr. Isacson said, adding, however, that it is crucial. Unless you have an A9 dopamine cell that can function, one that can take up and release dopamine, it is a futile effort. I'd rather you give L-dopa to the patient.

**Glioblastoma Therapy**

The first clinical uses of stem cells are likely to be in conditions with the worst prognosis - glioblastomas, for example. Standard treatment is surgical removal, followed by chemotherapy and radiation treatment. Nonetheless, patients usually die within a year from new tumor foci, no more than a centimeter away from what was removed, according to Xandra Breakefield, PhD, of the Neuroscience Center at Massachusetts General Hospital in Boston.

![Image](file:///Users/spohlman/Desktop/Neurology%20Today.htm)  
**Figure.** Dr. Xandra Breakefield focuses on treating glioblastomas by implanting therapeutic cells at the time of surgery.

Her research focuses on treating glioblastomas by implanting therapeutic cells at the time of surgery. The goal is to leave cells behind that can move out and try to target new tumor foci as they form, either to kill them or prevent them from growing.

The neural precursor cells (NPCs) she uses are somewhat differentiated, so they are not quite stem cells anymore. Importantly, though, they migrate specifically to areas of lesions and tumors in the brain.

Dr. Breakefield’s lab is studying the ability of NPCs to seek out glioblastomas in the rat brain. They implant tumor cells on one side of the brain, and NPCs on the other. Within two weeks, the NPCs cross the corpus callosum and infiltrate into the tumor. None remain at the injection site. They definitely have a homing mechanism for tumor foci, she said.

Intraventricular injection, followed by migration to the tumor, can also be successful. Indeed, this is currently the most efficient way to get cells to the tumor. However, at the moment only 5 percent of the injected cells reach the tumor, with many dying in the ventricles.

Having established that the cells would migrate to tumors, Dr. Breakefield and colleagues then armed the cells with a toxic protein. They chose S-TRAIL, a secreted apoptosis-inducing protein that is highly selective for tumors, owing to the S-TRAIL receptor, not found on most normal cells.

They used a glioblastoma line carrying a mutant epidermal growth factor receptor (EGFR), among the most malignant and fastest growing of all glioblastomas. Migrating NPCs invaded the tumor, secreted S-TRAIL, and caused some decrease in growth, but did not actually shrink the tumor. Despite this early disappointment, Dr. Breakefield said, we think this a promising strategy. For now, the researchers are backtracking a bit to assess the strategy in less aggressive tumors.

A key tactic for assessing their success is improvement in imaging modalities. The index of success when I started was survival of the animal, Dr. Breakefield said. This was the ultimate black box, and it was frustrating, because you couldn’t monitor the growth of the tumor.

By introducing a luciferase gene from a soft coral known as Renilla, it has been possible to track cells, although the signal was so weak that one hundred thousand cells were needed in a defined area to find them. Switching to a luciferase from another species improved resolution to ten thousand cells. When combined with a second, biotin-based label that can bind a variety of different agents, they can see as few as 100 cells. This will expand our ability to track both tumor cells and neural precursor cells, she said.

She noted that a lot of people are anxious to move to clinical trials. There are several immortalized human stem cells lines that are already clinical grade. Because they are immortalized, they have the potential to form tumors, but for a patient otherwise facing only a year of survival, that may not be so much of a concern, Dr. Breakefield said. Other types of stem cells are also being prepared for clinical use.

Ultimately it would be wonderful if we could enlist the patient’s own endogenous human stem cells, and modify them on site so they could migrate into the tumor and secrete a therapeutic protein, Dr. Breakefield said, but that’s in the future.

Stem cells can potentially be armed with a wide variety of other therapeutic molecules. The trick is to have them secrete something that is toxic to tumor cells, but not to themselves, such as S-TRAIL. Dr. Breakefield said. Pro-drug activating enzymes, cytokines that elicit an immune response to the tumor cells, other apoptosis inducers, and anti-angiogenic factors are all possibilities, and most of these have been at least preliminarily studied. And there’s no reason we can’t arm these cells with multiple agents, she said, as is done with viral vectors.

The next (and final article) in the reports from the AAN stem cell conference will focus on the politics, business, and ethics of stem cell therapy.