Chapter 5
Can We Use Human Embryonic Stem Cells to Treat Brain and Spinal Cord Injury and Disease?

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Abstract The potential use of human embryonic stem cells for the treatment of neurological disease and injury is discussed from the perspectives of two common disease scenarios. Spinal cord injury and diseases such as multiple sclerosis that affect specific cell types in the spinal cord represent a substantial proportion of all neuropathologies and are among the most heavily targeted by efforts to establish stem cell-based replacement therapies. Parkinson's disease selectively destroys a single type of neuron in a restricted region of the brain. For this reason it was the first neurological disease for which cell replacement therapy was attempted in humans and is considered one of the most amenable to treatment using stem cells. Although the replacement of a single cell type or the repair of a restricted lesion would appear to be relatively straightforward, several issues conspire to make stem cell-based replacement therapy in the brain and spinal cord substantially more challenging. These include the inherent complexity of neural circuits, the problems of ensuring the survival of stem cells and their derivatives after implantation and directing their differentiation into the appropriate cell types, and the increased refractoriness of chronic injury to treatment due to changes in the cellular environment. A layman's guide to the composition of brain and spinal cord tissue is provided, and an update of recent advances in basic neuroscience and stem cell research with relevance to these issues is presented.

Keywords Amyotrophic lateral sclerosis, demyelinating diseases, motoneuron diseases, multiple sclerosis, Parkinson's disease

5.1 Introduction

A highly profiled potential arena for the clinical use of stem cells is the treatment of neurological disease. This is because tissue regeneration is particularly limited in the nervous system, and loss of neurons and nerve fibers due to injury or disease

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can therefore lead to severe and irreversible loss of function. Using stem cells to overcome the inherently limited regenerative capacity of the nervous system would revolutionize neurology, and could bring tremendous benefit to large patient groups suffering from debilitating disorders such as spinal cord injury, demyelinating diseases, movement disorders, Alzheimer's disease, and stroke. But how promising is the stem cell approach? Can we use stem cells to treat any neurological disease, or are there limitations to the capacity of stem cells to recreate the essential neural connections that are lost in these pathologies? If the latter, can we expect to 'push the envelope' sufficiently both in our understanding of the molecular and cellular deficits involved and in our ability to manipulate stem cells so that rational and economically feasible stem cell-based treatments can eventually be designed for neuropathologies? These are pivotal questions on which the future of neural stem cell research hinges. The aim of this article is to provide non-neuroscientists with a perspective on the challenges facing this field, by focusing on the potential application of human embryonic stem cells to the treatment of two specific diseases: spinal cord injury and Parkinson's disease.

The nervous system is extremely complex. Not just in its gross anatomical structure, with a myriad of fiber tracts and highly branched peripheral nerves investing the body with innervation, but also in the degree of cellular interactions on which brain function depends. Each nerve cell, or neuron, is in isolation a powerful analog computational device, so complex that considerable computer power is required to simulate accurately the ability of just one neuron to generate and integrate chemical and electrical signals. The human brain and spinal cord contain on the order of 100 billion neurons and 10–50 times as many glia cells, and many tens or hundreds of thousands of these can be destroyed by a single small lesion. Moreover, neurons come in a bewildering variety of functional types, with characteristic branching shapes, biochemical signalling molecules, and electrical firing patterns. The complexity becomes unfathomable when one considers that each neuron is embedded in highly interconnected neural circuits whose specific connectivity patterns are decisive for proper function. Each neuron can make synaptic connections with hundreds of other neurons and receive connections from thousands or tens of thousands of other neurons. The total number of synaptic connections in the human brain has been estimated at 100–1,000 trillion.

Given this complexity, it might seem preposterous to imagine that injecting stem cells into an injured or diseased brain could reinstate functions that have been lost. But such pessimism is tempered by the fact that the brain's complexity also engenders it with a remarkable plasticity, a capacity to continuously strengthen and weaken, make and break connections and reorganize circuits so that new functions can be acquired. Increasing knowledge is being gained about how this plasticity is regulated and gives rise to memory and the ability to learn. If stem cells could be used to boost the brain's innate plasticity it could augment and accelerate recovery from neurological damage. Moreover, developmental neuroscientists are gaining increasing insight into how the brain's intricate pattern of connections is established to begin with during embryonic, fetal and early postnatal development. Armed with this information, manipulation of stem cells to generate specific neuron types and

form specific synaptic connections is becoming increasingly realistic. Add to this the proposition that in some cases of disease perhaps only a minority of lost connections need to be repaired to recoup function. On this backdrop, and given the imaginative uses of stem cells being pursued, stem cell treatments for damage and disease even of the scope of Alzheimer's and stroke should not be dismissed out of hand.

The use of human embryonic stem cells (hESCs) to treat neurological pathologies is only one of a broad spectrum of potential treatment strategies, all of which are being pursued actively in neuroscience research. It is important to be aware that other approaches may end up being just as powerful as or perhaps even more powerful than using stem cells. A comprehensive account of the issue is beyond the scope of this chapter, but in defining research goals and public policy the use of hESCs must be weighed against at least the following: (1) using exogenous adult (somatic) stem cells, (2) promoting the in situ proliferation and differentiation of endogenous neural stem cells, (3) promoting the inherent capacity for axon regrowth and synaptic plasticity, (4) developing new drugs, (5) developing more effective regimens of training and rehabilitation, (6) developing treatments based on the electrical or chemical stimulation of specific brain structures, such as deep brain stimulation for treating basal ganglia disorders, and (7) brain-machine interfaces and neural prosthetics. One of the great challenges facing modern medicine, in this case neurology, is to ascertain the cost-benefit ratios of the available options for each particular disease.

With respect to stem cells as a source of neurons and glia, both embryonic stem cells and somatic stem cells are being investigated actively world-wide. Embryonic stem cells have the greatest potential, but are subject to both ethical and technical problems. In particular, they pose difficulties with respect to tissue rejection (they are not intrinsically autologous) and tumorigenic potential (which could override the desired direction of differentiation). Somatic stem cells provide a source of autologous, non-tumorigenic cells but may be difficult to generate in sufficient quantities and too restricted in their differentiation potential for all applications. For both sources, highly creative strategies are being pursued to overcome the limitations, such as nuclear reprogramming and altered nuclear transfer.

For a discussion of the different types of stem cells (embryonic, somatic), their potential sources, and strategies for manipulating their properties, see the chapters by Funderud, Borge, Ølstørn et al., and Hurlbut.

5.2 Spinal Cord Injury

Spinal cord injury due to trauma is a leading cause of disability throughout the world, with an incidence of over 10,000 new cases per year in Europe alone. The incidence is unlikely to decline in the near future, as most cases arise in young adults through accidents, in particular traffic accidents. The rising use of automobiles in China and other rapidly developing Asian countries portends a huge number of new

cases in that part of the world during the coming decades. The palliative treatments currently available are costly. In the U.S., the lifetime cost for a single patient with a life expectancy of 60 years after injury has been estimated at 4–12 million dollars, depending on the severity. The economics provide a strong motivation for developing curative treatments among which stem cell strategies are prominent.

5.2.1 What is the Spinal Cord Made of?

The spinal cord is an extension of the brain (Fig. 5.1a) and as such contains the same kind of tissue with the same kinds of cells as does the brain. The general complexity is, however, somewhat lower, in the sense that the spinal cord contains neural circuits with relatively automatic functions that are better understood than many of the higher functions in the brain itself. For example, the spinal cord contains circuits that mediate the many reflexes that are important for reacting properly to the environment (for example the withdrawal reflex, in which you pull away from a painful stimulus, or the stretch reflex, which helps coordinate movements) and for regulating the state of the body (reflexes that control internal organs), as

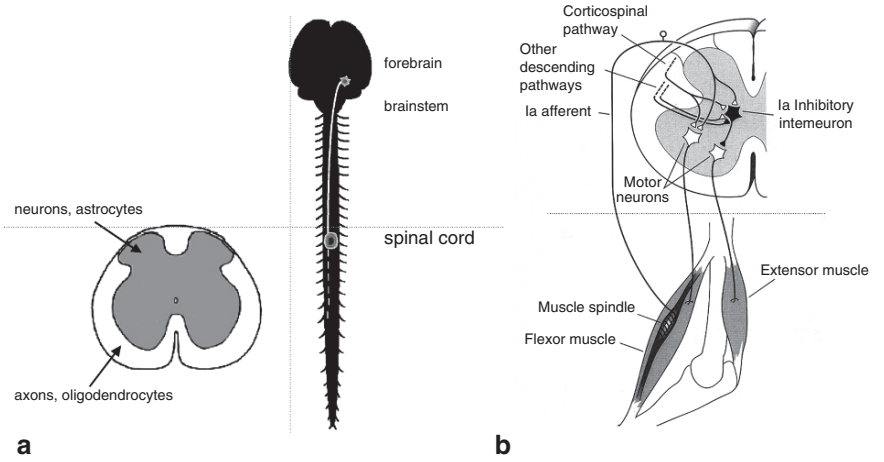

Fig. 5.1 **a.** A schematic view of the brain and spinal cord showing the forebrain (location of premotor neurons in the motor cortex, the so-called 'upper motoneurons'), the brain stem (location of many neuron populations with descending axons to the spinal cord), and the spinal cord (in which an injury affecting a neuron with a descending axon is shown). To the left, a transverse section through the spinal cord showing the core of grey matter (where virtually all neurons and astrocytes are found) and the surrounding white matter (where longtiudinal axons and the oligodendrocytes that invest them with myelin are found). **b**. A highly simplified schematic representation of a spinal reflex circuit that controls limb muscle contraction. Shown are the motoneurons that innervate the muscles, a sensory neuron (Ia afferent) that relays information about muscle contraction back into the spinal cord, and an interneuron and multiple descending synaptic inputs from the brain that regulate the activity of the motoneuron. Panel b from Principles of Neuroscience, 3rd edition, 1991, edited by Kandel et al., Springer, with permission.

well as circuits that generate relatively automatic and repetitive movements, such as walking. But even though the functions subserved are relatively easy to understand, many of the circuits involved are still complex enough to defy adequate description today. Much more research needs to be done before a full account of the types of neurons involved in these spinal cord circuits can be obtained. This is one of the main challenges facing stem cell strategies: not knowing precisely what needs to be replaced after an injury.

The neural circuits in the spinal cord are composed of three basic types of neuron (Fig. 5.1b). *Motor neurons*, sometimes called *motoneurons*, are special because they send their nerve fibers, also known as *axons*, out of the spinal cord to innervate muscles or peripheral neurons in the body. *Interneurons* are interconnected with each other and with motoneurons in such a way that they can drive the motoneurons in particular patterns of activity. For example, the interneurons that are responsible for the walking circuit are connected so that muscles in the two legs are activated in alternation, so that the legs make their steps in alternation. This activity pattern is imposed onto the motoneurons which then transmit it to the muscles (Kiehn 2006). Motoneurons and many interneurons also receive sensory input from *sensory neurons*, whose cell bodies lie outside the spinal cord but whose axons extend into the spinal cord. The signals which the sensory neurons transmit into the spinal cord convey information about what is happening in and around the body so that the activity of the interneurons and motoneurons can be modulated appropriately. For example, during walking, one might trip over a small unevenness in the sidewalk. The disruption of the intended movement is picked up by sensors in the legs and in the organs of balance and the information is transmitted by the sensory neurons to the interneurons and motoneurons in the spinal cord so that they change their activity patterns to compensate for the disturbance. This happens automatically (reflexively) so that the brain itself doesn't have to take conscious action (although the event would certainly be registered consciously as having happened).

Interneurons, like motoneurons, have axons, but unlike motoneuron axons these do not exit the spinal cord (although the axons of some interneurons, called *projection neurons*, extend all the way up into the brain). Some interneuron axons are short, others long, some stay on one side of the spinal cord, others cross to the other side, and some extend up the spinal cord while others extend down. The interneurons can be classified according to where their axons go, which other neurons (interneurons and motoneurons) they connect with, what kind of sensory information they listen to, what kind of intrinsic firing patterns and chemical messengers they employ, and even which genes they express (Goulding et al. 2002; Nissen et al. 2005). We do not yet know how many different types of interneurons exist in the spinal cord, but a conservative estimate based on the attributes listed above would be on the order of several tens to a hundred. These are distributed differentially within the spinal cord in an overall anatomical pattern that is still only partially understood (Goulding et al. 2002; Nissen et al. 2005). Nevertheless, the locations of certain circuits have been pinpointed, such that we know for example that the circuit that controls walking is located in the upper lumber segments of the spinal cord, even though we don't know exactly which interneurons make up that circuit (Kiehn 2006).

Motoneurons are functionally a much more homogeneous population of neurons than the interneurons, but they also differ according to whether they innervate muscles or peripheral neurons, and also according to which muscles they innervate. Their distribution is quite well characterized, so we know where the motoneurons that innervate a given muscle are located along the length of the spinal cord.

In addition to motoneurons and interneurons, another class of cells, the *glial cells,* are present in the spinal cord (Fig. 5.2). Glial cells have for many years been considered to provide structural and metabolic support for neurons, but more recent research indicates that some of them also send signals among themselves and in a limited fashion also to neurons, so the spectrum of functional roles these cells possess is being extended. There are three basic types of glial cells, each of which has specific, well-known functions. *Astrocytes* regulate the cellular environment around the nerve cells and contribute to the blood-brain barrier, *oligodendrocytes* form the electrical insulation called *myelin* around nerve fibers, and *microglia* participate in inflammatory reactions.

If one cuts transversely through the spinal cord and looks at the cut end, one sees a fundamental anatomical feature that is highly relevant for injury and disease scenarios, namely that there is a central area of slightly darker tissue that is surrounded by a lighter rind (Fig. 5.1a). The central core is called the grey matter and is where nearly all of the neurons and astrocytes are located, and the lighter, outer rind is called the white matter and is where most of the nerve fibers are located, including all the nerve fibers that carry information from the brain to the spinal cord and vice versa. The whiteness of the white matter is due to the high fat content of the myelin insulation around the nerve fibers. Because of this organization, trauma or disease that is limited to the white matter will affect primarily nerve fibers, whereas any insult to the grey matter will affect the neurons. Some injuries and diseases will of course impact on both.

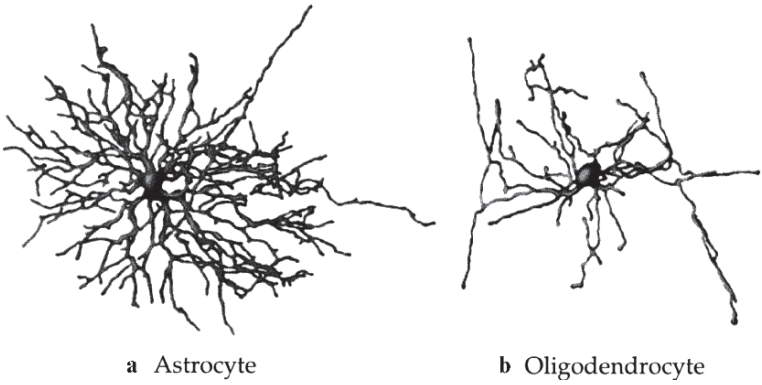

Fig. 5.2 The astrocytes and oligodendrocytes of the brain and spinal cord, revealed by immunohistochemical procedures that label the two glial cell types differentially. From 'Neuroscience', 1st edition, 1997, edited by Purves et al., Sinauer, with permission.

There are on the order of 100 to a few thousand motoneurons innervating each muscle in the body, depending among other things on the size of the muscle, and there are on the order of 650 muscles in the body (although not all are innervated by motoneurons in the spinal cord, some are innervated by motoneurons located in the brain stem) so the total number of motoneurons in the human spinal cord is not likely to exceed a million. In fact, in rats and mice the number of spinal motoneurons has been determined fairly accurately to be about half a million and a third of a million, respectively (Bjugn and Gundersen 1993; Bjugn 1993). In rats and mice there are believed to be 10–12 times as many interneurons as motoneurons. Estimates of the total number of neurons in the human spinal cord go as high as 13 million. On top of this there are about twice as many glial cells as neurons. The white matter of the spinal cord contains several million axons, some of which derive from spinal neurons but many of which derive from neurons in the brain.

The adult human spinal cord is a little under a half a meter long and about 1.5 cm in diameter. Given the numbers above, even a small nick in the white matter could sever many tens of thousands of axons and an injury that destroys a half a centimeter of the spinal cord could eliminate 100,000 neurons. It is therefore not surprising that spinal cord injuries can have such catastrophic effects.

5.2.2 What Happens?

What actually happens after a traumatic injury to the spinal cord? As in a wound to any tissue, the initial trauma will injure or kill cells at the trauma site. Thus, a certain number of neurons and glial cells will be eliminated, and a certain number of axons will be severed (but their parent neurons, which may be located quite some distance away, will very likely survive). But the trouble has really only just begun. Over the course of hours to a few days, secondary damage will spread from the primary injury site to affect surrounding tissue (Fig. 5.3). The secondary damage, which is most pronounced during the first 24 hours after injury, is due to several factors, including loss of blood supply and oxygen due to damaged blood vessels, pressure damage due

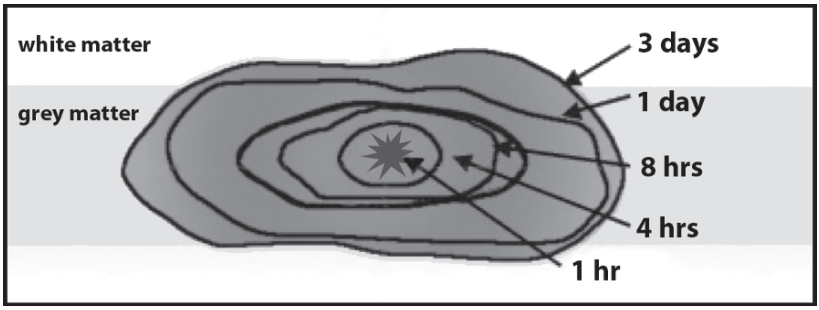

Fig. 5.3 The time course of the spread of secondary damage following a traumatic spinal cord injury

to intraspinal bleeding, the release of ions, neurotransmitters and chemical factors from damaged cells that can provoke injury and death in other cells in the vicinity, and the initial stages of inflammatory responses. Thus, an initial injury that destroys a small fraction of a cubic centimeter of the spinal cord can spread to destroy many cubic centimeters of tissue within a few days. For this reason, one of the main strategies being pursued for the acute treatment of spinal cord injury is to try to limit the secondary damage, for example by cooling, which has been used successfully to limit secondary damage in the brain after stroke and ischemic insults.

As the spinal cord injury progresses through the acute and subacute phases, further changes occur as the inflammatory response triggers astrocytes to enter a reactive state in which they proliferate and generate scar tissue, much as fibroblasts do in other tissues of the body. The astrocytic scar can be quite large and is one of the major impediments to the regeneration of axons in the injured spinal cord, since the scar tissue not only creates a mechanical barrier but is also directly inhibitory to axon growth.

Because of the spread of tissue damage due to secondary injury and the creation of astrocytic scar tissue, the outlook for treating spinal cord injury irrespective of treatment strategy is obviously best for acute injuries and worst for chronic injury cases. In animal experiments, it is commonly seen that success is greater when treatments are initiated soon after an injury (Thuret et al. 2006). Any attempts at using stem cells to treat spinal cord injuries in chronic patients will therefore have to deal simultaneously with the special problems that the chronic situation poses.

5.2.3 Other Types of Spinal Injury and Disease Relevant for Stem Cell-Based Treatment Strategies

A number of other pathological scenarios lead to spinal cord injury and disease in which neurons and glial cells are destroyed. Ischemic insults due to an interruption of blood supply and pressure exerted by tumor metastases can both destroy spinal cord tissue indiscriminately in the absence of trauma. Several well known diseases attack specific types of spinal cord cells. For example, demyelinating diseases such as multiple sclerosis (MS) destroy the oligodendrocytes that create the insulating myelin around axons, and motoneuron diseases such as spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS) destroy motoneurons.

5.2.4 Current Status of Efforts to Treat Spinal Cord Injury and Disease with Embryonic Stem Cells

Given the overall complexity of spinal cord tissue, it is not surprising that current efforts at using embryonic stem cells to treat spinal cord injury or disease are focused primarily on pathologies that affect only one type of cell. Chief among these are MS, which targets the oligodendrocyte, and diseases such as ALS, which

target the motoneuron. The hope is that if a stem cell-based treatment strategy can be developed for these relatively simple cell replacement scenarios, then this may pave the way for developing strategies for more complex scenarios, such as traumatic injuries that can affect all the cell types in the spinal cord.

5.2.4.1 Oligodendrocyte Replacement in Demyelinating Diseases

Among the several demyelinating diseases that have been described, MS is one of the best known among the general public, and one of the most prevalent diseases affecting the central nervous system, with an estimated 2.5 million MS patients world-wide. MS can strike at a variety of ages, as early as late childhood, but typically in young adults (mean age of onset is around 30 years). Women are affected about 50% more often than men. Although a chronic disease, MS need not exhibit incessant progression, but often goes through cycles of remission. It does not affect life span significantly. The basic histopathological scenario is a focal loss of the myelin sheaths that invest nearly all axons in the spinal cord, creating a distinctive plaque that can be observed in the living patient with non-invasive imaging techniques. The number and distribution of plaques determine the loss of function in any given patient. There is no known cure and treatments are purely palliative. Since MS appears to involve an autoimmune response, immune-suppression therapy is becoming an important strategy for controlling symptoms.

Clearly, since the myelin loss associated with MS is focal, replacement strategies can be envisioned in which oligodendrocyte-producing stem or progenitor cells are injected into a plaque, proliferate, and re-invest the area with myelin-producing oligodendrocytes. For those patients with a limited number of isolated plaques this kind of approach could alleviate the majority of symptoms. Since spinal cord injury due to trauma also leads to demyelination, such a treatment would also be beneficial for spinal cord injury patients. How close are we to establishing such a treatment?

During the course of embryogenesis, embryonic stem cells and their descendent lineages are instructed by precisely timed and localized molecular signals which direct differentiation into the proper cell types in the proper locations. In using embryonic stem cells to create a specific cell type such as the oligodendrocyte, it is therefore necessary to know which signals are involved and how these regulate the expression of the genes that characterize that cell type. Great progress has been made recently in understanding the molecular control of the genetic program of oligodendrocyte differentiation (Kitada and Rowitch 2006; Liu et al. 2007). Using this information, researchers have successfully treated hESCs in vitro with molecular factors that promote the generation of oligodendrocytes. Some studies report oligodendrocyte differentiation to over 90% purity, although this rate of success is controversial (Keirstead et al. 2005; Duncan 2005). Human ESC-derived oligodendrocyte progenitors injected into the injured spinal cords of adult rats survive, differentiate into oligodendrocytes, enhance remyelination of denuded axons and promote some recovery of motor function (Keirstead et al. 2005). Unfortunately,

this has only been seen in acutely, not chronically injured spinal cords. These findings clearly demonstrate the potential for using hESCs to replenish functional oligodendrocytes. Nevertheless, a number of challenges remain. These include the problem of scaling up from rats to humans (the volume of spinal cord lesions is substantially smaller in rats), the problem of allogenicity (hESC-derived cells could trigger immune reactions and tissue rejection), overcoming the refractoriness of chronic injuries to this and other treatments, and in particular the issue of potential heterogeneity in hESC-derived cells. Since ESCs can in principle generate any cell type, anything less than 100% purity of the desired cell type could introduce undesirable and potentially deleterious side effects. For example, ESCs can potentially generate tumor cells, which would absolutely contraindicate their use in cell replacement therapy.

Despite the obvious challenges, efforts are now being made to generate hESC-derived oligodendrocyte-producing progenitor cells in large numbers for human clinical trials, and within a few years it should become clear whether the promise demonstrated in animal experiments will bear fruit in a clinical setting.

5.2.4.2 Motoneuron Replacement in Motoneuron Diseases

Several diseases target motoneurons and lead to their destruction. SMA presents congenitally or in early childhood and has an incidence of about 1 per 5,000. Some forms of SMA are lethal within a few years whereas others permit survival to adulthood. Adult onset motoneuron diseases include ALS, which has an incidence of 1–2 per 100,000, and which affects not only motoneurons in the spinal cord and brain stem but also premotor neurons (the so-called upper motoneurons) in the motor cortex. ALS has high mortality, with few patients surviving more than 5 years.

Because motoneurons are a relatively homogeneous neuron population, they provide a particularly tractable target for stem cell-based therapies. As is the case for oligodendrocytes, great strides have been made recently in understanding the genetic program of motoneuron differentiation (Shirasaki and Pfaff 2002). Researchers have used this information to direct the differentiation of embryonic stem cells from mice into motoneuron progenitors and from these into functional motoneurons in vitro (Wichterle et al. 2002; Miles et al. 2004). When injected into the spinal cords of rats that have been infected with a virus that kills motoneurons, these embryonic stem cell-derived motoneurons can replace the missing motoneurons, and given appropriate manipulations to induce axon outgrowth, can reinnervate muscle and provide recovery of motor function (Deshpande et al. 2006). Although the overall treatment strategy is complex (new motoneurons must be injected, axon growth inhibitors must be counteracted with drugs, and axon attractants must be seeded into the limbs to coax the motoneuron axons to the muscles), these results clearly demonstrate the potential of using embryonic stem cells to produce functional motoneurons for replacement therapy.

As for oligodendrocytes, despite the promise, substantial challenges remain. First, the results obtained so far using mouse ESCs need to be replicated using

hESCs. Thereafter, a number of technical problems need to be solved before the same approach can be used in human patients. Motoneuron diseases, unlike the focal damage to oligodendrocytes caused by MS, can destroy motoneurons throughout the brain stem and spinal cord, and even premotor neurons in the cerebral cortex. One challenge will be to design replacement strategies that facilitate the injection of motoneurons and premotor neurons into such an extensive target. The complex treatment strategy necessary to induce the axons of ESC-derived motoneurons to grow from the spinal cord to the muscles needs to be scaled up, as the distances involved are much greater in humans than in rodents. A major challenge will be to direct the establishment of synaptic connections from interneurons onto the new motoneurons in patterns that produce appropriate function. In addition, some of the same general challenges as noted above for oligodendrocyte replacement (allogenicity, purity, tumorigenicity) need to be addressed. Nevertheless, the generation of motoneurons from hESCs appears likely to be realized in the near future and given the devastating outcome of ALS will almost certainly prompt clinical trials.

5.3 Parkinson's Disease

Parkinson's disease is one of a variety of diseases affecting the basal ganglia deep within the brain. The basal ganglia are large collections of neurons that are involved primarily in the learning, selection and regulation of voluntary movements (although they also contribute to emotional and cognitive functions). Diseases of the basal ganglia therefore present as movement disorders, either as too much movement (such as the uncontrolled movements of dystonias and Huntington's chorea) or too little movement (such as the bradykinesia of Parkinson's disease). Parkinson's disease is remarkable in that it selectively attacks a very specific population of neurons that use dopamine as neurotransmitter and which are located in a structure called the substantia nigra. The substantia nigra provides a diffuse dopaminergic innervation to structures in the basal ganglia, and the mere loss of this dopaminergic innervation creates a critical imbalance of basal ganglia activity that results in the classical symptoms of Parkinson's disease: bradykinesia, rigidity, and tremor (Fig. 5.4). Indeed, the principal treatment for Parkinson's disease today is the medicinal replacement of dopamine by ingestion of the dopamine precursor L-dopa (other treatments, such as surgical lesions or stimulation of specific sites within the basal ganglia with implanted electrodes, are also used). Parkinson's disease and forms of parkinsonism (insults that affect the substantia nigra less specifically and thus produce the same symptoms as Parkinson's disease along with other symptoms) have an incidence of about 1 per 1,000 overall, but this rises to 2–3 per 100 in the elderly (over 70 years of age).

There are only about 200,000 dopaminergic nigral neurons on each side of the brain. Their relative paucity and well-defined, restricted location in the substantia nigra made them a prime target for cell replacement therapy starting already in the late 1970s, when Swedish researchers began implanting embryonic substantia nigra tissue first into the

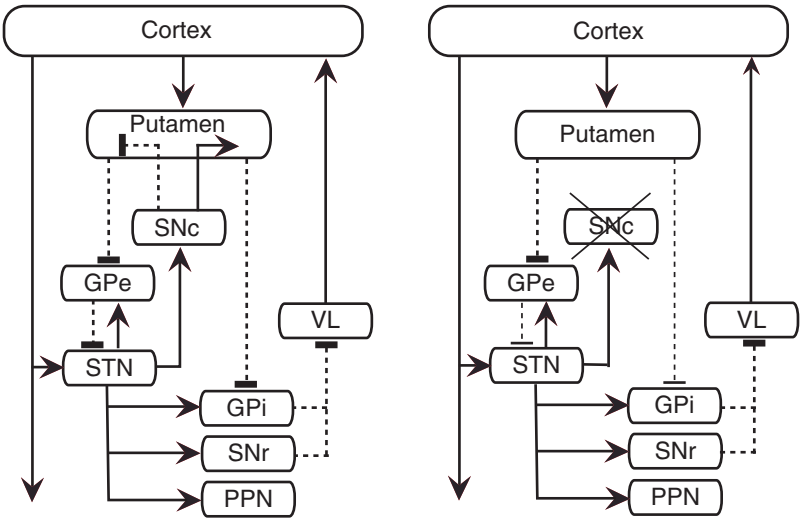

Fig. 5.4 A highly simplified schematic of the connections between different parts of the basal ganglia, the thalamus, and the cortex, along with the dopaminergic inputs to the basal ganglia from the substantia nigra. A. The normally functioning circuit embodies properly balanced excitation (solid lines) and inhibition (dotted lines) so that activity that regulates the selection and initiation of movement by the cortex is appropriate. B. Loss of the dopaminergic inputs to the basal ganglia (through lesion of the substantia nigra pars compacta, SNc) disrupts this balance, resulting in too much inhibition of the thalamus and thus too little activation of movement initiation centers in the cortex. GPe (globus pallidus pars externa), GPi (globus pallidus pars interna), STN (subthalamic nucleus), SNr (substantia nigra pars reticularis), PPN (pedunculopontine nuclei), VL (ventrolateral nucleus of the thalamus). Modified and adapted from Obeso et al. (2000), with permission.

brains of animals with experimentally-induced parkinsonism and then into humans with Parkinson's disease (Björklund and Lindvall 2000). In the animal models, the implanted tissue developed into dopaminergic neurons, some of which innervated the proper basal ganglia targets, and this resulted in the alleviation of the symptoms of the movement disorders exhibited by the animals. Similarly, in human patients treated with substantia nigra tissue from human embryos, signs of dopaminergic innervation have been detected using non-invasive imaging and in postmortem assessment, and clinical improvement above and beyond potential placebo effects have also been documented in some of the patients (Björklund and Lindvall 2000).

The 'proof-of-principle' established by the implantation of embryonic dopaminergic neuron precursors, as well as the ethical issues and practical limitations involved in using tissue directly from human embryos, has prompted intense research into the possibility of using embryonic stem cells to generate dopaminergic neurons. Here there has also been tremendous progress in identifying and characterizing the molecular determinants that establish the dopaminergic phenotype during development. This information has facilitated the recent generation of high-purity dopaminergic neurons with the correct substantia nigra character from mouse ESCs in vitro (Andersson et al. 2006). This advance extends previous work in which hESCs have

been induced to differentiate into dopaminergic neurons in vitro, albeit at substantially lower purity, through manipulation of the molecular environment (Taylor and Minger 2005). Implantation of hESC-derived dopaminergic neuron progenitors into animal models of parkinsonism has been shown to alleviate symptoms to some extent, but it has been unclear whether this is the result primarily of a reestablishment of dopaminergic innervation or other effects, such as graft-derived trophic factors or the stimulation of endogenous repair mechanisms. Moreover, survival of hESC-derived dopaminergic neuron progenitors in animal models has been low, indicating that much work remains to be done to ensure that the implanted cells can integrate and survive efficiently. Purity has also been a major issue. Different hESC lines exhibit different capacities for generating dopaminergic neurons, and contaminating cell types can even include non-neural cells that remain proliferative long after the implantation (Taylor and Minger 2005). The hope is that improved protocols, based on greater insight into the normal developmental program for dopaminergic differentiation, can be established for generating high-purity hESC-derived dopaminergic neuron progenitors and that these will provide a better source for implantation.

Thus, although dopaminergic neurons of the substantia nigra provide another attractive target for hESC-based cell replacement therapy, there are still substantial hurdles to cross before a clinically feasible approach is available. In addition to the general problems associated with ESCs, one of the principal difficulties is that the neural circuitry of the basal ganglia and surrounding structures is much less well understood than that of the spinal cord. Moreover, the role of dopamine in regulating basal ganglia function is complex, with different effects being elicited in different basal ganglia target neurons. Loss of dopamine can also trigger compensatory changes in the system (changes in dopamine receptor densities, synaptic rearrangement) that are difficult if not impossible to predict from patient to patient. Recreating a situation in which implanted dopaminergic neurons innervate the basal ganglia in the appropriate way will thus be a major challenge, compounded by the large volume of tissue the basal ganglia occupy in the human brain. Nevertheless, as for oligodendrocytes and motoneurons, it seems highly likely that the generation of pure dopaminergic neuron progenitors from hESCs will be realized. Given advances in our understanding of the way the basal ganglia function, and in how to promote the survival of implanted progenitors, cell replacement therapy will almost certainly become an available option. Whether this option will be competitive in the face of other treatment strategies, which are also advancing rapidly, remains to be seen.

5.4 Alternatives to Cell Replacement

Although the replacement of lost neurons and/or glia is a principal aim of stem cell research, embryonic stem cells may prove to be clinically useful in other, less direct ways. It has been noted in many animal experiments that functional improvement

can occur after stem cell implantation despite the lack of obvious neuronal differentation or synaptic integration. Embryonic stem cells and their progeny have been shown to release cytokines and growth factors that are involved in brain development and plasticity (Bentz et al. 2007; Kamei et al. 2007) and in this capacity might promote synaptic plasticity and reorganization without participating directly in the formation of new connections. Treatment with growth factors has been proposed as a potential approach to facilitate recovery from the widespread brain damage associated with stroke and Alzheimer's disease (Kuipers and Bramham 2006). Either as a genetically engineered source of human growth factors in vitro or as an implanted, local in vivo source for targeting growth factor delivery to specific sites in the brain, hESCs could contribute to the development of clinically viable growth factor therapies.

Embryonic stem cells have also been envisioned as a means to provide growth-promoting substrates for damaged axons. A great deal of effort has recently been directed towards using olfactory ensheathing cells for this purpose, including in clinical trials (Thuret et al. 2006). Despite their demonstrated capacity to support axon growth, it is unclear whether olfactory ensheathing cells are the best possible cell type for all areas of the brain and all injury and disease scenarios. Again, because embryonic stem cells can be induced to differentiate into any cell type, they could in principle be engineered to produce growth-promoting cells perfectly tailored to different brain regions and neuronal deficits.

Lastly, embryonic stem cells have been recognized as an ideal source of cells for testing drug therapies in vitro or in animal models prior to embarking on clinical trials. The great diversity of cell types in the brain poses a major challenge for the development of drugs for treating neurological disorders, because drug effects may vary across and within neuronal populations. The possibility of generating unlimited numbers of neurons of any given standardized phenotype for in vitro testing would facilitate a more rapid and reproducible assessment. The same cells could be implanted into animal models to provide testing within an in vivo context.

5.5 Summary

The potential of human embryonic stem cells to generate any cell type within the brain and spinal cord offers hope that cell replacement therapies for a variety of neurological disorders may someday be realized. Stem cell-based replacement strategies are nevertheless highly challenging, due to the complexity of neural tissue and the requirement for a highly controlled differentiation of stem cells. Advances in our understanding of how diverse types of neurons and glial cells differentiate normally are paving the way towards a pre-differentiation of embryonic stem cells into specific lineages prior to use. If the additional challenges of ensuring the survival and functional integration of stem cell-derived neural cells can be met while avoiding side effects, then stem cell-based replacement therapies are likely to become powerful tools in the treatment repertoire. Whether these tools will become

economically feasible or competitive in the face of alternative treatment strategies remains to be seen. Embryonic stem cells offer therapeutic promise in other ways as well, for example as potential sources of growth factors or substrates for damaged neurons and axons, and in particular as the source of standardized neural cell populations for drug testing. On this backdrop, continued research into the use of human embryonic stem cells is highly warranted.

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