One is to replace dopaminergic cells that die in Parkinson disease. When transplanted into the striatum, these neurons release dopamine onto their targets without the need to grow long axons or form elaborate synapses (Figure 50–14). Another is to transplant immature inhibitory interneurons from the ganglionic eminences in which they are produced (Chapter 46) to the cortex, where they mature and form synapses. By enhancing inhibition, these neurons attenuate the manifestations of disorders in which insufficient inhibitory drive plays a role, such as epilepsy and anxiety.

Unfortunately, application of these methods to human patients has been fraught with difficulties. One is the difficulty of obtaining and growing developing neurons in sufficient numbers and with sufficient purity. Second, it has been challenging to modify neurons by introducing new genes so as to improve their chances of functioning in a new environment. Third, in many cases, the grafted neurons are already too mature

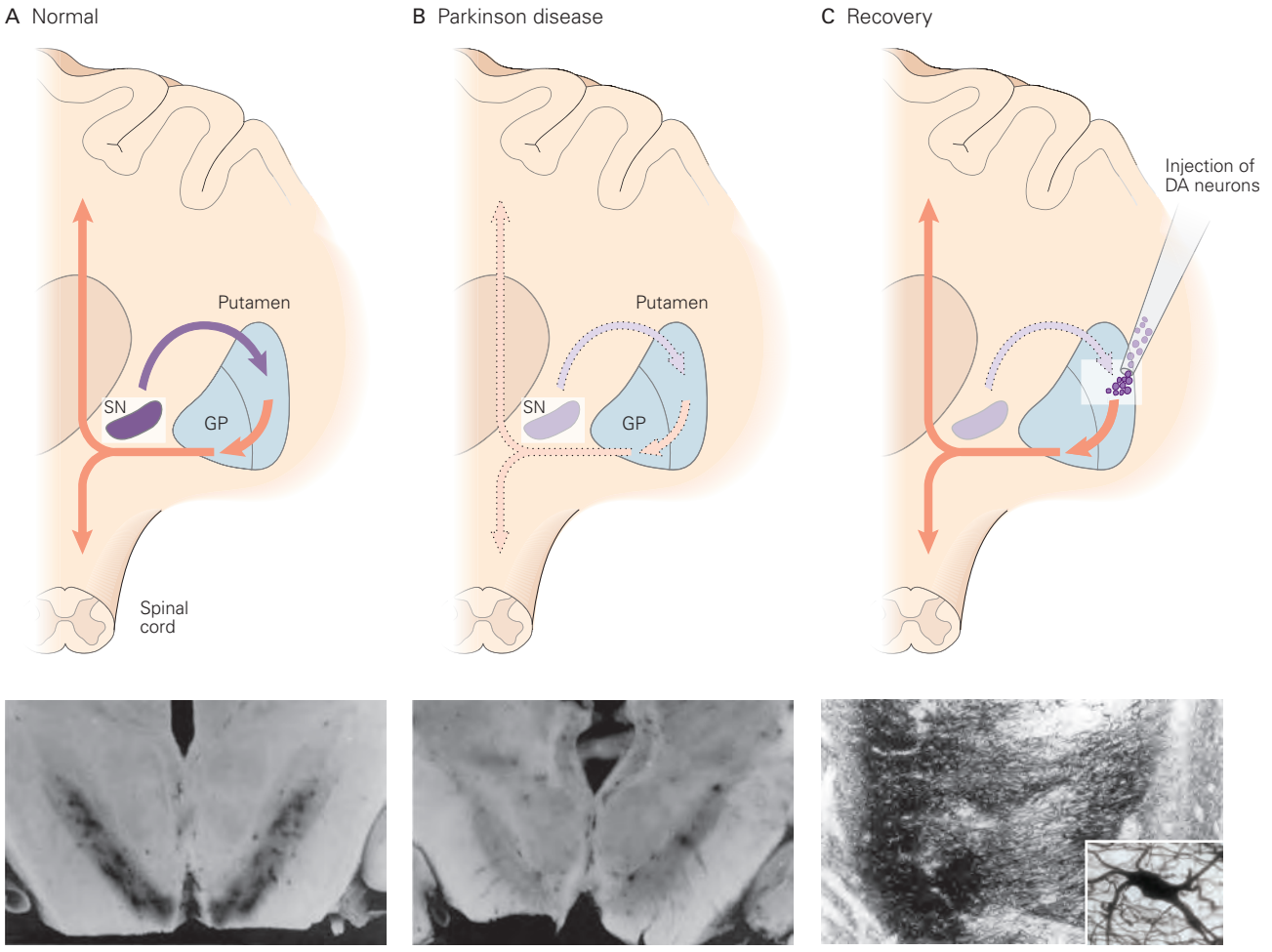

Figure 50–14 Loss of dopaminergic (DA) neurons in Parkinson disease can be treated by grafting embryonic cells into the putamen.

A. In the healthy brain, dopaminergic projections from the substantia nigra (**SN**) innervate the putamen, which in turn activates neurons in the globus pallidus (**GP**). Pallidal outputs to the brain and spinal cord facilitate movement. The image below shows melanin-rich dopaminergic neurons in human substantia nigra.

B. In Parkinson disease, the loss of dopaminergic neurons in the substantia nigra deprives the putamen–globus pallidus pathways of their drive. The image beneath the diagram shows the virtual absence of melanin-rich dopaminergic neurons in the substantia nigra of an individual with Parkinson disease.

C. Direct injection of embryonic dopaminergic neurons into the putamen reactivates the globus pallidus output pathways. The image below shows tyrosine hydroxylase expression in the cell bodies and axons of embryonic mesencephalic dopaminergic neurons grafted into the putamen of a human patient. (Image reproduced, with permission, from Kordower and Sortwell 2000. Copyright © 2000. Published by Elsevier B.V.)

to differentiate properly or to integrate effectively into functional circuits.

These obstacles can be overcome by transplanting neural precursors into the adult brain where they can go on to differentiate into neurons in a hospitable environment. Several classes of precursors have been transplanted successfully, including neural stem cells and committed precursors. Some initial success has been obtained with embryonic stem (ES) cells. These cells are derived from early blastocyst stage embryos and can give rise to all cells of the body. Because they can divide indefinitely in culture, large numbers of cells can be generated, induced to differentiate, and then engrafted.

More recently, this technology has been enhanced by the molecular reprogramming of skin fibroblast cells to create induced pluripotent stem (iPS) cells (Figure 50–15). These cells have a distinct advantage over ES cells; embryos are not required for their production, effectively bypassing a minefield of practical, political, and ethical concerns that have hindered research using human ES cells. Another advantage of iPS cells is that they can be generated from an individual patient's own skin cells, neatly avoiding issues of immunological incompatibility. It is also possible to genetically modify the iPS cells in culture by repairing a defective gene before transplantation.

Because ES and iPS cells have the potential to generate any cell type, it is essential that their differentiation be guided along specific pathways in culture before they are transplanted. Methods for generating specific classes of neural precursors, neurons, and glial cells from ES and iPS cells have now been devised (Figure 50–15).

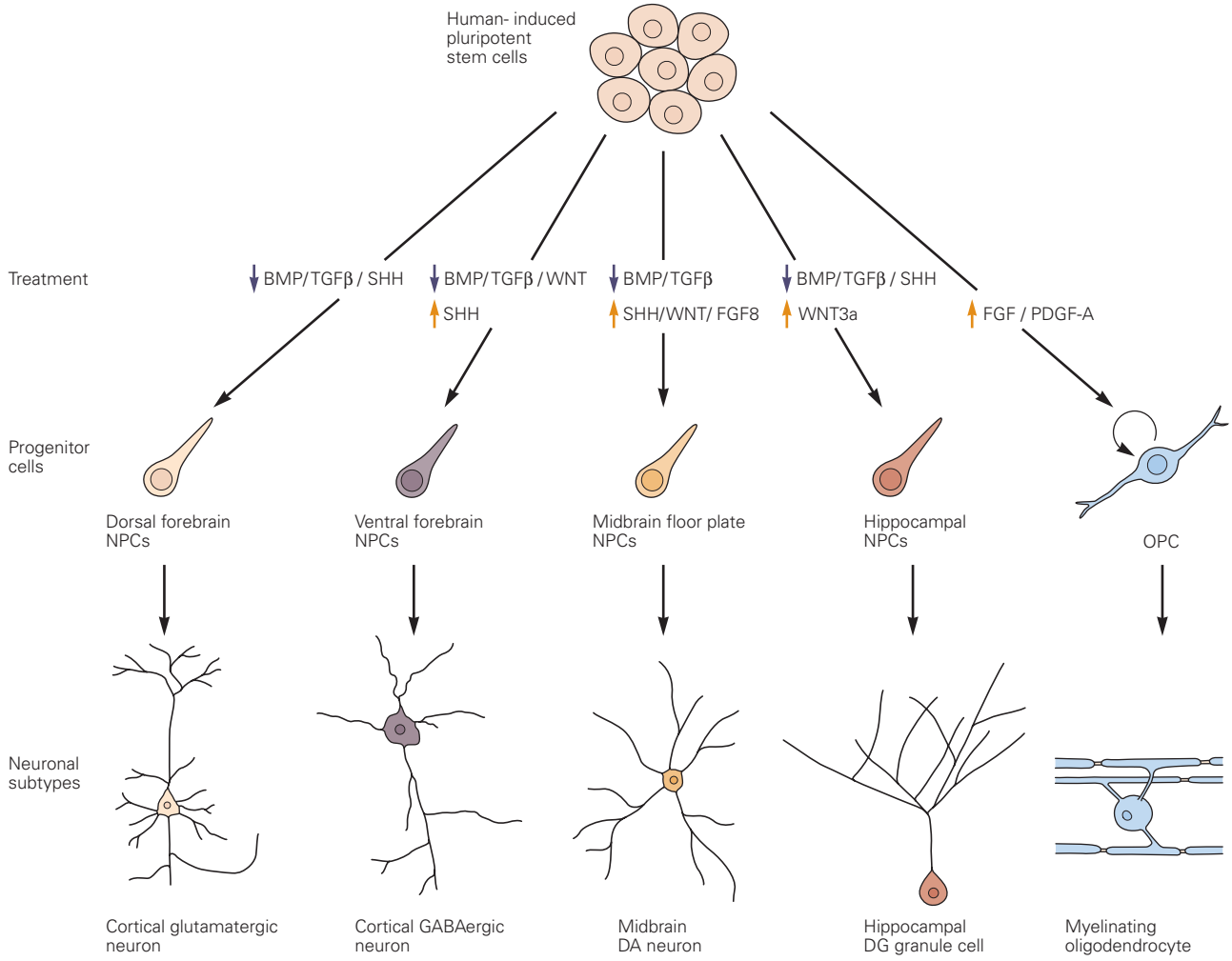

Figure 50–15 Induced pluripotent stem cells can be reprogrammed to generate precursors of many neuronal and glial types. The precursors can then be transplanted into the brain or spinal cord, where cells complete their differentiation and integrate into functional circuits. (Abbreviations: **DA**, dopamine; **DG**, dentate gyrus; **NPC**, neural progenitor cell; **OPC**, oligodendrocyte progenitor cell.) (Adapted, with permission, from Wen et al. 2016. Copyright © 2016 Elsevier Ltd.)

For example, it is possible to generate neurons that possess many or all of the properties of the spinal motor neurons that are lost in amyotrophic lateral sclerosis (Figure 50–16) or to generate the dopaminergic neurons lost from the striatum in Parkinson disease and then to engraft such neurons into the spinal cord or brain.

Although many hurdles need to be overcome, clinical trials using ES and iPS cell-derived neurons are underway. In addition, these cells are being used in chemical screens to identify compounds that counteract the cellular defects that underlie human neurodegenerative disease.

Stimulation of Neurogenesis in Regions of Injury May Contribute to Restoring Function

What if, following injury in adults, endogenous neuronal precursors could be stimulated to produce neurons capable of replacing those that have been lost? Two sets of recent findings suggest that this idea is not so far-fetched.

First, precursors capable of forming neurons in culture have been isolated from many parts of the adult nervous system, including the cerebral cortex and spinal cord, even though neurogenesis in adults is ordinarily confined to the olfactory bulb and hippocampus. This diversion of cell fate led to the idea that neurogenesis in the adult occurs in only a few sites, because only they contain appropriate permissive or stimulatory factors. This hypothesis has spurred a search for such factors, in the hope that they could be used to render a larger range of sites capable of supporting neurogenesis.

Second, in a few cases, the generation of new neurons can be stimulated by traumatic or ischemic injury (akin to stroke), even in areas such as the cerebral cortex

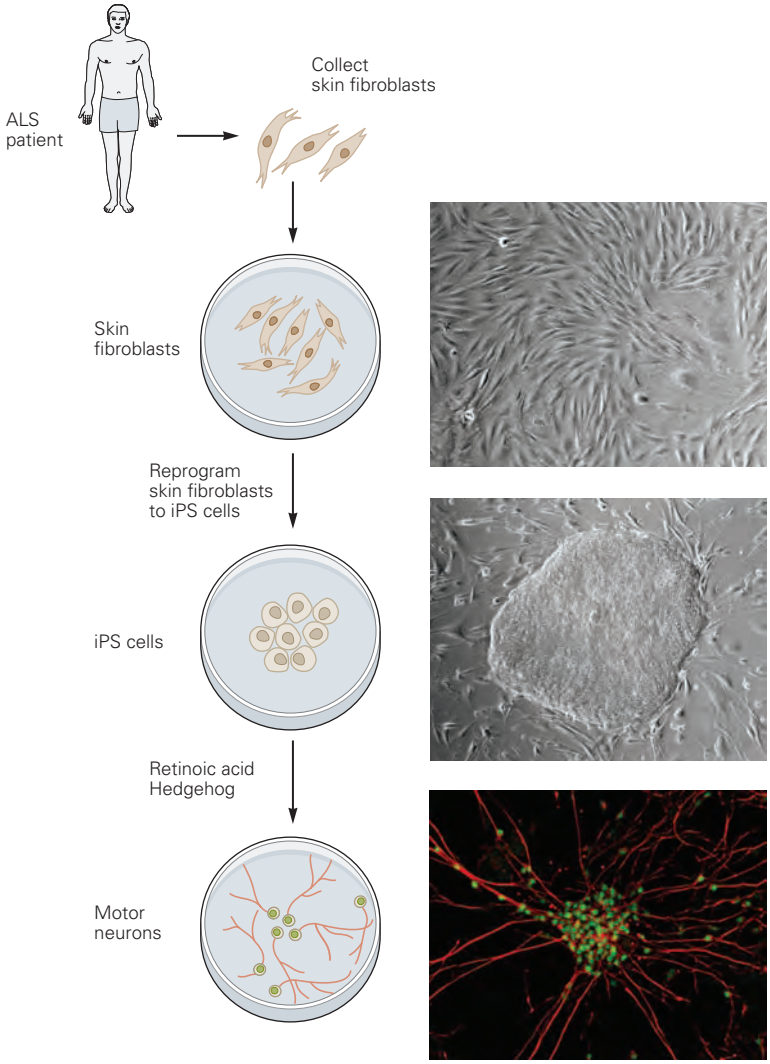

Figure 50–16 **Induced pluripotent stem cells derived from an individual with amyotrophic lateral sclerosis (ALS) can differentiate into spinal motor neurons.** Fibroblasts from the skin of a patient with ALS were used to generate induced pluripotent stem (**iPS**) cells, which were then directed to a motor neuron fate (see Figure 50–15). These cells can be used to analyze mechanisms that underlie motor neuron loss in ALS. The images at right show (from top to bottom) cultured fibroblasts, an iPS cell clump, and differentiated motor neurons expressing characteristic nuclear transcription factors (**green**) and axonal proteins (**red**). (Micrographs reproduced, with permission, from C. Henderson, H. Wichterle, G. Croft, and M. Weygandt.)

or spinal cord in which neurogenesis normally fails to occur. The fact that recovery after stroke and injury is poor demonstrates that spontaneous compensatory neurogenesis, if it occurs in humans, is insufficient for tissue repair. However, injury-induced neurogenesis has been enhanced in experimental animals in several ways. In one, administration of growth factors promotes neuronal production from progenitors grown in culture. In another, glial cells that retain the capacity to divide, such as Müller glia in the retina or astrocytes in the cortex, are reprogrammed to differentiate into neurons. If such interventions could be adapted to humans, the range of neurons subject to replacement would be greatly increased.

Transplantation of Nonneuronal Cells or Their Progenitors Can Improve Neuronal Function

Cells other than neurons are lost after brain injury. Among the most profound losses are those of oligodendrocytes, the cells that form the myelin sheath around central axons. The stripping of myelin continues long after traumatic injury and contributes to progressive loss of function of axons that may not have been injured directly.

Although the adult brain and spinal cord are capable of generating new oligodendrocytes and replacing lost myelin, this production is insufficient to restore function in many cases. Since several common neurological diseases, most notably multiple sclerosis, are accompanied by a profound state of demyelination, there is strong interest in providing the nervous system with additional oligodendrocyte precursors in order to augment remyelination.

Neural stem cells, multipotential progenitors, ES cells, and iPS cells can give rise not only to neurons but also to nonneural cells, including oligodendrocytes and their direct precursors. Indeed, at present, human ES cells are being channeled into oligodendrocyte progenitor cells and implanted into injured spinal cords of experimental animals. Transplanted cells that differentiate into oligodendrocytes enhance remyelination and substantially improve the locomotor ability of experimental animals (Figure 50–17).

Restoration of Function Is the Aim of Regenerative Therapies

We need to bear in mind that efforts to replace central neurons or to enhance the regeneration of their axons

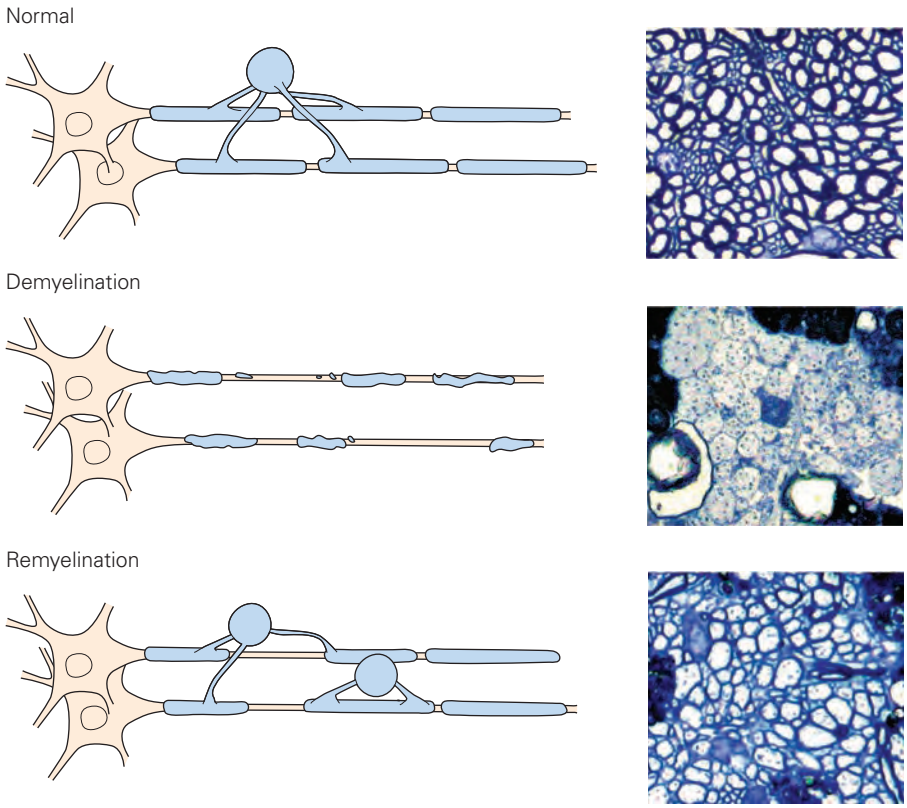

Figure 50–17 Restoration of myelination in the central nervous system by transplanted oligodendrocyte stem cells. In rodents with demyelinated axons, grafts of oligodendrocyte precursor cells can restore myelination to near normal. Sections through central nerve tracts are shown in the images at right. (Adapted, with permission, from Franklin and ffrench-Constant 2008. Copyright © 2008 Springer Nature.)

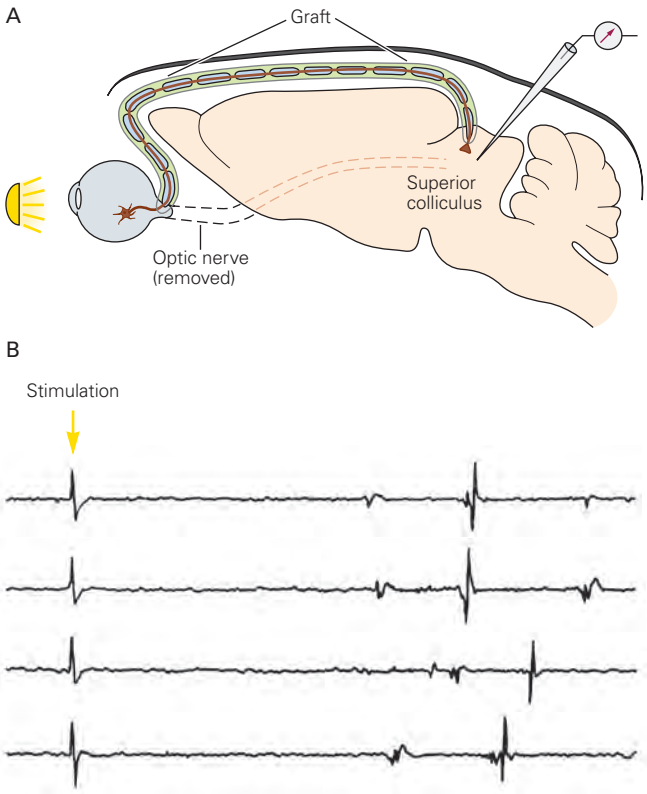

Figure 50–18 Regenerated retinal ganglion axons in the optic nerve can form functional synapses. (Adapted, with permission, from Keirstead et al. 1989. Copyright © 1989 AAAS.)

A. A segment of optic nerve in an adult rat was removed, and a segment of sciatic nerve was grafted in its place. The other end of the sciatic nerve was attached to the superior colliculus. Some retinal ganglion cell axons regenerated through the sciatic nerve and entered the superior colliculus.

B. Once the axons of the retinal ganglion neurons had regenerated, recordings were made from the superior colliculus. Flashes of light delivered to the eye elicited action potentials in collicular neurons, demonstrating that at least some regenerated axons had formed functional synapses.

would be of little use if these axons were unable to form functional synapses with their target cells. The same fundamental questions asked about axon regeneration in adults therefore apply to synaptogenesis: Can it happen, and if not, why not?

It has been difficult to address these questions because axonal regeneration following experimentally induced injury is usually so poor that the axons never reach appropriate target fields. However, several of the studies discussed earlier in this chapter offer hope that synapse formation is possible within the dense adult neuropil. In fact, axon branches that regenerate following injury can form synapses on nearby targets. For example, Aguayo and his colleagues found that retinal axons were able to regrow into the superior colliculus when they were channeled through a peripheral nerve that had been grafted into the optic nerve (Figure 50–18A). Remarkably, some collicular neurons fired action potentials when the eye was illuminated, showing that functional synaptic connections had been reestablished (Figure 50–18B). More recent studies have promoted regeneration of severed axons by enhancing their intrinsic growth programs, as described above, and observed some restoration of function.

Likewise, neurons that arise endogenously or are implanted by investigators can form and receive synapses. Thus, there is reason to believe that if injured axons can be induced to regenerate, or new neurons supplied to replace lost ones, they will wire up in ways that help restore lost functions and behaviors.

Highlights

1. When axons are transected, the distal segment degenerates, a process called Wallerian degeneration. The proximal segment and cell body also undergo changes, as do the injured neuron's synaptic inputs and targets.
2. It was long thought that Wallerian degeneration was a passive and inevitable consequence of the distal segment being deprived of sustenance from the cell body, but it is not known to be an active, regulated process. Genes called *NMNAT* and *SARM1* are key components of a core signaling pathway that controls the process. Intervention in the pathway can slow or even halt degeneration.
3. Axons can regenerate and form new synapses following injury, but in mammals, regeneration is far more widespread and effective in peripheral axons than in central axons.

4. A key factor in the differential response of peripheral and central axons is that the environment confronting injured central axons is poor at supporting growth. It both lacks nutritive factors present in the pathway of peripheral nerves and contains growth-inhibitory factors absent from peripheral nerves.
5. Structures that inhibit regeneration include myelin fragments that persist following Wallerian degeneration and astrocytes that form glial scars at injury sites. Inhibitory factors in myelin include Nogo and myelin-associated glycoprotein. Inhibitory factors secreted by astrocytes include chondroitin sulfate proteoglycans.
6. Central regeneration is also hindered by intrinsic decreased ability of adult central neurons to grow, due to downregulation of growth programs active during development. Interventions that restore or disinhibit growth pathways, such as JAK/STAT and mTOR signaling, enable regeneration.
7. However, it is important to note that the failure of regeneration following injury may be related to the stabilization of connections that occurs at the end of critical periods. For example, myelination, which occurs largely at the end of a critical period, may have the secondary effect of preventing further, large-scale rearrangement of synaptic connections. Thus, caution will be needed to ensure that treatments aimed at fostering recovery following injury do not end up promoting formation of maladaptive circuits.
8. Another approach for restoring function following damage is to harness the ability of intact axons to form new connections, generating adaptive circuits that can compensate to some extent for those lost to injury.
9. The traditional view that all neurogenesis occurs during or shortly after gestation has now been modified by the discovery that new neurons are born throughout life in a few brain areas. These neurons arise from resident stem cells and can integrate into functional circuits.
10. Cells capable of forming new neurons are also present in many other areas of the brain and spinal cord but remain quiescent. Attempts to activate them by providing growth factors or introducing growth-promoting genes (transcriptional reprogramming) could harness their potential following injury or in neurodegenerative disease.
11. Another approach to neuronal replacement is to implant developing neurons. Although fetal neurons are sometimes used for this purpose in experimental animals, a more useful source may be neurons derived from ES or iPS cells. They can be grown in large quantities, genetically modified if necessary, and treated to differentiate into specific neuronal types. Clinical studies using this approach are now beginning.

Joshua R. Sanes

Selected Reading

Benowitz LI, He Z, Goldberg JL. 2017. Reaching the brain: advances in optic nerve regeneration. Exp Neurol 287:365–373.

Dell'Anno MT, Strittmatter SM. 2017. Rewiring the spinal cord: direct and indirect strategies. Neurosci Lett 652: 625–634.

Gerdts J, Summers DW, Milbrandt J, DiAntonio A. 2016. Axon self-destruction: new links among SARM1, MAPKs, and NAD+ metabolism. Neuron 89:449–460.

He Z, Jin Y. 2016. Intrinsic control of axon regeneration. Neuron 90:437–451.

Magnusson JP, Frisén J. 2016. Stars from the darkest night: unlocking the neurogenic potential of astrocytes in different brain regions. Development 143:1075–1086.

McComish SF, Caldwell MA. 2018. Generation of defined neural populations from pluripotent stem cells. Philos Trans R Soc Lond B Biol Sci 373:pii: 20170214.

Zhao C, Deng W, Gage FH. 2008. Mechanisms and functional implications of adult neurogenesis. Cell 132:645–660.

References

Alilain WJ, Horn KP, Hu H, Dick TE, Silver J. 2011. Functional regeneration of respiratory pathways after spinal cord injury. Nature 475:196–200.

Altman J. 1969. Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol 137:433–457.

Altman J, Das GD. 1965. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol 124:319–335.

Bareyre FM, Kerschensteiner M, Raineteau O, Mettenleiter TC, Weinmann O, Schwab ME. 2004. The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. Nat Neurosci 7:269–277.

Bei F, Lee HHC, Liu X, et al. 2016. Restoration of visual function by enhancing conduction in regenerated axons. Cell 164:219–232.

Beirowski B, Berek L, Adalbert R, et al. 2004. Quantitative and qualitative analysis of Wallerian degeneration using restricted axonal labelling in YFP-H mice. J Neurosci Methods 134:23–35.

Bradbury EJ, McMahon SB. 2006. Spinal cord repair strategies: why do they work? Nat Rev Neurosci 7:644–653.

Bradbury EJ, Moon LD, Popat RJ, et al. 2002. Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 416:636–640.

Caroni P, Schwab ME. 1988. Antibody against myelin-associated inhibitor of neurite growth neutralizes nonpermissive substrate properties of CNS white matter. Neuron 1:85–96.

Conforti L, Gilley J, Coleman MP. 2014. Wallerian degeneration: an emerging axon death pathway linking injury and disease. Nat Rev Neurosci 15:394–409.

David S, Aguayo AJ. 1981. Axonal elongation into peripheral nervous system "bridges" after central nervous system injury in adult rats. Science 214:931–933.

Dimos JT, Rodolfa KT, Niakan KK, et al. 2008. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science 321:1218–1221.

Duan X, Qiao M, Bei F, Kim IJ, He Z, Sanes JR. 2015. Subtype-specific regeneration of retinal ganglion cells following axotomy: effects of osteopontin and mTOR signaling. Neuron 85:1244–1256.

Essuman K, Summers DW, Sasaki Y, Mao X, DiAntonio A, Milbrandt J. 2017. The SARM1 toll/interleukin-1 receptor domain possesses intrinsic NAD+ cleavage activity that promotes pathological axonal degeneration. Neuron 93:1334–1343.

Ferri A, Sanes JR, Coleman MP, Cunningham JM, Kato AC. 2003. Inhibiting axon degeneration and synapse loss attenuates apoptosis and disease progression in a mouse model of motoneuron disease. Curr Biol 13:669–673.

Franklin RJ, ffrench-Constant C. 2008. Remyelination in the CNS: from biology to therapy. Nat Rev Neurosci 9:839–855.

Galtrey CM, Fawcett JW. 2007. The role of chondroitin sulfate proteoglycans in regeneration and plasticity in the central nervous system. Brain Res Rev 54:1–18.

Gerdts J, Brace EJ, Sasaki Y, DiAntonio A, Milbrandt J. 2015. SARM1 activation triggers axon degeneration locally via NAD^+ destruction. Science 348:453–457.

Gerdts J, Summers DW, Sasaki Y, DiAntonio A, Milbrandt J. 2013. Sarm1-mediated axon degeneration requires both SAM and TIR interactions. J Neurosci 33:13569–13580.

Goldman SA, Kuypers NJ. 2015. How to make an oligodendrocyte. Development 142:3983-3995.

Guo Z, Zhang L, Wu Z, Chen Y, Wang F, Chen G. 2014. In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model. Cell Stem Cell 14:188–202.

Imayoshi I, Sakamoto M, Ohtsuka T. 2008. Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. Nat Neurosci 10:1153–1161.

Jorstad NL, Wilken MS, Grimes WN, et al. 2017. Stimulation of functional neuronal regeneration from Müller glia in adult mice. Nature 548:103–107.

Keirstead HS, Nistor G, Bernal G, et al. 2005. Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. J Neurosci 25:4694–4705.

Keirstead SA, Rasminsky M, Fukuda Y, Carter DA, Aguayo AJ, Vidal-Sanz M. 1989. Electrophysiologic responses in hamster superior colliculus evoked by regenerating retinal axons. Science 246:255–257.

Kordower J, Sortwell C. 2000. Neuropathology of fetal nigra transplants for Parkinson's disease. Prog Brain Res 127:333–344.

Lim DA, Alvarez-Buylla A. 2016. The adult ventricular-subventricular zone (V-SVZ) and olfactory bulb (OB) Neurogenesis. Cold Spring Harb Perspect Biol 8:pii: a018820.

Lois C, Alvarez-Buylla A. 1994. Long-distance neuronal migration in the adult mammalian brain. Science 264: 1145–1148.

Mack TGA, Reiner M, Beirowski B, et al. 2001. Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat chimeric gene. Nat Neurosci 4:1199–1206.

Magavi SS, Leavitt BR, Macklis JD. 2000. Induction of neurogenesis in the neocortex of adult mice. Nature 405:951–955.

Magnusson JP, Göritz C, Tatarishvili J, et al. 2014. A latent neurogenic program in astrocytes regulated by Notch signaling in the mouse. Science 346:237–241.

Maier IC, Schwab ME. 2006. Sprouting, regeneration and circuit formation in the injured spinal cord: factors and activity. Philos Trans R Soc Lond B Biol Sci 361:1611–1634.

Osterloh JM, Yang J, Rooney TM, et al. 2012. dSarm/Sarm1 is required for activation of an injury-induced axon death pathway. Science 337:481–484.

Schwab ME, Thoenen H. 1985. Dissociated neurons regenerate into sciatic but not optic nerve explants in culture irrespective of neurotrophic factors. J Neurosci 5:2415–2423.

Schwegler G, Schwab ME, Kapfhammer JP. 1995. Increased collateral sprouting of primary afferents in the myelin-free spinal cord. J Neurosci 15:2756–2767.

Smith PD, Sun F, Park KK, et al. 2009. SOCS3 deletion promotes optic nerve regeneration in vivo. Neuron 64:617–623.

Sohur US, Emsley JG, Mitchell BD, Macklis JD. 2006. Adult neurogenesis and cellular brain repair with neural progenitors, precursors and stem cells. Philos Trans R Soc Lond B Biol Sci 361:1477–1497.

Southwell DG, Nicholas CR, Basbaum AI, et al. 2014. Interneurons from embryonic development to cell-based therapy. Science 344:1240622.

Takahashi K, Tanabe K, Ohnuki M, et al. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861–872.

Takahashi K, Yamanaka S. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663–676.

Tavazoie M, Van der Verken L, Silva-Vargas V, et al. 2008. A specialized vascular niche for adult neural stem cells. Cell Stem Cell 3:279–288.

Thuret S, Moon LD, Gage FH. 2006. Therapeutic interventions after spinal cord injury. Nat Rev Neurosci 7: 628–643.

Torper O, Ottosson DR, Pereira M, et al. 2015. In vivo reprogramming of striatal NG2 glia into functional neurons that integrate into local host circuitry. Cell Rep 12:474–481.

Wen Z, Christian KM, Song H, Ming GL. 2016. Modeling psychiatric disorders with patient-derived iPSCs. Curr Opin Neurobiol 36:118–127.

Wernig M, Zhao JP, Pruszak J, et al. 2008. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc Natl Acad Sci U S A 105:5856–5861.

Winkler C, Kirik D, Bjorklund A. 2005. Cell transplantation in Parkinson's disease: how can we make it work? Trends Neurosci 28:86–92.

Yiu G, He Z. 2006. Glial inhibition of CNS axon regeneration. Nat Rev Neurosci 7:617–627.

Zhou FQ, Snider WD. 2006. Intracellular control of developmental and regenerative axon growth. Philos Trans R Soc Lond B Biol Sci 361:1575–1592.

51

Sexual Differentiation of the Nervous System

Few words are more loaded with meaning than the word "sex." Sexual activity is a biological imperative and a major human preoccupation. The physical differences between men and women that underlie partner recognition and reproduction are obvious to all of us, and their developmental origins are well understood. In contrast, our understanding of behavioral differences between the sexes is primitive. In many cases, their very existence remains controversial, and the origins of those that have been clearly demonstrated remain unclear.

In this chapter, we first briefly summarize the embryological basis of sexual differentiation. We then discuss at greater length the behavioral differences between the two sexes, focusing on those differences or dimorphisms for which some neurobiological basis has been found. These dimorphisms include physiological responses (erection, lactation), drives (maternal behavior), and even more complex behaviors (gender identity). In analyzing these dimorphisms, we will discuss three issues.

First, what are the genetic origins of sexual differences? Human males and females have a complement of 23 chromosomal pairs, and only one differs between the sexes. Females have a pair of X chromosomes (and are therefore XX), whereas males have one copy of the X chromosome paired with a Y chromosome (XY). The other 22 chromosome pairs, called *autosomes*, are shared between males and females. We will see that the initial genetic determinants arise from a single gene on the Y chromosome, while later ones arise indirectly from sex-specific patterns of expression imposed upon other genes as development proceeds.

Second, how are sexual differences initiated by the Y chromosome translated into differences between the brains of men and women? We will see that key intermediates are the sex hormones, a set of steroids that