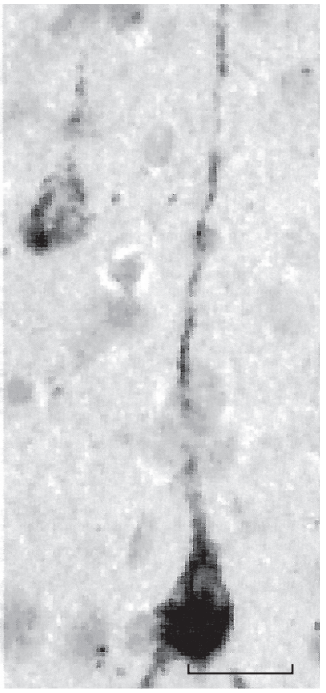
SECTION 5

APPLICATION OF BIOREGENERATIVE ENGINEERING TO ORGAN SYSTEMS

13

NERVOUS REGENERATIVE ENGINEERING



τ-protein-based tangles found in the inferior temporal gyrus of the human brain in Alzheimer's disease, stained with an antibody against the *N*-terminal sequences of the τ protein. Scale bar: 20 μm (Reprinted with permission from Horowitz PM et al: Early N-terminal changes and caspase-6 cleavage of τ in Alzheimer's disease, *J Neurosc* 24:7895–902, copyright 2004.) See color insert.

Bioregenerative Engineering: Principles and Applications, by Shu Q. Liu

The nerve system controls the activity and function of peripheral tissues and organs. The disorder of the nerve system often leads to the malfunction of not only the nervous system but also the peripheral tissues and organs. Neural disorders can be induced by a variety of factors, ranging from mechanical injury to gene mutation. In spite of extensive investigations, the mechanisms of most neural disorders remain poorly understood. Few effective approaches have been established for the treatment of neural diseases. Recent work in neural regenerative medicine and engineering has provided a basis for the establishment of molecular and cell regenerative engineering approaches for the treatment of neural disorders. Although most of these approaches have not been tested in clinical trials, preliminary investigations have demonstrated the potential of using these approaches for therapeutic purposes. In this chapter, we will focus on the organization and function of the nerve system, the pathogenic mechanisms and conventional treatment of common neural disorders, as well as recent development in nerve regenerative engineering.

ANATOMY AND PHYSIOLOGY OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEMS [13.1]

Neural Cells

The nerve system is composed of neurons and nonneuronal cells. *Neurons* are cells that receive and process signals and emit signals to control the activity and function of effector cell types. *Nonneuronal cells* are known as glial cells, which provide support and protection to the neurons.

Neurons. A neuron is composed of several parts: a cell body, short processes known as dendrites, and a long process known as the *axon*, which is also referred to as a *nerve fiber* (Fig. 13.1). The cell body contains typical cellular components such as the nucleus,

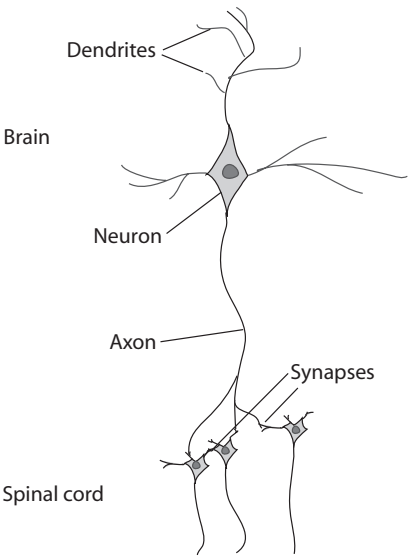


Figure 13.1. Schematic representation of the structure of neurons.

endoplasmic reticulum, Golgi apparatus, endosomes, mitochondria, and cytoskeletal filaments, including actin filaments, neurofilaments, and microtubules. Note that the neurofilaments are intermediate filaments in the neuron. *Dendrites* are short processes that are extensions from the cell body and are responsible for communication with other neurons and transmission of signals from and to the cell body. The *axon* is a long process extended from the cell body, and transmits signals (action potentials) from the cell body to peripheral effector cells via synapses (Fig. 13.2). Chemical substances such as acetylcholine and norepinephrine (Fig. 13.3), known as neurotransmitters, are required for signal transmission through the synapses. Action potentials from the presynaptic terminal stimulate the release of a neurotransmitter, which in turn activates the postsynaptic cell membrane to elicit action potentials. In the central nervous system, synapses are found between different neurons. In the peripheral systems, synapses can be found between peripheral neurons as well as between neurons and skeletal muscle cells. An axon is enclosed within a myelin sheath formed by a glial cell type known as *Schwann cells* in the peripheral systems.

On the basis of function, neurons are classified into sensory neurons, motor neurons, and interneurons. The *sensory neurons*, also known as *afferent neurons*, receive and transmit signals from the peripheral systems to the central nervous system. The *motor neurons*, known as *efferent neurons*, process received signals and emit signals that control peripheral cells such as skeletal muscle cells and gland cells. The *interneurons* transmit signals between different neurons.

Based on the structure of the cell, neurons can be classified into three types: unipolar, bipolar, and multipolar neurons. *Unipolar neurons* exhibit only a single process from the

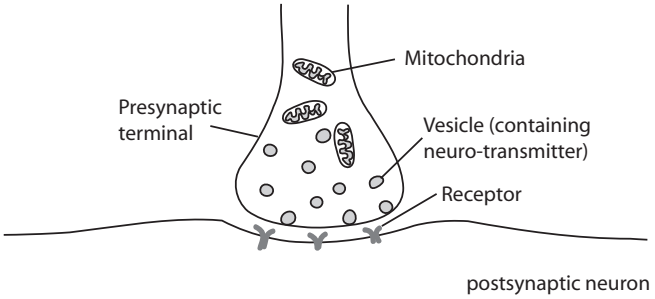
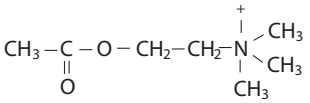


Figure 13.2. Schematic representation of the neuronal synapse. Based on bibliography 13.1.

Acetylcholine



Norepinephrine

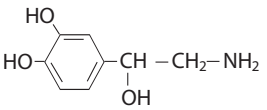


Figure 13.3. Chemical structure of acetylcholine and norepinephrine.

cell body. The process is divided into two branches; one reaches a peripheral tissue with its end structures serving as sensory receptors and the other branch transmits sensory signals to the central nervous system. These neurons often serve as sensory neurons. *Bipolar neurons* possess an axon and a dendrite. The dendrite receives signals from a peripheral tissue and the axon transmits the signals to the central nerve system. This type of neuron also serves as sensory neuron. *Multipolar neurons* possess one axon and more than one dendrites. These are the most popular neurons and are found in the brain and spinal cord.

Glial Cells. Glial cells support and protect the neurons. There are four types of glial cells: astrocyte, microglia, oligodendrocyte, and ependymal cell. *Astrocytes* are glial cells with multiple processes and are often distributed around the neurons and capillaries. These cells participate in the regulation of the brain fluid composition. *Microglial cells* are macrophages of the central nervous system and are capable of phagocytosing and degrading microorganisms, cell debris, and harmful substances in the brain. *Oligodendrocytes* are cells that wrap axons and form axon myelin sheaths in the central nervous system (note that the cell type that wraps peripheral nerve axons is known as the *Schwann cell*). *Ependymal cells* are cells that line the ventricle cavities of the brain and the central canal of the spinal cord. These cells secrete cerebrospinal fluid in the brain ventricles.

Organization of the Nervous System

The nervous system is composed of two subdivisions: the central and peripheral nervous systems. The *central nervous system* includes the brain and spinal cord, whereas the *peripheral nerve system* contains neural cells and structures external to the central nervous system, including peripheral sensory receptors, ganglia, and nerve fibers.

Brain. The brain is composed of four major parts: cerebrum, diencephalon, cerebellum, and brainstem and is the center of the nervous system.

Cerebrum. The *cerebrum* includes the left and right hemispheres. Each hemisphere is composed of a number of lobes, including the *frontal lobe* (controlling thinking, motivation, mood, judgment, and the voluntary motor function), *parietal lobe* (controlling the integration of sensory information except for smelling, hearing, and vision), *occipital lobe* (controlling the integration of visual information), and *temporal lobe* (controlling memory and the integration of hearing and smelling).

Each lobe is composed of three distinct structures: the cortex, cerebral medulla, and cerebral nuclei. The *cortex* is the external layer of cerebrum known as the *gray matter*, which contains primarily neurons. The *cerebral medulla* is the structure between the cortex and the cerebral nuclei, known as *white matter*, and is composed of nerve fibers that link various parts of the central nervous system. The nerve fibers that connect neurons within the same hemisphere are defined as *association fibers*, those that connect the two hemispheres are *commissural fibers*, and those that connect the cerebrum and other parts of the brain are *projection fibers*. Each hemisphere contains a set of *basal nuclei*, which are neuron-containing gray matter, located laterally in regions inferior to the cerebrum and diencephalon. These nuclei include the lentiform nucleus, caudate nucleus, subthalamic nucleus, and substantia nigra. The lentiform and caudate nuclei together are known as the *corpus striatum*. The main function of the basal nuclei is to control motor activities.

The cerebrum also includes a limbic system. The *limbic system* is composed of certain cortical regions, certain nuclei such as the anterior nucleus of the thalamus and the habenular nucleus of the epithalamus, certain basal nuclei, the hypothalamus, the olfactory cortex, and nerve fibers that link various cortical regions and nuclei. The limbic system controls survival functions such as reproduction, memory, and nutrition-related activities.

Diencephalon. The *diencephalon* is a structure located between the cerebrum and the brainstem. It is composed of four parts: thalamus, subthalamus, epithalamus, and hypothalamus. The *thalamus* includes a group of nuclei: the medial geniculate, lateral geniculate, ventral posterior, ventral anterior, ventral lateral, lateral dorsal, and lateral posterior nucleus. The overall function of thalamus is to receive and process sensory information from the peripheral nervous system. Auditory information goes to the medial geniculate nucleus and visual information goes to the lateral geniculate nucleus. The ventral anterior and lateral nuclei participate in the regulation of motor activities. The lateral dorsal nucleus is linked to the cerebral cortex and other thalamus regions and is related to the control of emotion. The lateral posterior nucleus is responsible for the integration of sensory inputs from the peripheral nervous system.

The *subthalamus* is a structure below the thalamus and is composed of subthalamus nuclei and several nerve fiber bundles. The subthalamus participates in the regulation of motor activities. The *epithalamus* is a structure located above the thalamus and is composed of habenular nuclei and the pineal body. The habenular nuclei participate in the processing of smell and odor inputs. The pineal body may be involved in the regulation of the sleep cycle.

The *hypothalamus* is composed of several nuclei and nerve fiber bundles. The nuclei include the anterior, supraoptic, paraventricular, dorsomedial, posterior, and ventromedial nucleus, and the mammillary body. These nuclei play critical roles in the regulation of several functions, including cardiac contractility, bloodflow control, urine release, ion balance, sexual activity, sweat production, food and water intake, feeling and emotion, and sleep cycle. One of the most important functions for hypothalamus is to control hormone secretion from the *pituitary gland*, a structure located below the hypothalamus and connected to the hypothalamus via the infundibulum. The hypothalamus produces and releases a number of hormones, including growth hormone-releasing hormone, growth hormone-inhibiting hormone, gonadotropin-releasing hormone, corticotropin-releasing hormone, and thyroid-releasing hormone. These hormones can act on hormone-generating cells in the pituitary gland and control the secretion of the pituitary gland. The growth hormone-releasing hormone stimulates the secretion of growth hormone from the pituitary gland, whereas the growth hormone-inhibiting hormone exerts an opposite effect. The gonadotropin-releasing, corticotropin-releasing, and thyroid-releasing hormones stimulate the release of corresponding hormones from the pituitary gland. The pituitary hormones play a critical role in regulating a variety of functions, ranging from growth, water absorption, thyroid hormone secretion from the thyroid gland, glucocorticoid hormone secretion from the cortex of the adrenal gland, and follicle maturation and estrogen secretion in ovaries and sperm cell production in testes.

Cerebellum. The *cerebellum* is a structure located below the cerebrum and behind the brainstem. As the cerebrum, the cerebellum contains gray cortex, nuclei, and white medulla. The cerebellum also contains nerve fiber bundles that connect the cerebellum

to other parts of the brain, including the cerebrum, brainstem, and diencephalon. The cerebellum is divided into three parts: the flocculonodular lobe, vermis, and two symmetric lateral hemispheres. The flocculonodular lobe is involved in the regulation of body balance and eye movement. The vermis and part of the lateral hemispheres are responsible for the control of the posture, movement, and motion coordination. The remaining lateral hemispheres, together with the cerebrum, control the process of learning complex skills.

Brainstem. The *brainstem* is a structure that connects the spinal cord to the brain and is composed of the medulla oblongata, pons, and midbrain (Fig. 13.4). The brainstem is responsible for the regulation of physiological activities that are critical to the survival of the body, such as cardiac contraction, the maintenance of bloodflow and pressure, respiration, swallowing, and vomiting. The *medulla oblongata* is the lower part of the brainstem and contains a number of medullary nuclei. These nuclei serve as central nerve regulatory centers that are composed of neuron-containing gray matter and control the activity of the heart and blood vessels. The medulla oblongata also contains ascending and descending nerve fiber bundles, which connect the spinal cord to the brain. It is important to note that the medullary descending nerve fibers originated from the left and right hemispheres are projected to the opposite side and cross at the lower part of the medulla oblongata. Thus, nerve fibers from the left hemisphere control the activities of the right body, whereas those from the right hemisphere control the activities of the left body. The brainstem also contains nuclei that control the body balance and motion coordination.

The *pons* is a structure located in the middle of the brainstem and contains a number of nuclei and ascending and descending nerve bundles. Some of the nuclei serve as the central nerve centers that control respiration and the sleep cycle. Other nuclei mediate the communication between the cerebrum and cerebellum. A number of cranial nerve nuclei, including those for nerve VI (abducens), VII (facial), VIII (vestibulocochlear), and IX (glossopharyngeal), are located in the pons (see below).

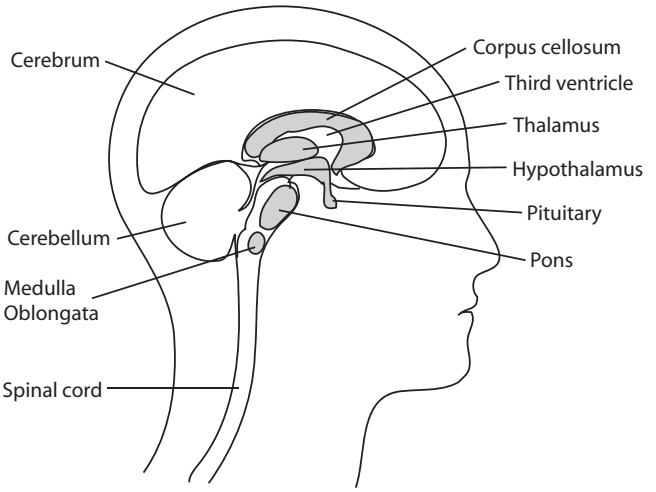


Figure 13.4. Schematic representation of the midbrain. Based on bibliography 13.1.

The *midbrain* is a structure located above the pons and contains the nuclei of cranial nerve III (oculomotor), IV (trochlear), and V (trigeminal). These nerve fibers are described in the following section. The midbrain also contains nuclei that receive and process hearing information and serve as the control center for the auditory system. The ascending and descending nerve fiber bundles pass through the midbrain. In addition, there are scattered neurons throughout the brainstem. These neurons constitute a structure called reticular formation or system. This system controls the consciousness and sleep–wake cycles.

Cranial Nerves. Cranial nerves are nerve fiber bundles that arise directly from the brain, instead of the spinal cord. There are 12 pairs of cranial nerves (Fig. 13.5). Some of these nerves are responsible for the transmission of sensory and somatic motor information, while others transmit information for the parasympathetic system, which regulates the function of the heart, large blood vessels, and glands.

Cranial nerves I and II arise from the lower part of the cerebrum and are responsible for receiving and processing smell and visual information, respectively. These nerves are known as olfactory and optic nerves. The remaining 10 nerves are from the brainstem. *Cranial nerve III* is known as oculomotor nerve and is responsible for regulating the activities of the eye. This nerve contains parasympathetic nerve fibers, which regulate the contractility of smooth muscle cells in the eye and therefore control the size of the pupil and the shape of the eye lens. *Cranial nerve IV* (trochlear nerve) controls the contraction of eyeball muscles and thus the eyeball movement. *Cranial nerve V* (trigeminal nerve) controls the contraction of ear and throat muscles and also controls the proprioceptive and cutaneous sensory function. *Cranial nerve VI* (abducens), together with nerve IV, controls the movement of the eyeball. *Cranial nerve VII* (facial nerve) controls the contraction of facial, ear, and throat muscles. *Cranial nerve VIII* (vestibulocochlear nerve) is responsible for receiving and processing hearing and balancing information from the ear. *Cranial nerve IX* (glossopharyngeal nerve) controls the contraction of pharynx muscles and the secretion of salivary glands. *Cranial nerve X* (vagus nerve) is a parasympathetic, motor, sensory nerve, controls the muscle contraction of the pharynx/larynx, regulates the cardiac activity, and mediates the contractility of smooth muscle cells in the blood vessel wall and the gastrointestinal wall. *Cranial nerve XI* (accessory nerve) is composed of cranial

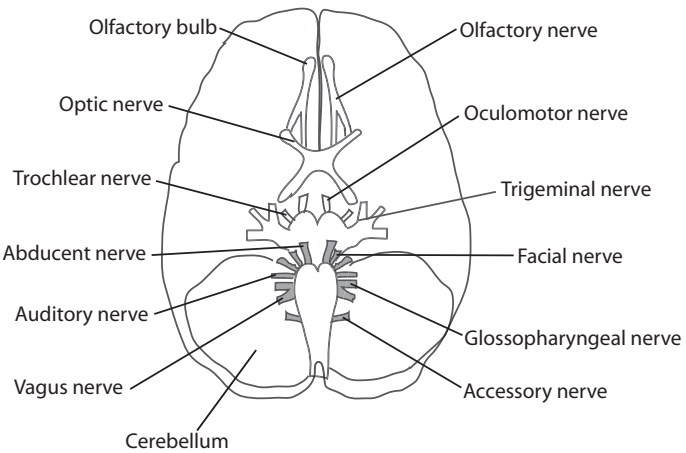


Figure 13.5. Schematic representation of the cranial nerves. Based on bibliography 13.1.

and spinal nerve fibers. The cranial nerve fibers contribute to the vagus nerve and regulate the contractility of smooth muscle cells. The spinal fibers control the movement of the neck and shoulder muscles. *Cranial nerve XII* (hypoglossal nerve) controls the contraction of the tongue and the geniohyoid muscles.

Cerebral Circulation. There is a rich network of blood vessels in the brain. The main arteries that supply blood to the brain are the left and right *common carotid arteries*, which originate from the aortic arch, and the left and right *vertebral arteries*, which originate from the subclavian arteries. Each common carotid artery is divided into the internal and external arteries before entering the cranial cavity. The *internal carotid arteries* enter the cranial cavity and supply blood to the brain, whereas the *external carotid arteries* supply blood to the facial tissues. The vertebral arteries enter the cranial cavity and join together to form the *basilar artery.* The internal carotid arteries and the basilar artery join together to form the *cerebral arterial circle.* Branches from the cerebral arterial circle supply blood to the brain. The cortex of each hemisphere is supplied by the anterior, middle, and posterior cerebral arteries from the cerebral arterial circle.

Arteries are eventually divided into numerous capillaries. The endothelium of the capillaries in the brain possesses well-developed tight junctions, constituting the *blood–brain barrier.* This barrier allows only lipid-soluble substances, such as CO_2, CO, ethanol, and nicotine, to freely pass. Small water-soluble substances, such as amino acids and glucose, can be transported across the blood–brain barrier via active energy-consuming processes. Large molecules and particles, such as viruses and bacteria, cannot pass the blood–brain barrier under physiological conditions.

Blood from the brain is collected by small veins from the capillary network. The small veins conduct blood to a number of cranial venous sinuses. There are several small and medium-sized sinuses, including the superior sagittal, inferior sagittal, straight, cavernous, and occipital sinuses. Venous blood from these sinuses converges to larger sinuses, including the *transverse* and *sigmoid sinuses*, which drain blood into the *internal jugular vein*. Venous blood eventually returns to the right atrium via the subclavian and brachiocephalic veins and the superior vena cava.

Meninges and Cerebral Ventricles. Meninges are membranes of connective tissue, which cover and protect the brain and spinal cord. There are three meningeal layers: the dura mater/periosteum, arachnoid mater, and the pia mater. The *dura mater* is the outmost layer, which folds and extends into the brain fissues at three locations: the falx cerebri (between the two cerebral hemispheres), tentorium cerebelli (between the cerebrum and cerebellum), and falx cerebelli (between the two cerebellar hemispheres). The dura mater is attached to the periosteum of the cranial cavity, forming an integrated functional layer. The *arachnoid mater* is a thin membrane, which is separated from the dura mater by the subdural space filled with serous fluid. The *pia mater* is a membrane that is tightly attached to the brain. The pia mater is separated from the arachnoid mater by the subarachnoid space, which is filled with fluid and contains blood vessels.

The brain contains a number of cavities. These include the two *lateral ventricles* (one in each cerebral hemisphere), the *third ventricle*, and the *fourth ventricle*. The four ventricles are connected via two channels. The two lateral ventricles and the third ventricle are connected by a short channel known as the *interventricular foramina*. The third and fourth ventricles are connected by a long channel called the *cerebral aqueduct*. The fourth ventricle is continuous with the subarachnoid space and the spinal cord.

The brain ventricles, spinal cord, and subarachnoid space are filled with *cerebrospinal fluid,* which is similar in composition to the serum except that the cerebrospinal fluid contains a very low concentration of proteins. The cerebrospinal fluid is produced in the ventricles by a structure known as the *choroid plexus*, which contain capillaries and connective tissue covered by ependymal cells. The cerebrospinal fluid flows through the ventricles, leaves the fourth ventricle via several apertures, including the median aperture and two lateral apertures, flows through the subarachnoid space, and enters the dural venous sinuses, where cerebrospinal fluid joins the blood.

Spinal Cord. The *spinal cord* is a part of the central nervous system and extends from the brainstem to the vertebral column. At each transverse level, the spinal cord is composed of central gray matter and peripheral white matter. The *gray matter* consists of neurons, glial cells, and nerve axons, whereas the *white matter* consists of nerve axons. *Spinal nerves* arise from a large number of rootlets of the gray matter symmetrically at two dorsal and two ventral sites. Nerve fibers from several rootlets combine to form a nerve bundle, which leaves the spinal cord through the vertebral column. The spinal cord is vertically composed of several segments, known as the *cervical*, *thoracic*, *lumbar*, and *sacral segments*. The spinal cord gives rise to 31 pairs of spinal nerve bundles. Each pair of nerve bundles leaves the vertebral column at a corresponding vertebra. These nerves innervate peripheral tissues and organs.

At each level, the gray matter of the spinal cord is divided into two functional units: sensory and somatic motor control units. The sensory unit is located in the two dorsal sites symmetrically, whereas the somatic motor control unit is located in the two ventral sites. The dorsal nerve bundles contain input nerve fibers that transmit sensory signals from the peripheral receptors to the dorsal sensory ganglia, whereas the ventral nerve bundles contain output nerve fibers that transmit signals from the ventral motor control centers to the peripheral muscles. While the somatic motor control neurons are located within the gray matter, the sensory receiving and processing neurons are located in the dorsal root ganglia, which is located outside the spinal cord. The sensory neurons transmit signals from the ganglia to the dorsal gray matter of the spinal cord.

The Peripheral Nervous System. The peripheral nerve system is composed of neurons and nerve bundles outside the brain and spinal cord. A peripheral nerve bundle contains several structures: Schwann cell-enclosed nerve axons, blood vessels, and connective tissue. Each Schwann cell/axon bundle is enclosed with a connective tissue membrane known as endoneurium. A group of axons is enclosed within a perineurium membrane, forming a nerve fascicle. A number of axon fascicles are enclosed within an epineurium sheath, forming a nerve bundle. The connective tissue membranes protect the nerve fibers from injury.

The peripheral nerve fibers from the spinal cord are divided into various groups, based on the origin of the nerves and region of innervation. Each group is defined as a plexus, which contains several nerve bundles from a number of vertebrae. Major nerve plexuses include the cervical, brachial, thoracic, lumbosacral, coccygeal plexuses. The cervical plexus contains nerves from cervical vertebrae 1–4 and innervates the head and neck skin, muscle, and tissue. The brachial plexus originates from cervical vertebra 5 and thoracic vertebra 1, and innervates the upper limbs and part of the head. The thoracic plexus originates from thoracic vertebrae 1–12, and innervates the chest skin, muscle, and tissue. The lumbosacral plexus is from lumbar vertebrae 1–4 and sacral vertebrae 1–4, and innervates

the abdomen and lower limbs. The coccygeal plexus is from sacral vertebrae 4 and 5, and innervates the muscles of the pelvic floor and the coccyx.

Functional Integration of the Nervous System

Sensory Input. The nervous system has a number of sensory functions: hearing, visualizing, touching, smelling, tasting, sensing temperature, and sensing pain. All sensory activities are triggered by environmental stimulations. To sense and react to a stimulus, it is necessary to have a complete sensory system, including the *sensory receptors* that sense the environmental stimulus and covert the stimulus to action potentials, *sensory input nerves* that transmit the action potentials to the central nerve system, *sensory centers* that receive and process the input signals, and *neuronal centers* that respond to the sensory centers and generate signals for the awareness of the environmental stimulation and for corresponding responsive actions (Fig. 13.6).

Sensory receptors are the ending structures of the axon and dendrites. There are various types of sensory receptors. Based on the location, sensory receptors can be divided into three groups: exteroreceptors, visceroreceptors, and proprioceptors. The *exteroreceptors* are distributed in the superficial tissues such as the epidermis and dermis, and are responsible for sensing external stimulations. The *visceroreceptors* are found in the viscera and internal organs, and are responsible for sensing internal stimulations. The *proprioceptors* are found in connective tissues, such as the joint, tendon, and bone, and provide information about the body position and movement.

Based on the function, sensory receptors can be classified into mechanoreceptors, chemoreceptors, thermoreceptors, photoreceptors, and pain receptors. The

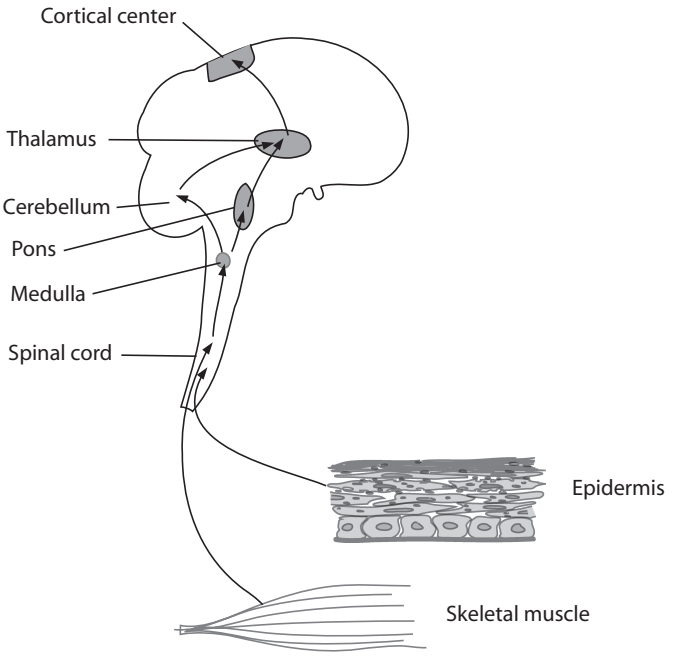


Figure 13.6. Schematic representation of the pathways of sensory signals. Based on bibliography 13.1.

mechanoreceptors respond to mechanical stimuli, such as tension, compression, and shearing, and are found in the epidermal tissue, muscle, inner ear, and internal organs. These receptors are responsible for the sensing of mechanical contacts and air vibrations (sounds). The *chemoreceptors* sense chemical stimuli, are responsible for the activities of smelling and tasting, and are found in the epidermal tissue. The *thermoreceptors* are responsible for the sensing of temperature and are found in the epidermal tissue. The *photoreceptors* sense light and are found in the retina. The *pain receptors* sense mechanical, chemical, and thermal stimuli and are found in almost all types of tissues.

The sensory signals sensed by the receptors are transmitted to the central nerve system via sensory nerves. There are various sensory pathways in the spinal cord and brainstem. These pathways are composed of neurons that are specific to sensory receptors. Each type of neuron is responsible for the transmission of a specific sensory signal. There are several ascending sensory pathways that transmit signals from the spinal cord to various parts of the brain. These include the spinothalamic pathway (transmitting pain, temperature, light, and mechanical signals from the spinal cord to the thalamus), the dorsal-column/medial–lemniscal pathway (transmitting proprioception, pressure, and vibration signals from the spinal cord to the brainstem), the spinocerebellar pathway (transmitting proprioception signals from the spinal cord to the cerebellum), and the spinoreticular pathway (transmitting tactile signals from the spinal cord to the reticular formation of the brainstem).

Sensory signals from the peripheral nerve system and the spinal cord are eventually converged to the primary sensory areas of the cerebral cortex. Each type of sensory input is projected to a designated cortical area. For instance, the somatic cortical area is located in the middle region of the cerebral cortex. Other regions are illustrated in Fig. 13.7. This area is organized in relation to the distribution of the peripheral tissues and organs. The sensory inputs from the head and face are transmitted to the inferior cortical area, whereas the sensory inputs from the lower limbs are projected to the superior cortical area. The sensory inputs from other organs are projected to corresponding areas between the head and the lower limbs.

Motor Control. The nervous motor system controls the contractile activities of the skeletal muscle cells by emitting action potentials and thus regulates the movement, posture, and balance of the body. There are two types of movements: involuntary and voluntary. An *involuntary movement* is controlled by the spinal cord motor centers and includes

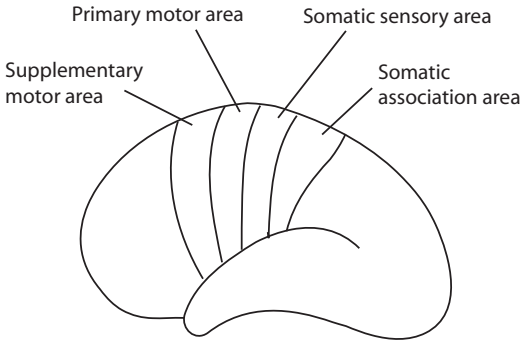


Figure 13.7. Schematic representation of the distribution of the motor and sensory control regions in the cortex. Based on bibliography 13.1.

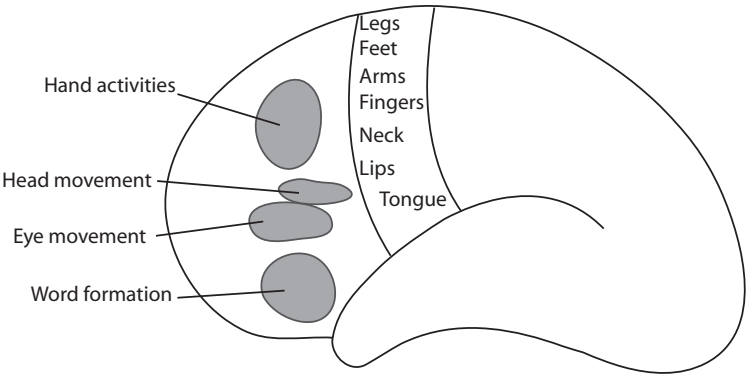


Figure 13.8. Schematic representation of the distribution of the cortical motor control regions. Based on bibliography 13.1.

primarily reflexes of the skeletal muscle system. The reflex movement is not initiated through conscious judgment by the cortex. It should be noted that the heart beating, gastrointestinal motion, and blood vessel contraction are also involuntary movements, but these are controlled by the parasympathetic nerve system, not by the motor system. In contrast, a *voluntary movement* is consciously initiated and controlled by the cortex to achieve specified goals, such as running, walking, eating, writing, and talking.

Voluntary movements are induced in response to the action potentials initiated from the *primary motor area* located anterior to the primary sensory region of the cortex (Fig. 13.8). The primary motor area is organized in relation to the distribution of the peripheral tissues and organs. The action potentials initiated from the superior region of the primary motor area controls the muscles of the lower limbs, whereas the action potentials from the inferior area of the cortex controls the muscles of the head, face, and upper limbs. There is another cortical area, called *premotor area*, which is located anterior to the primary motor area and regulates the coordination of various muscles, ensuring accurate movements. The third important motor-control area is the *prefrontal area*, which is responsible for the regulation of mood- and emotion-related movements.

The motor-control signals or action potentials are transmitted from the cortical motor control centers to the brainstem or spinal cord, and then to the peripheral skeletal muscles via motor nerves (Fig. 13.9). The motor nerves are divided into direct and indirect descending nerves. The direct nerves are responsible for the control of accurate muscle contraction, muscle tone, skilled movements, and emotion-related facial movements. The indirect nerves control less accurate muscle activities related to overall body movements. The direct nerves exist only in mammals.

Autonomic Control of Vital Activities. Vital activities include the heartbeat, regulation of bloodflow and pressure, and temperature regulation. These activities are primarily controlled by two nervous systems: the sympathetic and parasympathetic nervous systems.

Sympathetic Nervous System. The *sympathetic nerve system* is composed of a number of control units. A typical sympathetic control unit consists of preganglionic neurons and postganglionic neurons (Fig. 13.10). The *preganglionic neurons* are located in the gray matter of the spinal cord in the region from thoracic vertebra 1 to lumbar vertebra 2. The *postganglionic neurons* are located in either a structure near the vertebrate column known

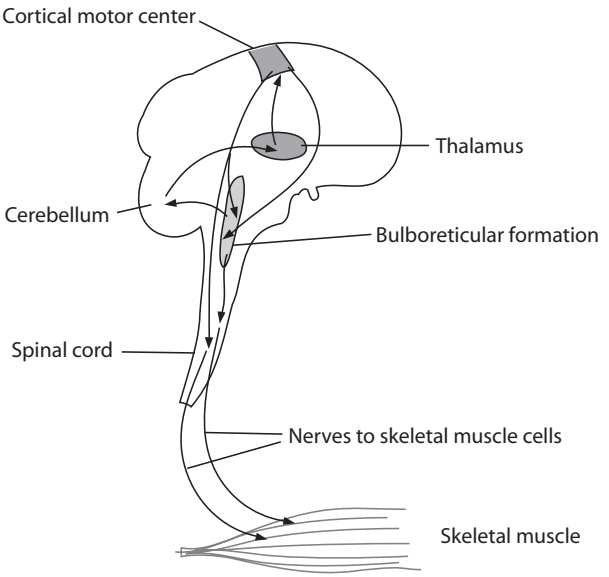


Figure 13.9. Schematic representation of the pathways of motor control signals. Based on bibliography 13.1.

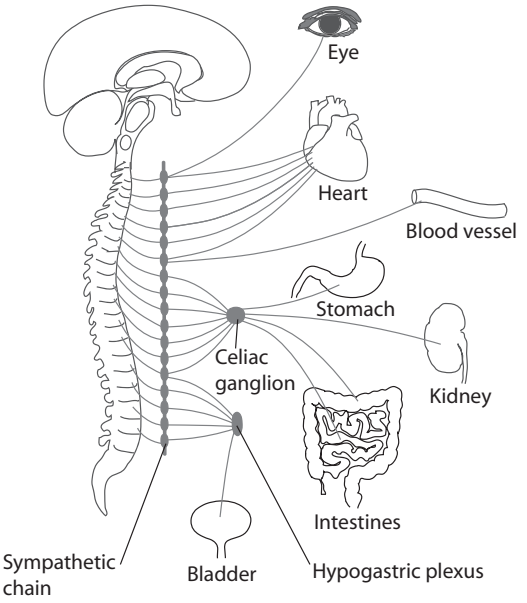


Figure 13.10. Schematic representation of the sympathetic nervous system. Based on bibliography 13.1.

as the *sympathetic chain ganglia* or a structure near the target tissues known as the *collateral ganglia*. The axons of preganglionic neurons join the spinal nerves, leave the spinal cord, and extend to the *sympathetic chain ganglia*. In the sympathetic chain ganglia, the sympathetic nerves are organized into four different groups: the spinal, sympathetic, splanchnic, and adrenal gland nerves.

For the *spinal group*, the preganglionic axons synapse with the postganglionic neurons in the sympathetic chain ganglia. The postganglionic axons leave the sympathetic chain ganglia, join the spinal nerves, and innervate peripheral tissues and organs. For the *sympathetic group*, the preganglionic axons synapse with the postganglionic neurons in the sympathetic chain ganglia. The postganglionic axons leave the sympathetic chain ganglia, form sympathetic nerves, and innervate peripheral tissues and organs. The heart is a major target of the sympathetic nerves. Activation of the sympathetic nerves induces an increase in cardiac contractility and the heartbeat. For the *splanchnic group*, the preganglionic axons enter the sympathetic chain ganglia, but do not form synapses there. They leave the sympathetic chain ganglia, form the splanchnic nerves, extend to the peripheral organs, and enter another sympathetic structure known as the *collateral ganglia*, where they synapse with postganglionic neurons. The postganglionic axons innervate target tissues, including the stomach and intestines. The *adrenal gland nerves* are different form others. The preganglionic axons do not synapse with postganglionic neurons, but directly extend to the adrenal gland and synapse with the adrenal medullar cells, stimulating the secretion of epinephrine and norepinephrine.

Parasympathetic Nervous System. This is another nervous system that controls the vital activities. As the sympathetic nervous system, the parasympathetic nervous system is composed of a number of control units. Each unit consists of preganglionic and postganglionic neurons (Fig. 13.11). The *preganglionic neurons* are located in the brainstem, and the postganglionic neurons are located in a structure near or within the target tissues or organs, known as the *terminal ganglia*. The preganglionic axons leave the brainstem, extend to and enter the terminal ganglia, where they synapse with the postganglionic

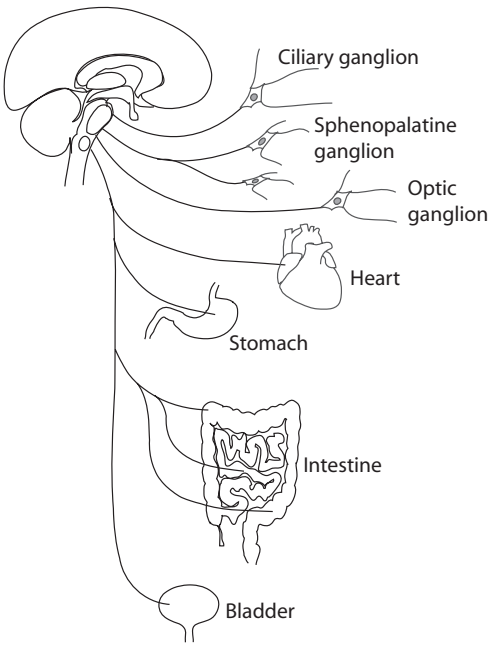


Figure 13.11. Schematic representation of the parasympathetic nervous system. Based on bibliography 13.1.

neurons. The postganglionic axons leave the terminal ganglia and innervate target tissues and organs.

Autonomic Regulation of Cardiovascular Functions. The activities of the heart and blood vessels are controlled by the sympathetic and parasympathetic nervous systems in a coordinated manner. The heart is innervated with both sympathetic and parasympathetic (vagus) nerves. Arteries and veins are primarily innervated with sympathetic nerves. Certain structures, such as the baroreceptors, which attach to the carotid arteries, are innervated with parasympathetic vagus nerves and play a critical role in regulating the cardiovascular activities.

The regulation of the cardiovascular functions is accomplished via autonomic reflexes, or nerve-controlled actions in response to peripheral inputs. In general, the sympathetic nerve system activates, whereas the parasympathetic system inhibits the cardiovascular activities. The two systems regulate the cardiovascular activities in coordination and counterbalance each other's effects. An increase in the activity of the sympathetic system will activate the parasympathetic system via a feedback mechanism, and vice versa. Thus the cardiovascular activities are maintained within a relatively stable range, which is defined as the *physiological range*.

A typical example is baroreceptor-mediated regulation of arterial blood pressure. The baroreceptors detect changes in blood pressure. An increase in blood pressure beyond the physiological level stimulates the baroreceptors. Signals from the baroreceptors are transmitted to the brainstem via the vagus nerve and activate the parasympathetic cardiovascular control neurons. The parasympathetic signals are transmitted to the heart, inducing a decrease in the heartbeat and contractility. As a result, arterial blood pressure decreases. In contrast, a decrease in arterial blood pressure to a level below the physiological level will reduce stimulation to the baroreceptors as well as to the parasympathetic control neurons, resulting in a situation with relatively dominant sympathetic activities. Thus, heartbeat and cardiac contractility both increase simultaneously, leading to an increase in blood pressure.

NERVOUS DISORDERS

Nerve Injury

Etiology, Pathology, and Clinical Features [13.2]

Brain Injury. Brain injuries can be caused by head trauma and skull fractures resulting from physical impacts or penetrations. An injury beyond the tolerant level can cause changes in the structure and function of the brain at the injury site. Open trauma can cause hemorrhage, which significantly compromises the brain function at the hemorrhage site, often leading to dysfunction of the corresponding peripheral systems. Another common type of brain injury is concussion, which is defined as mild reversible brain injury with transient amnesia and loss of consciousness. This type of injury is often caused by blunt impacts on or sudden deceleration of the brain. Usually, concussion is diagnosed when no visible brain damage and hemorrhage are observed. The pathogenic mechanisms for the loss of consciousness are related to the dysfunction of the reticular system in the brainstem due to sudden rotation or movement of the brain. The mechanisms for amnesia are related to the injury of the cerebrum.

Severe head impact or deceleration causes brain contusion, which is often associated with various degrees of apparent brain injury, ranging from small superficial hemorrhage to large area necrotic destruction of the brain structure. The site and degree of brain injury can be detected by CT scan or MRI. This type of injury is often associated with prolonged loss of consciousness and amnesia. Clinical signs are dependent on the injury location and size. In certain cases, diffusive edema may occur within a short period following the trauma due to alterations in blood pressure (hypertension) and microcirculatory function. In addition, glial cells and leukocytes migrate to the injury and hemorrhage sites within hours to days and generate extracellular matrix components, eventually resulting in the formation of fibrous tissue or scars. The scars in the brain are often the causes of post-traumatic epilepsy.

Cranial Nerve Injury. Head trauma, especially basilar skull fractures, is often associated with injury of the cranial nerves, such as the optic, trochlear, olfactory, trigeminal, facial, and auditory nerves. Nerve injuries are often induced by shearing deformation of the skull. In severe cases, the nerve fibers can be completely severed. Nerve injury often induces degradation of the distal nerve fibers in association with biochemical changes, such as reduction in the expression of synaptophysin, which is an axonal marker (Fig. 13.12). Clinical signs are dependent on the type of nerve that is injured and the degree of the injury. For instance, minor injury of the optic nerves may cause blurring of vision, whereas bruising and transecting of the optic nerves will result in partial or complete blindness.

Spinal Cord Injury. Spinal cord injury can be induced by damage to, fracture, or dislocation of the vertebral column. The thoracic spinal cord is often injured by vertical compression. The cervical spinal cord can be injured by flexion. Spinal cord injury can be detected by imaging approaches such as X-ray and MRI. Spinal cord injuries result in functional changes in peripheral sensation and motor control. In severe cases, paralysis occurs in areas below the injured spinal cord.

Immediately following spinal cord injury, hemorrhage may occur, which significantly affect the function of the spinal cord neurons. Other changes include regional spinal cord edema and ischemia during the early period (about 4 h). Peripheral signs, such as demyelination of the peripheral nerves (Fig. 13.13) and regional loss of sensory input and movement, may be observed during this period. Spinal cord injury is usually reversible during the early period. Global infarction or necrosis occurs at the injury site about 8 h after spinal cord injury. Such injury is associated with complete paralysis below the injury site. Complete spinal cord transection or global spinal cord infarction is usually irreversible. At the injury site, glial cells and leukocytes are often activated. These cells can migrate to the damaged tissue and generate extracellular matrix, eventually forming fibrous tissue or scars.

EXPERIMENTAL MODELS OF SPINAL CORD INJURY. Spinal cord injury is often created in small rodents and used to investigate the mechanisms of nerve injury and regeneration. Several types of experimental models have been developed and reported in the literature. These include complete and partial spinal cord transection, blunt contusion, and compression. In the spinal cord *transection model*, the spinal cord is completely transected at a selected location, or selected nerve tracts in the spinal cord are transected. Neuronal growth and axonal regeneration can be observed after transection. In the blunt *contusion model*, a weight impactor can be dropped from a desired height to a segment of exposed

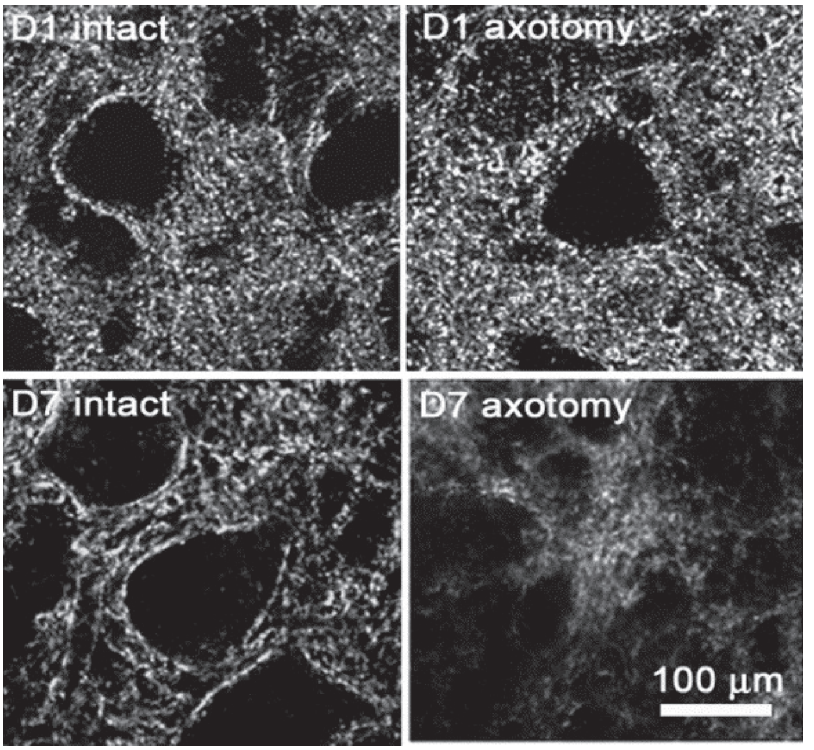


Figure 13.12. Influence of axotomy on the structure of neurons. Confocal microscopy images of facial nuclei at the intact (left) and axotomy (right) sides. D1 and D7 are days 1 and 7, respectively, after axotomy. The specimens were labeled with an antibody against mouse synaptophysin, an axonal marker. The expression level of synaptophysin is apparently reduced at the axotomy side. (Reprinted from Ikeda R, Kato F: Early and transient increase in spontaneous synaptic inputs to the rat facial motoneurons after axotomy in isolated brainstem slices of rats. *Neuroscience* 134:889–99, 2005, copyright 2005, with permission from Elsevier.)

spinal cord, causing blunt injury. The degree of injury can be controlled by altering the weight and height of the impactor. Electronically controlled mechanical impactors can be developed and used to cause blunt injury based on a similar principle. In the *compression model*, a mechanical clip or band can be applied to an exposed spinal cord to induce circumferential compression. The degree of injury can be controlled by altering the level of compression. This model simulates spinal cord injury due to the compression of vertebral columns.

An important issue in spinal cord injury modeling is to estimate the degree of injury or whether the nerve tracts are completely severed. Axonal tracers are often used to achieve such a goal. The nerve axons are capable of conducting retrograde transport of substances toward the cell body. A tracer can be applied to a site distal to the nerve injury. The completeness of transection can be judged by observing the appearance of the tracer in the cell body. Among the three types of model described above, the contusion and compression models usually result in incomplete injury. Because of the complexity of the spinal cord, it is often difficult to estimate the degree of nerve regeneration by using these incomplete injury models.

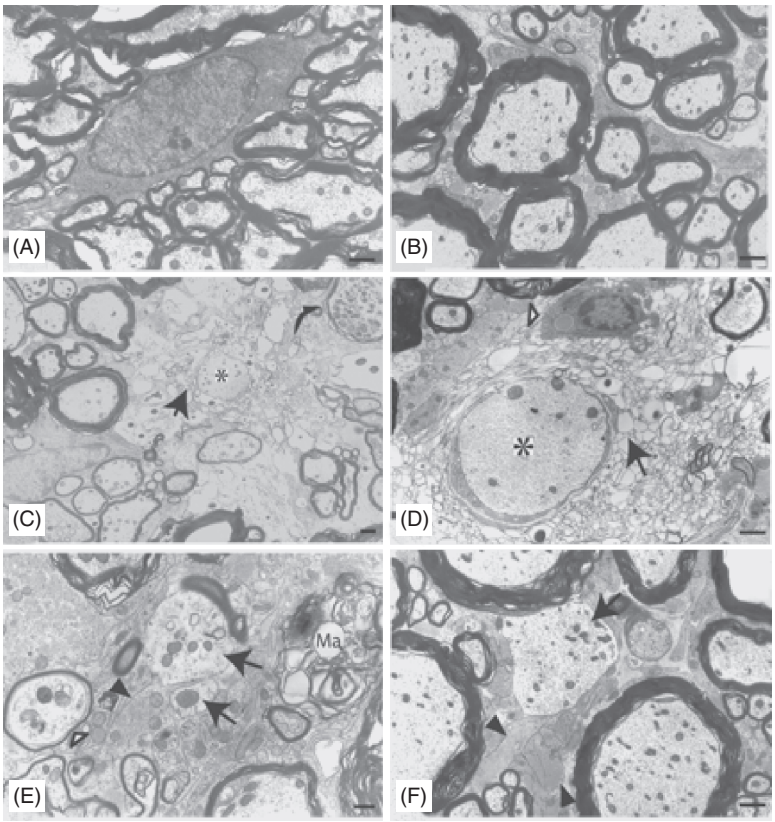


Figure 13.13. Histological micrographs showing demyelination after contusive spinal cord injury. (A,B) Normal myelin sheaths in the ventrolateral funiculus of the adult spinal cord. These sheaths surround axons. (C,D) Demyelination 1 week after contusive spinal cord injury in the ventrolateral funiculus. Many myelin sheaths degenerated and numerous intramyelinic vacuoles were seen (arrows). A macrophage was observed in close relation to degenerating myelin (open arrowhead in panel D). The axons surrounded by degenerating myelin appeared morphologically normal (asterisks). (E,F) Some demyelinated axons survived for at least 1 month after injury (arrows). (E,F) Healthy-appearing demyelinated axons were observed in the ventrolateral funiculus of spinal cord at the injury epicenter (E) and also a few millimeters away from the epicenter (panel F, arrow). Scale bars: 2.5 µm. (Reprinted with permission from Cao Q et al: *J Neurosci* 25:6947–57, copyright 2005.)

Peripheral Nerve Injury. Peripheral nerve injury is largely induced by trauma resulting from accidents and surgery. The peripheral nerves are capable of recovering from injury when they are not completely severed. However, when the nerves are completely severed, the distal axons are usually disintegrated into segments within several days, followed by further degradation of the nerve segments. At the same time, the myelin sheaths of the Schwann cells that enclose the axons are also degraded. The degraded axon and myelin are phagocytosed by macrophages.

The proximal neurons are able to regenerate and extend their axons and restore the lost distal axonal segment (Fig. 13.14). However, the outcome depends on the severity of the injury, especially the distance between the two ends of the severed axon. When the two

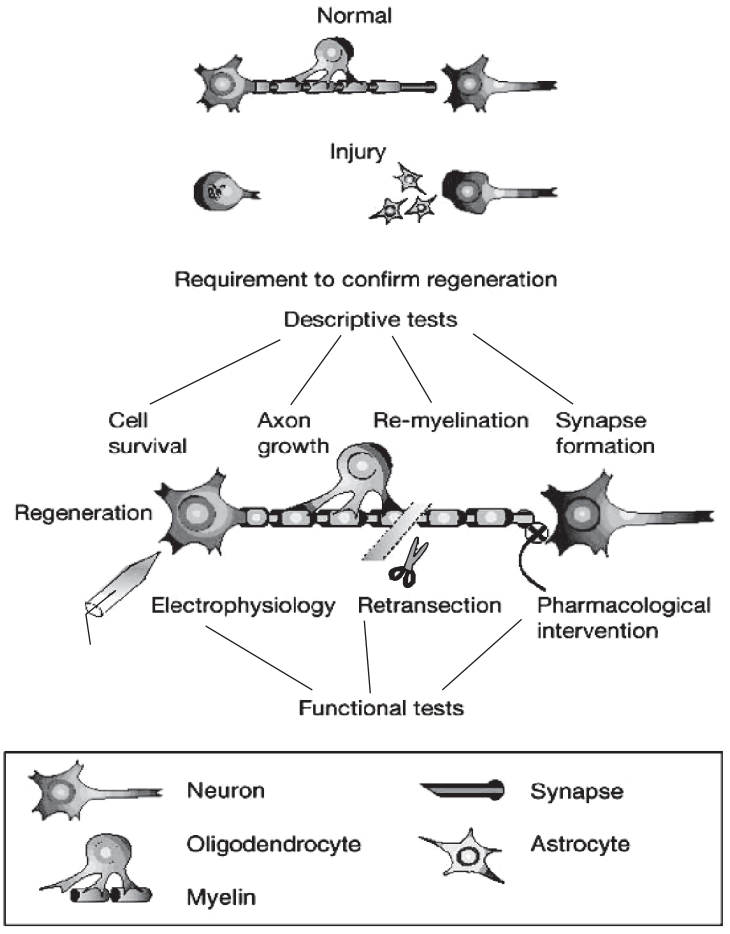


Figure 13.14. Nerve regeneration after nerve injury. A number of descriptive and functional tests have been designed and used for evaluating the outcome of nerve regeneration after nerve injury. Descriptive tests, as shown in the figure, can be used to determine the survival and integrity of the injured system, whether axonal regeneration is present, and if appropriate synaptic connections and remyelination have occurred. Functional tests, incldung electrophysiological and pharmacological intervention, can be used to assess the function and specificity of the regenerated pathway. (Reprinted by permission from Macmillan Publishers Ltd.: Horner PJ, Gage FH: *Nature* 407:963–70, copyright 2000.)

ends are close to each other and well aligned, nerve regeneration and reconnection can occur. In contrast, when the two ends of a severed nerve are separated and not aligned, the injured nerve segment may not be restored. The residual Schwann cells play a critical role in nerve regeneration. These cells start to enlarge and undergo mitosis when the proximal axon starts to regenerate. The Schwann cells are aligned along the injured axons, preparing for the regeneration and reconnection between the proximal and distal axons. The proximal axons that encounter the Schwann cells are able to restore the injured distal axons. However, the proximal axons that are not escorted by Schwann cells cannot reach the severed distal axons.

Nerve regeneration is a slow process. It usually takes about several weeks for the proximal regenerating nerves to reach the injured distal nerves. The end of each proximal nerve may form several sprouts. The sprouts that encounter the Schwann cells develop into axons and replace the severed distal nerves. The remaining sprouts are gradually disintegrated. Following the growth of the proximal axons into the Schwann cells, the regenerating axons are enclosed by newly generated myelin sheaths.

Conventional Treatment of Nerve Injury. For the injury of the central nerve system due to craniocerebral trauma, several approaches can be used for the treatment depending on the severity of the injury. For patients with concussion (transient unconsciousness), no special treatment is needed. Patients are usually given analgesics such as aspirin or acetaminophen for headache, and antidepressants for anxious depression, if any. For severe head injury, especially when patients are unconscious, several treatments should be carried out: (1) check to ensure that the airways are functional and are able to supply adequate ventilation (artificial respiration should be applied, if necessary); (2) stop bleeding, if any; (3) restore blood pressure, if hypotension exists (note that hypertension may exist with severe head injury); (4) restore the cardiac function, if necessary; (5) measure and reduce intracranial pressure when intracranial pressure is too high; (6) supply nutrients via a nasogastric tube, if coma persists; and (7) resort to surgical intervention to stop hemorrhage, relieve intracranial pressure, repair fractured or distorted bones, and/or remove coagulated blood, if necessary. For severed peripheral nerves due to trauma, simple surgical reconnection or grafting of the injured nerves with an autologous nerve segment, if necessary, can be performed. Such an approach usually gives a satisfactory result.

Molecular Nerve Regenerative Engineering [13.3]

Strategies of Molecular Nerve Regenerative Engineering. Principal strategies for the treatment of nerve injury are to promote neuronal survival and regeneration, prevent neuronal death, protect the neurons from secondary injury, stimulate axonal adhesion, extension and reconnection, reduce fibrosis and scar formation, enhance synaptic formation. In conventional medicine, there are few available methods that can be used to effectively achieve these strategies. The recovery of injured neurons and nerve fibers is very much dependent on the self-regeneration capability. For the past decade, regenerative engineering approaches have been developed at the molecular, cellular, and tissue levels to facilitate the regeneration of injured nerves. At the molecular level, mitogenic proteins and genes can be used to promote the survival and regeneration of injured neurons and to prevent neuronal death. At the cellular level, various cell types can be used and transplanted to the injury site to assist the recovery of injured neurons. At the tissue level, various biological and mechanical devices have been constructed to serve as guidance for the regeneration of injured nerve axons. Although most of these engineering approaches have not been used in clinical therapy, experimental investigations have demonstrated potential for clinical applications.

For the central nerve system, because the function of many structures has not been completely understood and the system is difficult to access, it is often a challenge to "engineer" the brain and spinal cord. Thus, engineering treatment for injured brain and spinal cord is limited. In contrast, the peripheral nerve fibers are relatively easy to access, it is not technically difficult to manipulate these fibers. Here, the principle of molecular and cell regenerative engineering for central and peripheral nerve injuries is discussed.

Molecular regenerative engineering approaches can be established and used for facilitating the regeneration of injured neurons and nerve fibers. There are several strategies for the application of molecular engineering approaches to nerve regeneration: preventing cell death, promoting cell survival, and enhancing the differentiation of stem and progenitor cells to neurons and supporting cells. There exist several types of growth factors that are known to promote cell survival and growth, and prevent cell death. Thus, growth factors and their genes are candidate therapeutic factors for the treatment of nerve injury. At the protein level, growth factors can be applied directly to the site of nerve injury to stimulate nerve cell regeneration. At the gene level, selected growth factor genes can be manipulated to enhance the level of gene expression, resulting in an increase in the production of encoded growth factors.

There are several challenges for the application of molecular regenerative engineering approaches to nerve regeneration. These include the selection of therapeutic growth factors and/or genes, the establishment of appropriate techniques for the delivery of the therapeutic factors, and precise delivery of the therapeutic factors to the injury site. The selection of therapeutic factors and genes is dependent on the nature of therapeutic molecules, the degree of nerve injury, and the type of the injured cells. While certain types of growth factors promote nerve growth and regeneration, other types may exert an opposite effect. Fr instance, neurotrophic factors and receptors stimulate nerve regeneration, whereas epidermal growth factor receptor (EGFR) inhibits nerve regeneration. EGFR can be activated or phosphorylated in response to the stimulation of chondroitin sulfate proteoglycans and activates signaling mechanisms that suppress axonal regeneration. Other therapeutic factors and genes are discussed for different cases in the following sections.

There are several approaches for the delivery of therapeutic factors and genes to the site of nerve injury:

1. Therapeutic proteins can be delivered to the nerve system via direct injection to the injury site of the central nervous system. This method gives a local delivery of highly concentrated therapeutic factors. However, it is difficult to find the precise site of injury for injection. Furthermore, direct injection induces nerve injury.
2. Therapeutic factors and genes can be injected into the spinal cavity. This cavity is connected to the four cerebral ventricles and the arachnoid space that cover the cerebrum (see page 507). The delivered factors can reach the cranial cavity via diffusion, where they are taken up by epithelial cells and transported to the nearby nerve cells. This approach is suitable for delivering therapeutic factors to the brain and spinal cord. Compared to the direct injection method, the spinal cavity delivery is a safe and reliable method, although it may require a high dose of growth factors or genes to reach an effective concentration at the injury site.
3. Selected cell types, such as glial cells or fibroblasts, can be cultured and transfected with a therapeutic gene, rendering the cells to express a high level of a desired factor. These cells can be delivered to the site of nerve injury to release the selected therapeutic factor. Direct injection is an effective method for cell delivery.

It should be noted that the bloodstream may be considered an alternative delivery route. Therapeutic factors and genes can be injected into the blood, taken up by endothelial cells, and transported to the target tissues. However, due to the presence of the blood–brain barrier, it may be difficult to deliver therapeutic proteins and genes to the brain system with high efficiency.

Enhancement of Neuron Survival. There are several growth factors that can be used for the enhancement of neuronal cell survival and regeneration. These factors include brain-derived neurotrophic factor (BDNF), neurotrophin (NT)3, neurotrophin 4, nerve growth factor (NGF), and glial cell-derived growth factor (GDNF) (Table 13.1). The functions of these growth factors are briefly discussed as follows.

BRAIN-DERIVED NEUROTROPHIC FACTOR [13.4]. Brain-derived neurotrophic factor is a 247 amino acid protein and a member of the nerve growth factor family. The brain-derived neurotrophic factor gene is mapped to chromosome 11 and locus 11p13 in humans. This factor shares a nucleotide sequence considerably similar to the nerve growth factor gene. The brain-derived neurotrophic factor gene is similar among different mammals. Brain-derived neurotrophic factor is expressed primarily in the cortical neurons. These neurons produce the precursor of brain-derived neurotrophic factor. The precursor is released into the extracellular matrix and cleaved by several enzymes, including serine protease plasmin, matrix metalloproteinases (MMP)7 and MMP3, resulting in the formation of mature brain-derived neurotrophic factor.

Brain-derived neurotrophic factor is necessary for the survival of neurons in the central nervous system. During embryonic development, brain-derived neurotrophic factor plays a critical role in the differentiation and proliferation of neuronal cells and contributes to the development of germ cells. In response to nerve injury, the expression of the BDNF gene is often upregulated, an important mechanism for the self-repair of injured nerves (Fig. 13.15). A treatment with BDNF in cultured dorsal root ganglion explants enhances neurite outgrowth (Fig. 13.16). In neural degenerative disorders, such as Alzheimer's and Huntington's diseases, the expression of brain-derived neurotrophic factor is downregulated, which contributes to the development of these disorders. In transgenic model of brain-derived neurotrophic factor deficiency induced by homologous recombination or site-specific gene targeting in embryonic stem cells, sensory neurons are lost in various areas of the central nerve system. However, the motor neurons are not significantly affected by the deficiency of the brain-derived neurotrophic factor gene.

Brain-derived neurotrophic factor can be released on neuron depolarization, resulting in the activation of intracellular signaling pathways. In dopamine neurons, brain-derived neurotrophic factor stimulates the expression of dopamine D3 receptor during development and adulthood, contributing to the control of animal behavior. In serotonergic neurons, brain-derived neurotrophic factor regulates the expression of several postsynaptic serotonin receptors in the frontal cortex, hippocampus, and hypothalamus. The deficiency of brain-derived neurotrophic factor induces a reduction in the expression of these serotonin receptors, which is associated with behavioral abnormalities such as increased aggressiveness and hyperphagia. These observations suggest that brain-derived neurotrophic factor plays a critical role in the regulation of the development, function, and regeneration of dopaminergic and serotonergic neurons.

NEUROTROPHIN 3 [13.5]. Neurotrophin 3, also known as neurotrophic factor 3, is a growth factor that is similar in structure to nerve growth factor and brain-derived neurotrophic factor. All these factors are members of the nerve growth factor family. Neurotrophin 3 is composed of 258 amino acids and is expressed in the human brain and placenta. The gene of neurotrophin 3 is localized to chromosome 12 and locus 12p13. The gene sequence of neurotrophin 3 is well conserved among mammals. The primary function of

TABLE 13.1. Characteristics of Selected Nerve Growth-Related Factors*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Brain-derived neurotrophic factor	BDNF	247	28	Central nervous system (cortex, retina, and spinal cord), fetal testis	Regulating the survival of neurons, stimulating embryonic development
Ciliary neurotrophic factor	CNTF	200	23	Brain	Promoting neurotransmitter synthesis and neurite outgrowth and regulating the survival of neurons and oligodendrocytes
Glial cell line-derived neurotrophic factor	Astrocyte-derived trophic factor 1 and glial cell-derived neurotrophic factor	211	24	Nervous system, kidney	Promoting the survival and differentiation of dopaminergic neurons and preventing apoptosis of motor neurons

*Based on bibliography 13.4 and 13.8.

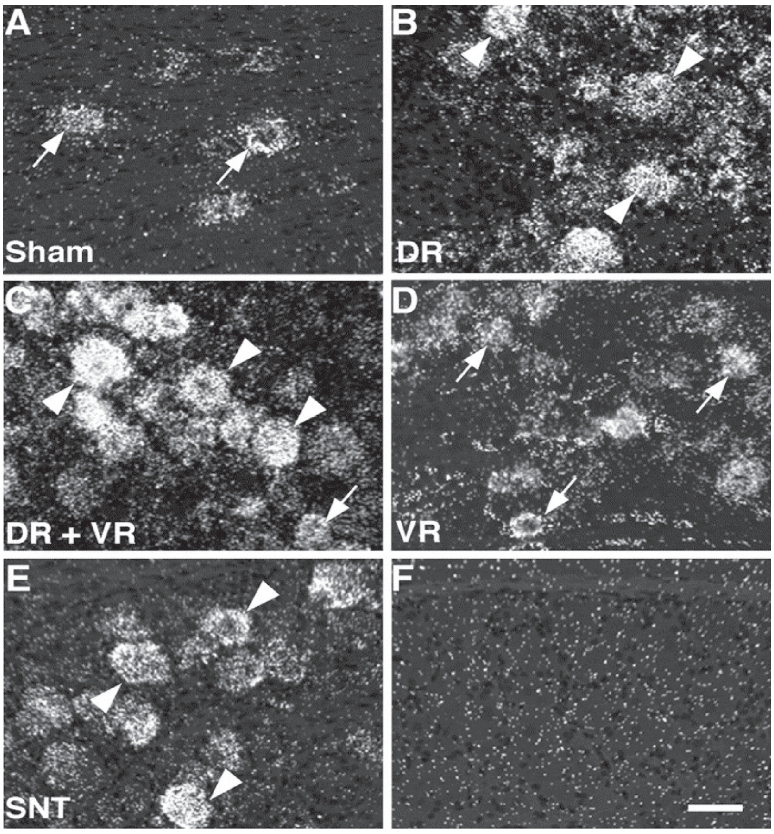


Figure 13.15. Expression of brain-derived neurotrophic factor (BDNF) in injured neurons. (A–E) Expression of BDNF mRNA in the ipsilateral L5 dorsal root ganglion (DRG) by in situ hybridization histochemistry in the sham control (A), dorsal rhizotomy only (B), dorsal rhizotomy + ventral rhizotomy (C), ventral rhizotomy only (D), and spinal nervous transection (E) groups at 7 days after surgery. Arrowheads indicate large-size sensory neurons positive for BDNF, whereas arrows indicate small-to-medium-diameter neurons. These observations show that nerve and spinal cord injury induces upregulation of the BDNF gene. (F) Hybridization with a sense probe for BDNF mRNA showed no positive signals. Scale bar: 50 μm. (Reprinted from Obata K et al: The effect of site and type of nerve injury on the expression of brain-derived neurotrophic factor in the dorsal root ganglion and on neuropathic pain behavior, *Neuroscience* 137:961–70, copyright 2006, with permission from Elsevier.)

neurotrophin 3 is to promote neuronal proliferation, differentiation, and survival as well as the neurite outgrowth during development.

Neurotrophin 3 can interact with the neurotrophin receptor p75 in neurons, exerting a stimulatory effect on neuronal mitogenic activities. In a rat model of spinal cord injury, the transfer of the neurotrophin 3 gene into the spinal cord stimulates the regeneration of injured axons (Fig. 13.17). Neurotrophin 3 (NT3) can also promote axonal growth of injured neurons into neural stem cell (NSC) grafts in injured spinal cord (Fig. 13.18). Furthermore, neurotrophin 3 plays a critical role, together with brain-derived neurotrophic factor, in the regulation of myelin formation by developing and regenerating Schwann

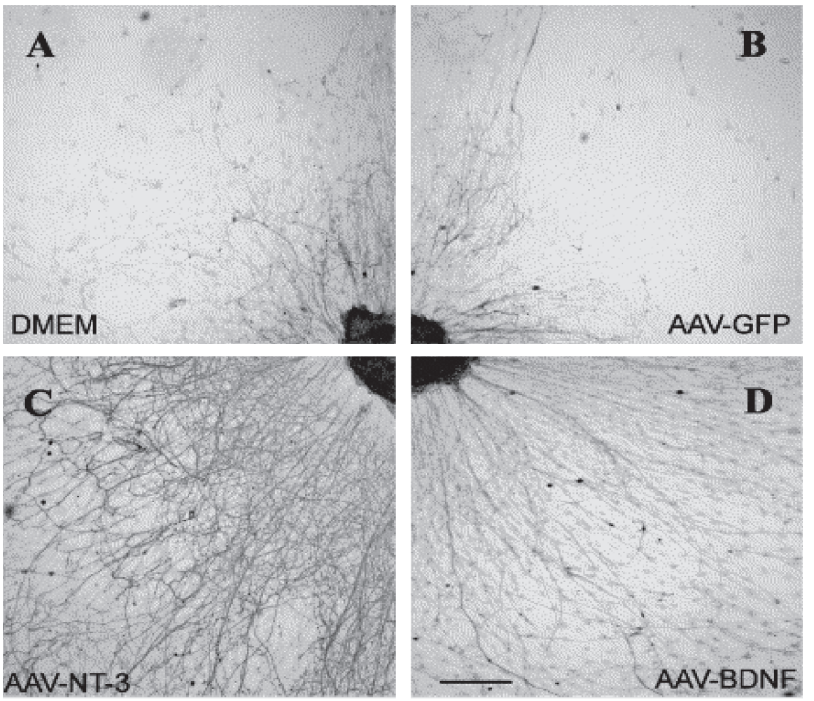


Figure 13.16. Enhancement of neurite outgrowth by neurotrophin-3 (NT3) and brain-derived neurotrophic factor (BDNF). Dorsal root ganglion explants were cultured with conditioned media collected from four groups of cells: (A) mock-infected 293 cells; (B) 293 cells transfected with adeno-associated viral vector (AAV) containing the GFP gene; (C) 293 cells transfected with AAV containing the NT3 gene; (D) 293 cells transfected with AAV containing the BDNF gene. Explant specimens were prepared and stained for neurofilaments using the RT97 antibody. In the controls (A and B), a low level of neurite outgrowth was observed. With AAV–NT3- and AAV–BDNF-conditioned medium, the rate of neurite outgrowth is significantly higher than the controls. Scale bar: 1 mm. (Reprinted from Blits B et al: Adeno-associated viral vector-mediated neurotrophin gene transfer in the injured adult rat spinal cord improves hind-limb function, *Neuroscience* 118:271–81, copyright 2003, with permission from Elsevier.)

cells. Neurotrophin 3 is also required for the outgrowth and innervation of sympathetic axons. In transgenic mice, the deficiency of neurotrophin 3 results in the loss of peripheral sensory and sympathetic neurons, while the motor neurons are not significantly affected. These defects are associated with the loss of peripheral muscular sensors, movement disorder, and death shortly after birth. Thus, neurotrophin 3 is a candidate factor for the treatment of nerve injury. It is interesting to note that neurotrophin 3 regulates not only the development and regeneration of the central and peripheral nervous system, but also the development of the heart. Neurotrophin 3 is required for the development of the cardiac atria and ventricles. The deficiency of neurotrophin 3 induces several major cardiac disorders, including atrial and ventricular septal defects and Fallot tetralogy, common forms of congenital cardiac defects. These disorders often result in perinatal death of transgenic mice.

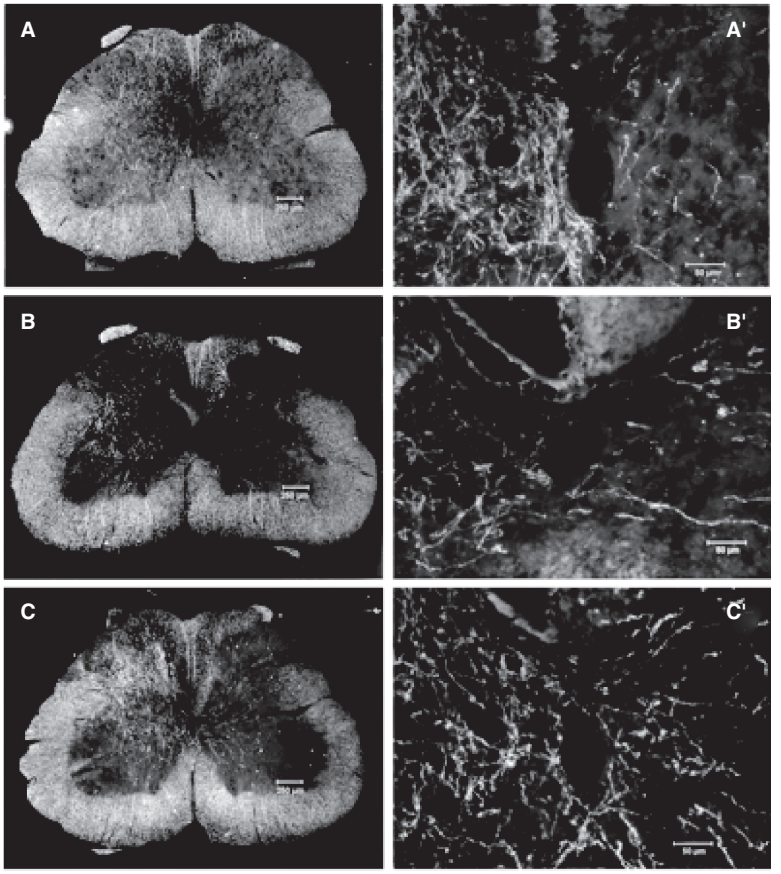


Figure 13.17. Enhancement of axonal growth by neurotrophin-3 (NT3) gene transfer to injured spinal cord. Rats with unilateral lesion of the corticospinal tract (CST) were transfected with Adv.EF α-NT3 or Adv.EF α-*LacZ*. The unlesioned corticospinal tract was labeled with BDA. (A) Section from a sham control rat. (B) Section from an Adv.EF α-*LacZ*-transfected rat. (C) Section from an Adv.EF α-NT3-transfected rat. (A′–C′) Higher-magnification photomicrographs of the regions around the central canal. Note that in panel C, BDA-labeled corticospinal tract neurites can be seen arising from the intact corticospinal tract, traversing the midline, and growing into the gray matter of the lesioned side of the spinal cord. (Reprinted with permission from Zhou L et al: *J Neurosci* 23:1424–31, copyright 2003.)

NEUROTROPHIN 4 [13.6]. Neurotrophin 4 is a 210-amino acid neurotrophic factor (13–14 kDa) expressed in the nervous system. It is also known as neurotrophin 5 or neurotrophin 4/5. The term neurotrophin 4 was derived based on the fact that the *Xenopus* neurotrophin 4 gene was used to isolate the human counterpart gene. However, the human neurotrophin 4 exhibits more diverse features and activities than the *Xenopus* neurotrophin 4. Thus, this growth factor was also termed *neurotrophin 5*. Some investigators prefer the term neurotrophin 4/5 to reflect the similarities and differences between the mammalian and *Xenopus* forms. The gene of neurotrophin 4 is localized to chromosome 19q13.3. Neurotrophin 4 plays a critical role in regulating the survival, proliferation, and differen-

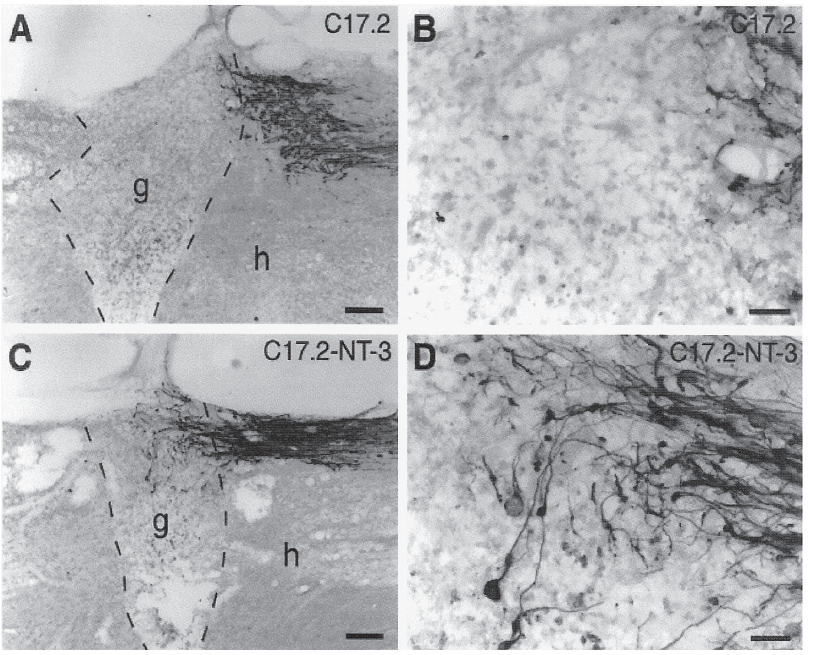


Figure 13.18. Neurotrophin-3 (NT3) enhances axonal growth of injured neurons into neural stem cell (NSC) grafts in injured spinal cord. Neural stem cell (mouse clone C17.2) grafts were transplanted to the lesion site of injured rat spinal cord. The growth of the dorsal column ascending sensory axons to the NSC graft was measured. (A) The ascending sensory axons rarely grow into the grafts of C17.2 NSCs without NT3 gene transfer. (B) Image from panel A with a higher magnification. (C) The ascending sensory axons could penetrate the graft of NSCs with NT3 gene transfer. (D) Image from panel C with a higher magnification. g: graft; h: host; dashed lines indicate host–graft interface. Scale bars: 177 μm in A,C; 44 μm in B,D. (E) Measurements of axon density within C17.2 grafts and C17.2–NT3 grafts (*$P < 0.05$). (Reprinted from Lu P et al: Neural stem cells constitutively secrete neurotrophic factors and promote extensive host axonal growth after spinal cord injury, *Exp Neurol* 181:115–29, copyright 2003, with permission from Elsevier.)

tiation of neurons. This factor also acts on the hippocampal neurons and contributes to the development of long-term memory. Furthermore, neurotrophin 4 is expressed in the fetal testis, contributing to the regulation of germ cell development and morphogenesis.

NERVE GROWTH FACTOR [13.7]. Nerve growth factor is a protein complex composed of four subunits, including one α unit, two β units, and one γ unit. The molecular weight of the complex is about 130 kDa. The genes that encode these subunits are mapped to chromosome 1 and locus 1p13.1. The gene sequence is similar between the human and mouse. Nerve growth factor subunits are produced at first as precursor proteins. The precursors are released into the extracellular matrix and cleaved by matrix metalloproteinases (MMP)7, MMP3, and serine protease plasmin. The cleavage activates the protein subunits. It is interesting to note that the precursor of nerve growth factor may exert an effect that is opposite to that of the mature form. In the rat and mouse models of brain injury, the precursor of nerve growth factor, produced and released by injured neurons, can

bind to the neurotrophin receptor p75 and induce apoptosis. The blockade of the interaction of the nerve growth factor precursor with the neurotrophin receptor reduces apoptosis.

The expression of nerve growth factor is regulated by several factors, including endothelin-1 (EDN1), endothelin receptor, protein kinase C, the Src family protein tyrosine kinases, mitogen-activated protein kinases, and activator protein 1 (AP1). Upregulated endothelin 1 can interact with and activate its receptor, which in turn induces the activation of the intracellular signaling protein kinases. The deficiency of endothelin 1 in transgenic mice results in a reduction in the expression of nerve growth factor, which is associated with the loss of neurons during the late embryonic stage and a reduction in the level of cardiac norepinephrine and sympathetic innervation. The overexpression of nerve growth factor in endothelin-1-deficient mice enhances the production of norepinephrine and sympathetic innervation.

Nerve growth factor is produced and released by neurons and plays a critical role in the regulation of growth and differentiation of sympathetic and sensory neurons of the nerve system. For instance, in a rat model of spinal cord dorsal root injury, a treatment with nerve growth factor induces the regeneration of injured dorsal axons and functional reconnection of injured synapses.

GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR [13.8]. Glial cell line-derived neurotrophic factor (GDNF) is a member of the transforming growth factor-β superfamily. This is a 211 amino acid protein produced by the neural glial cells. The gene of glial cell line-derived neurotrophic factor is localized to chromosome 5p13.1–p12. Glial cell line-derived neurotrophic factor exists in the form of disulfide-bonded homodimer, which is usually glycosylated.

The primary function of glial cell line-derived neurotrophic factor is to promote the survival, proliferation, and differentiation of neurons in the central nervous system. In particular, this neurotrophic factor is present in the midbrain and enhances the survival and proliferation of dopaminergic neurons. In the embryonic midbrain, glial cell line-derived neurotrophic factor plays a critical role in regulating the differentiation and morphogenesis of neuronal cells as well as the uptake of dopamine. It is important to note that glial cell line-derived neurotrophic factor exerts a specific effect on the dopaminergic neuron. It does not significantly influence the mitogenic activity of other types of neurons such as γ-aminobutyric-containing and serotonergic neurons. The dopaminergic neuron-specific features render this factor a potential therapeutic agent for the treatment of Parkinson's disease, which is possibly caused by progressive degeneration of the midbrain dopaminergic neurons.

Glial cell line-derived neurotrophic factor has been shown to mediate the survival and innervation of the motor neurons during development. The deficiency of glial cell line-derived neurotrophic factor in transgenic mice induces a reduction in motor axonal innervation to skeletal muscle cells. The overexpression of glial cell line-derived neurotrophic factor enhances the innervation of the motor axons. Furthermore, glial cell line-derived neurotrophic factor may be involved in the regulation of motor neuron pattern formation. In the deficiency of glial cell line-derived neurotrophic factor, motor neurons are mispositioned within the spinal cord in association with reduced innervation into the skeletal muscle tissue. These observations suggest the importance of glial cell line-derived neurotrophic factor in regulating the development of the peripheral nervous system.

Glial cell line-derived neurotrophic factor also plays a role in regulating the development of other systems. For instance, during the embryonic development, glial cell line-derived neurotrophic factor is expressed in the metanephric mesenchyme and acts on the ureteric bud epithelium, contributing to the regulation of ureter outgrowth and epithelial branching of the excretory system of the embryo (see Chapter 8 for the excretory system of the embryo).

Prevention of Cell Death. Nerve cell death is often induced by injury and injury-associated complications, such as ischemia and hypoxia, and is the most critical pathological event that deteriorates the function of the nervous system. In central nerve injury, most cells die in the injury site because of injury-associated complications. As reported in several studies, about 25–50% of the neurons die near the site of spinal cord axotomy within 4 weeks following the occurrence of the injury. The survival cells are highly atrophic with reduced capability of survival and proliferation.

Conventional approaches, as described on page 519 of this chapter, can be used to improve blood and oxygen supply in nerve injury, the most important step for the prevention of continuous cell death. However, nerve cells, especially injured nerve cells, may continue to commit to death even after the reestablishment of bloodflow. Thus, it is necessary to apply therapeutic approaches to injured nerve cells. Recent work has demonstrated that molecular engineering approaches can be used for such a purpose. It is now well established that cell death is mediated by extracellular ligands, including tumor necrosis factor α and Fas ligand, and intracellular death signaling molecules and caspases (see page 304). Extracellular stimuli, such as ischemia and hypoxia, may activate these factors and induce cell death. Molecular engineering approaches can be used to reduce cell death by suppressing the activity of cell death-promoting factors and by enhancing the activity of cell death-inhibiting factors. For instance, antibodies may be developed and used to block the extracellular cell death inducers TNF-α and Fas ligand. Genes encoding cell death inhibitors, such as Bcl2, can be used to transfect injured neuronal cells to prevent cell death. Furthermore, the nerve growth-stimulating factors, such as brain-derived neurotrophic factor, neurotrophin-4/5, nerve growth factor, and glial-derived neurotrophic factor, can prevent cell injury and death. These factors can be directly delivered to the site of nerve injury. The genes that encode these neurotrophic factors can be used to transfect injured nerve cells. These approaches have been shown to be effective for the prevention of neuronal death.

Prevention of Secondary Nerve Injury. Secondary injury is defined as disorders induced by pathological alterations initiated by the original nerve injury. Injury-induced pathophysiological alterations include, but are not limited to, hemorrhage (open wound), hypoxia (reduction in oxygen supply), inflammation (bacterial or nonbacterial), release of harmful enzymes (proteinases), and disorder of ion fluxes. These alterations significantly worsen the original injury and induce secondary injury of the nerve system, resulting in cell death and tissue disintegration. Thus, immediately after the initial nerve injury, it is critical to control these pathophysiological changes and protect the nerve system from secondary injury. Conventional approaches for the treatment of secondary injury have been described on page 519. Several molecular engineering approaches have been developed and tested for the prevention of secondary nerve injury. Strategies for preventing secondary injury include prevention of the disorder of ion transport and excitotoxicity.

PREVENTION OF ION FLUX [13.9]. The flux of ions, including sodium and calcium, is increased in nerve injury and contributes to secondary nerve injury (Fig. 13.19). Under physiological conditions, sodium is concentrated in the extracellular fluid. The concentration of sodium in the extracellular matrix is significantly higher than that in the cytoplasm. The concentration gradient for the sodium across the cell membrane is maintained by the

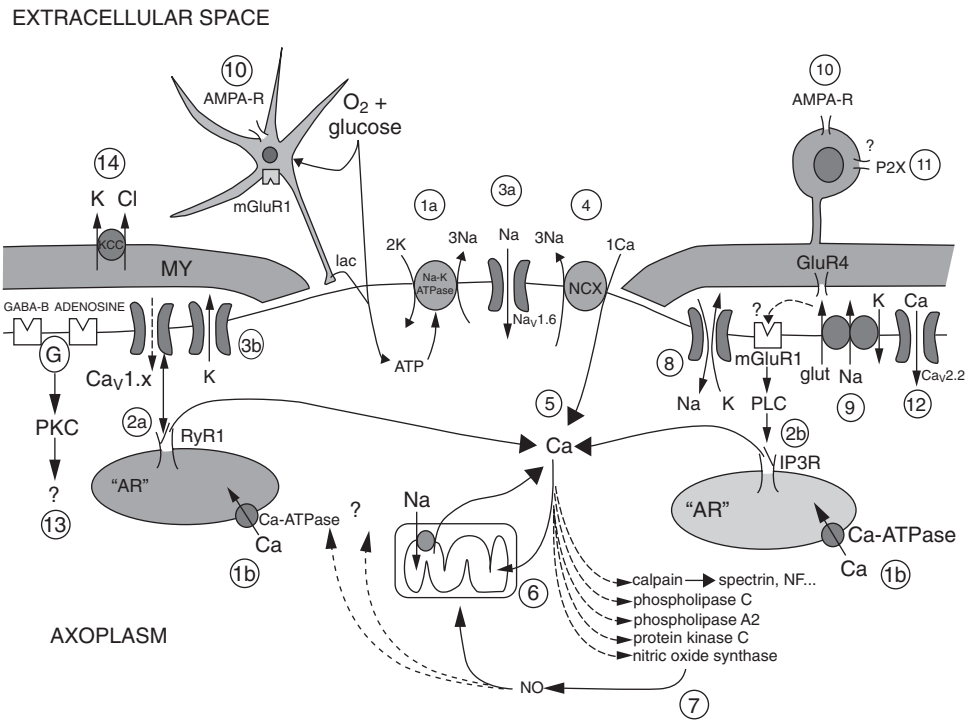


Figure 13.19. Mechanisms of ion-induced nerve injury. An energy deficit and/or excess demand impairs ATP-dependent pumps such as the Na–K ATPase (1a) and Ca ATPase (1b). Internal stores of Ca may contribute significantly to axonal Ca accumulation, triggered by depolarization via L-type Ca channels (2a) and/or generation of IP3 (2b). The rise in flux through noninactivating Na^+ channels (3a) will increase $[Na]_i$ and, together with depolarization caused by K efflux through a variety of K channels (3b), will stimulate the Na–Ca exchanger to operate in the reverse Ca import mode (4). This Ca accumulation (5) promotes destructive events including mitochondrial Ca overload (especially during reoxygenation) (6), and overactivation of several Ca-dependent enzyme systems (7). NO will inhibit mitochondrial respiration and alter other cellular proteins. Some Na influx may occur through Na/K-permeable inward rectifier channels (8). Glutamate is also released through reversal of Na-dependent glutamate transport (9), causing cellular injury from activation of ionotropic glutamate receptors (10). ATP-activated P2X purinergic receptors may cause Ca-dependent oligodendroglial injury (11). A component of Ca influx into damaged axons directly through voltage-gated Ca channels is also likely (12). GABA and adenosine release may play an "autoprotective" role (13). Anion transporters such as the K—Cl cotransporter participate in volume dysregulation in glia and the myelin sheath, contributing to conduction abnormalities (14). The locations of the various channels and transporters are drawn for convenience and do not necessarily reflect actual distributions. (Reprinted from Stys PK: General mechanisms of axonal damage and its prevention, *J Neurol Sci* 233:3–13, copyright 2005, with permission from Elsevier.)

continuous action of the sodium pumps in the cell membrane. Calcium is stored in the endoplasmic reticulum (ER) system in normal cells. The concentration of calcium in the cytoplasm is much lower than that within the ER. The calcium concentration gradient across the ER membrane is established and maintained by the calcium pumps in the ER membrane.

In nerve injury, especially in the presence of ischemia, cell membrane exhibits enhanced permeability to ions, resulting in an increase in ion flux. Such a change influences the level of membrane potential as well as the initiation and transmission of the action potential of the neurons, thus reducing the function of the nerve system. Ion channel blockers, including sodium channel blockers (QX-314, tetrodotoxin, and procaine) and calcium channel blockers (diltiazem, verapamil, and ω-conotoxin GVIA), have been developed and used to reduce ion flux. Such an approach has been shown to be effective for the prevention of secondary nerve injury. The ion channel blockers can be directly delivered into the spinal fluid. Alternatively, the blockers can be injected into the blood. Since the blockers are relatively small molecules, they can pass the blood–brain barrier and reach the injured nerve cells and axons.

PREVENTION OF EXCITOTOXICITY [13.10]. Central nerve injury is associated with excitotoxic activities mediated by glutamate, which contributes to secondary nerve injury. Glutamate-mediated secondary injury is often found in the nerve axon-concentrated white matter of the spinal cord. As discussed above, nerve injury induces disorder of sodium influx, which in turn influences the efflux of potassium. These alterations induce the disruption of the concentration gradient for sodium and potassium. Such a disruption activates the sodium-dependent glutamate transporter, resulting in the release of endogenous glutamate. Released glutamate can interact with and activate the α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA)/kainate receptor. This receptor mediates calcium release and transport, resulting in cytosolic accumulation of calcium. Calcium is known to activate various enzymes, such as calpains and phospholipases. These enzymes can cause irreversible injury of the axons. A treatment with a glutamate antagonist (e.g., kynurenic acid) or a selective AMPA antagonist (e.g., GYKI52466) significantly reduces secondary axonal injury. The inhibition of the sodium-dependent glutamate transport with L-*trans*-pyrrolidine-2,4-dicarboxylic acid can also protect the axons from injury. These substances may be potentially used for the prevention of secondary nerve injury.

Stimulation of Stem Cell Differentiation [13.11]. One of the strategies for inducing neuronal regeneration is to enhance the differentiation of the neural stem cells to neurons. Neurons have long been considered well-differentiated cells that exhibit a very low level of regenerative activity. However, recent work has demonstrated that there exist neuronal stem cells in a number of locations in the adult central nerve system, including the caudal portion of the subventricular zone, olfactory bulb, hippocampus, striatum, optic nerve, corpus callosum, spinal cord, cortex, retina, and hypothalamus. A typical type of stem cell is the neuroepithelial cells at these locations. The neuroepithelial cells are able to differentiate into neurons or glial cells to replace injured and dead cells. Thus, a conceivable therapeutic approach in nerve regenerative engineering is to promote the differentiation of the stem cells. Furthermore, neurons can extend their axons and replace injured and severed axonal segments. The enhancement of axonal growth is another approach for the treatment of nerve injury.

An effective method for stimulating the differentiation of neural stem cells is to deliver neurotrophic factors to injured nerve tissues. Neurotrophic factors have long been known to stimulate neural stem cell proliferation and differentiation. In the nerve system, there are a number of growth factors, also known as neurotrophic factors. These include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3), and neurotrophin-4/5 (NT4/5). These neurotrophic factors are required for the expression of the nerve regeneration-associated genes and stimulate neuronal regeneration. The neurotrophic factors also directly stimulate axonal regeneration and enhance structural and functional reconnection between injured neurons. The functions of these regeneration-stimulating factors are discussed on page 521 of this chapter.

It is important to address that neuronal regeneration is regulated not only by stimulatory neurotrophic factors but also by inhibitory factors. The inhibitory factors suppress the regeneration of neuronal cells. The understanding of the inhibitory effect helps to clarify the mechanisms of controlling neuronal regeneration. A typical inhibitory factor is growth and differentiation factor 11 (GDF11), a member of the transforming growth factor (TGF)β protein superfamily. This factor is expressed in the olfactory neuroepithelial cells, which are thought to include neural stem cells. When upregulated, GDF11 exerts an inhibitory effect on olfactory neurogenesis by activating an inhibitory factor p27, which is implicated in the induction of cell cycle arrest. The knockout of the GDF11 gene results in an increase in neurogenesis. The inhibitory effect of GDF11 represents a mechanism by which the density of neurons is checked and maintained at a certain level. GDF11 may be activated when excessive neurogenesis is induced, whereas GDF11 is suppressed when neurogenesis is reduced. This is a typical example of the feedback regulatory mechanism.

Neuronal regeneration is also inhibited by bone morphogenetic protein 2 (BMP2), a member of the BMP family, which are involved in the development of the nervous system. A treatment with BMP2 in cultured neural stem cells induces a significant reduction in the formation of a certain type of nerve cell that expresses the neuronal marker microtubule-associated protein 2, but enhances the formation of other type of nerve cells that express a glial cell marker S100β. These observations suggest that BMP2 may stimulate the transformation of neural stem cells to glial cells, while inhibiting the formation of neuronal cells. Further investigations have demonstrated that BMP7, another member of the BMP family, is upregulated in spinal cord injury. This factor may stimulate the regeneration of glial cells in injured spinal cord. Taken together, these investigations suggest that, in nerve regenerative engineering, both the stimulatory and inhibitory mechanisms should be taken into account.

Enhancement of Axonal Extension, Adhesion, and Reconnection [13.12]. Under appropriate conditions, the neuron is capable of regenerating its axon when the axon is severed. It is interesting to note that axon transection can serve as a stimulus for the initiation of axon regeneration. Such an injury can induce the expression of regeneration-associated genes, including c-jun and c-fos. The protein products of these genes enhance the expression of several factors that are involved in axon extension, including the Tα1-tubulin and nerve cell adhesion molecule (NCAM). The Tα1-tubulin is a cytoplasmic growth cone protein and is involved in growth cone guidance. The nerve cell adhesion molecule is a factor that regulates cell attachment and migration. These molecules promote axonal regeneration. Nerve regeneration fails in the absence of these factors. For therapeutic purposes, the genes of these factors can be used to construct recombinant genes, which

can be transferred to the injury site of the central nerve system by using methods described on page 436. The enhancement of gene expression by gene transfer facilitates the extension of injured axon.

Cell adhesion and attachment to substrate are critical processes for nerve axonal extension and regeneration. A number of adhesion molecules, such as growth-associated protein 43 (GAP43), cell adhesion molecule L1, N-cadherin, have been found to modulate neuronal adhesion. For instance, the overexpression of growth-associated protein 43 or cell adhesion molecule L1 in the Purkinje neurons of transgenic mice stimulates axonal sprouting of the Purkinje neurons into nerve grafts compared to wildtype mice, which do not significantly express growth-associated protein 43 and cell adhesion molecule L1 in the Purkinje neurons. It is interesting to note that growth-associated protein 43 and cell adhesion molecule L1 promote neuronal growth and axonal sprouting in a synergistic manner. When both molecules are overexpressed in the Purkinje neurons by genetic modulation, the rate of neuronal growth and axonal extension into nerve grafts is significantly enhanced compared to the transgenic model with overexpression of only a single molecule (either growth-associated protein 43 or cell adhesion molecule L1). These observations suggest that genetic upregulation of neuronal adhesion molecules via gene transfer can enhance neuronal regeneration and axonal growth in nerve injury. It is important to address that axonal outgrowth is regulated by opposing stimulatory and inhibitory factors. For instance, a protein known as netrin 1 significantly enhanced the axonal outgrowth of dopaminergic neurons in the embryonic ventral midbrain. In contrast, a protein known as slit-2 suppresses the axonal outgrowth of the dopaminergic neurons. These observations suggest that opposing regulatory factors may coordinately control the neuronal outgrowth in nerve injury. Thus, the inhibitory factors ought to be considered in the treatment of nerve injury.

Prevention of Fibrous Scar Formation [13.13]. Nerve injury is associated with glial cell proliferation, fibrosis, and scar formation. The scar tissue is composed of microglial cells, astrocytes, oligodendrocytes, and extracellular matrix. The scar tissue obviously imposes a physical barrier to the regeneration of axons. In addition, the cells in the scar tissue can secret a number of molecules, including neurocan (a major chondroitin sulfate proteoglycan or CSPG in the nerve system), proteoglycan phosphocan, brevican, tenascin, semaphorins, and ephrins. These molecules exist in the form of either membrane-bound molecules or soluble molecules and exert an inhibitory effect on axonal regeneration. Antagonistic molecules and pharmacological substances that inhibit the activity of these molecules can be used to enhance axonal regeneration in nerve injury. For example, urokinase has been used to degrade proteoglycans in injured nerve tissues and shown to promote axonal regeneration. Antibodies can be developed and used to neutralize the activity of the inhibitory molecules. Such a strategy can be potentially applied to the treatment of nerve injury.

Recent investigations have shown that nuclear factor (NF)κB plays a critical role in secondary inflammatory reactions and the formation of scar tissue after nerve injury. NFκB is a transcription factor, which activates the expression of genes encoding proinflammatory factors, such as CXCL10, CCL2, and transforming growth factor β2. Furthermore, NFκB stimulates the formation of chondroitin sulfate proteoglycans. When NFκB is selectively suppressed in a transgenic mouse model, inflammatory reactions and scar formation are significantly inhibited after contusive spinal cord injury. These observations show that selective inhibition of NFκB in glial cells exerts a protective effect on injured nerve axons.

There exist a number of central nerve myelin-associated inhibitory factors, including NI35, NI250, and several glycoproteins. These factors directly inhibit neuronal axon regeneration. The neutralization of these inhibitory factors with antibodies results in enhanced sprouting of injured spinal neurons. These experiments confirm the inhibitory role of the myelin-associated factors and suggest therapeutic applications of the antibodies.

Enhancement of Synaptic Formation [13.14]. Nerve injuries are often associated with the discontinuation of nerve synapses. An important strategy in nerve regeneration is to restore the nerve synapses. Several molecules, such as neurotrophins (NT), agrin, and s-laminin, have been shown to promote synaptic formation. For instance, neurotrophins, especially neurotrophin 3, stimulate not only spinal axonal outgrowth, but also the reconnection of transected axons to terminal muscular cells via the regeneration of synapses. Most neurotrophic factors exert a promoting effect on axonal outgrowth after injury. Thus, synapse-stimulating proteins and their genes can be used to treat nerve injury to enhance the restoration of injured synapses.

Nerve Cell Regenerative Engineering. Cell regenerative engineering is to identify, collect, modulate, and transplant functional cells to injury or lesion sites of the nerve system to replace lost cells and improve nerve regeneration. As discussed in the last section, molecular regenerative engineering can be potentially applied to the central nervous system to facilitate the regeneration of injured neurons and axons. However, in most cases, neuronal regeneration and axon outgrowth are hindered by pathological changes, such as glial proliferation, fibroblast infiltration, and fibrosis. These lesions encapsulate injured neurons and axons, preventing neuronal regeneration and axonal outgrowth. Thus, it is necessary to develop strategies to overcome these detrimental conditions. A potential strategy is to transplant stem and progenitor cells as well as supporting cells to enhance neuronal regeneration and axonal outgrowth. Candidate cell types may include embryonic and fetal stem cells, Schwann cells, and olfactory ensheathing cells. These cells may promote neuronal and axonal growth and regeneration, while protecting them from detrimental effects. It is expected that cell regenerative engineering, together with molecular regenerative engineering, may enhance the regeneration of injured neurons and the outgrowth of injured axons.

Embryonic and Fetal Stem Cells [13.15]. Embryonic and fetal stem cells are the primary cell candidates for the treatment of nerve injury. As discussed on page 381, embryonic stem cells are capable of differentiating into all specified cell types. Under the stimulation of appropriate environmental cues for nerve cell development, these stem cells may be induced to differentiate into neurons or glial cells. Fetal neural stem cells may also be used to generate neurons. In experimental models, stem cell transplantation has been shown to reduce neuronal cell death, enhance neuronal survival, and promote axonal outgrowth after nerve injury. Clinical trials for treating posttraumatic syringomyelia with fetal spinal cord grafts have demonstrated the feasibility of cell regenerative engineering for treating nerve injury. Although investigations with stem cell transplantation have provided promising results, embryonic and fetal cells may not be used until related ethical issues are resolved.

Adult Neural Stem Cells [13.16]. There exist adult stem and progenitor cells that can differentiate to neuronal and glial cells. These cells are neuroepithelial cells and can be

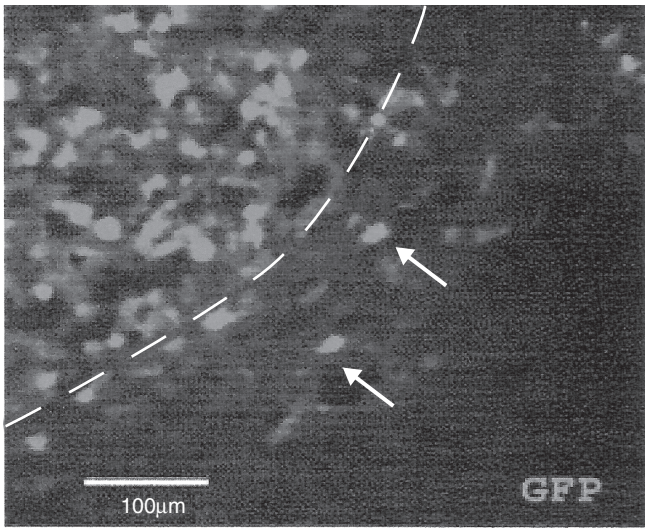


Figure 13.20. Fluorescent micrograph showing transplantation and engraftment of olfactory progenitor cells to the spinal cord of the rat. Neurosphere-forming progenitor cells were prepared from human adult olfactory epithelium, transfected with a GFP gene, and transplanted into traumatized rat spinal cord. At 1 week, specimens were collected from the injured spinal cord and prepared for observing GFP-labeled neurosphere-forming progenitor cells at the lesion site. The dashed line indicates the host–graft interface. Several transplanted GFP-positive cells appear with the host spinal cord bridging the injury site (arrows). (Reprinted from Xiao M et al: Human adult olfactory neural progenitors rescue axotomized rodent rubrospinal neurons and promote functional recovery, *Exp Neurol* 194:12–30, copyright 2005, with permission from Elsevier.)

found in the central nerve system as well as in the bone marrow. In the adult brain, neural stem and progenitor cells are present in several regions, including the caudal portion of the subventricular zone, olfactory bulb, hippocampus, striatum, optic nerve, corpus callosum, spinal cord, cortex, retina, and hypothalamus. Cells collected from these regions can be induced to differentiate to neurons, astrocytes, and oligodendrocytes. Figure 13.20 shows that olfactory cells transplanted into the rat spinal cord can engraft and integrate into the host tissue.

Stem and progenitor cells have been shown to express specific proteins. For instance, neuronal progenitor cells express the NeuN protein, whereas glial progenitor cells express GFAP and S100β. These proteins can be used as markers to identify neuronal and glial progenitor cells. For therapeutic purposes, neural stem and progenitor cells can be identified, collected, expanded in vitro, and transplanted to the site of nerve injury to enhance nerve regeneration. See page 395 for characteristics of neural stem and progenitor cells.

Bone Marrow Cells [13.17]. The bone marrow contains stem and progenitor cells for a variety of specified tissue types, including the nerve system. Several investigations have demonstrated that bone marrow-derived stem cells, when cultured in the presence of EGF or BDNF, can transform to cells that express the neural progenitor cell marker nestin, neuron-specific nuclear protein (NeuN), and glial fibrillary acidic protein (GFAP). When injected into the venous system of myeloablated animals, bone marrow cells can engraft to the brain and form cells that express neuronal protein markers such as NeuN, 200-

kilodalton neurofilaments, and class III β-tubulin in the brain. In human studies, transplanted bone marrow cells can transform to neurons in the cerebellum and cerebrum. Bone marrow-derived neurons account for about 1% of all neurons.

In a chicken spinal cord injury model, the implantation of human hematopoietic CD34+ stem cells into the injured spinal cord induces the formation of neuron-like cells that express the neuronal markers NeuN and MAP2. These neuron-like cells can extend their axons into the white matter of the spinal cord with synaptic terminals. Furthermore, these cells demonstrate spontaneous synaptic action potentials characteristic of functional neurons. These observations suggest that bone marrow-derived cells can transform to neuron-like cells and can be potentially used to treat spinal cord injury.

Bone marrow cells can also differentiate into glial cells. When bone marrow cells are injected to a demyelinated spinal cord, these cells can transform to oligodendrocytes that express myelin protein and induce remyelination. Bone marrow cells have been used to treat spinal cord injury, resulting in functional improvement of the spinal cord. However, there are several aspects that remain to be investigated. First, the morphology of the neural cells derived from bone marrow cells has not been thoroughly studied. It remains poorly understood whether bone marrow-derived cells can form axons. Second, the function of bone marrow-derived cells has not been systematically characterized. It is not clear whether bone marrow cells can develop into fully functional neurons or glial cells. Furthermore, the use of bone marrow cells for nerve regeneration remains a controversial topic. A recent study has demonstrated that the bone marrow hematopoietic stem cells do not contribute significantly to neuronal regeneration. Further investigations are necessary to clarify this issue.

Neuronal Supporting Cells [13.18]. Neuronal supporting cells include glial and Schwann cells, which can be used to serve as substrate for neuronal regeneration and axonal outgrowth. Schwann cells can be easily collected and cultured. These cells have been used to transplant to the site of nerve injury and to serve as bridges for axonal outgrowth and regeneration. To enhance the growth of transplanted cells, neurotrophic factors and/or their genes can be delivered, together with cell transplantation, to nerve injury sites. Schwann cells have been transplanted together with brain-derived neurotrophic factor (BDNF) and neurotrophin (NT)-3, in experimental spinal cord injury. Such a combination has been shown to promote axonal outgrowth. Furthermore, transplanted Schwann cells can guide axonal extension, a critical process for the reinnervation of axons to peripheral skeletal muscle cells.

Another type of neuronal supporting cells is the olfactory ensheathing cells. These cells are glial cells and are found in the nerve system in association with the olfactory neurons and axons. The primary function of olfactory ensheathing cells is to escort axons from the peripheral to the central nerve system. These cells are also capable of promoting axonal outgrowth. Such a property may be utilized to bridge the gap of nerve injury and enhance the myelination of newly generated axons. The role of olfactory ensheathing cells in regulating axonal regeneration has been observed in numbers of investigations. These observations demonstrate the potential of using olfactory glial cells for neural cell regenerative engineering.

Transgenic Cell Lines [13.19]. For the treatment of nerve injury, it is critical to promote nerve regeneration as rapidly as possible. To achieve such a goal, transgenic cell lines have been established by transfecting selected cell types with neurotrophic factor genes, such

as nerve growth factor, neurotrophin 3, brain-derived neurotrophic factor, and basic fibroblast growth factor genes. The transgenic cells can be used for transplantation into injured nerve tissues. These cells can express and release the transfected neurotrophic genes at a high rate, rapidly stimulating the regeneration of injured neurons and axons. Candidate cell types for such a purpose include embryonic stem cells, glial cells, Schwann cells, olfactory ensheathing cells, and fibroblasts. Preliminary investigations have demonstrated promising results for the use of genetically modified cells for the regeneration of injured neurons and axons.

Tissue Regenerative Engineering for Nerve Injury. Tissue regenerative engineering is to construct and provide nerve matrix scaffolds, which serve to enhance and guide neuronal regeneration and axonal outgrowth. Compared to molecular and cell regenerative engineering, which can be applied to both the central and peripheral nerve systems, tissue regenerative engineering has been primarily used for the treatment of peripheral nerve injury. This is attributed to the difficulty of accessing the central nerve system and the possibility of inducing nerve injury by tissue scaffold implantation. For peripheral nerve engineering, several strategies have been developed and used, including guidance of axonal regeneration and outgrowth, stimulation of neuronal proliferation by graft-based assistance, and improvement of neuronal function.

Stimulation of Neuronal Regeneration and Guidance of Axonal Outgrowth by Graft-Based Assistance [13.20]. As discussed on page 517 of this Chapter, severed nerve axons can regenerate and establish reconnection between the severed segments. However, such reconnection requires appropriate guidance from the Schwann cells, which form a sheath along the severed axons. When the severed proximal axon is relocated away from the distal axon, it is difficult for the proximal axon to reconnect to the severed distal axon. In conventional treatment, severed nerve bundles are reconnected surgically. However, when a segment of a nerve bundle is severely damaged or removed, it is impossible to reconnect the severed proximal to the distal nerve bundle. Under such as circumstance, it is necessary to provide guidance to the injured axons, allowing axonal extension along a desired path. For the past decade, a number of biological and synthetic materials have been developed and used to construct nerve scaffolds for the stimulation of axonal sprouting and the guidance of axonal extension. These materials include autologous nerve tissue grafts, allogenic (from the same animal species) and xenogeneic nerve tissue grafts (from different animal species), extracellular matrix components (laminin, fibronectin, collagen, and fibrin), and synthetic polymeric materials [poly (L-lactic acid), poly-L-lactide-ε-caprolactone, polyurethane, poly(2-hydroxyethyl methacrylate-co-methyl methacrylate), and polypyrrole-hyaluronic acid]. Nerve grafts based on these materials are briefly discussed here.

AUTOLOGOUS NERVE TISSUE GRAFTS [13.21]. Autologous nerve tissue specimens can be collected from the patient who receives grafts. Autologous nerve tissue is an ideal material type for constructing nerve grafts. Certain subcutaneous nerves, such as the branches of the saphenous nerve, can be used as nerve grafts. A nerve branch can be isolated and grafted into a severed nerve bundle. Such a graft usually provides effective support and guidance to the injured nerve and significantly enhances neuronal regeneration and axonal outgrowth. Autologous nerve tissue is usually considered the gold standard for constructing nerve grafts. Other types of natural tissue, such as autologous blood vessels, skeletal

muscle, and soft connective tissues, have also been used as nerve grafts. These tissues can be tailored into the shape of nerve bundles and grafted into severed nerves. However, the effectiveness of these tissues is limited compared to the autologous nerve grafts.

ALLOGENIC AND XENOGENEIC TISSUE GRAFTS [13.22]. Allogenic grafts are those collected from different individuals of the same animal species. Xenogeneic grafts are from individuals of different animal species. Various types of allogenic and xenogeneic tissue, such as nerve tissue, soft connective tissue, intestinal submucosa, and amnion, have been used as nerve grafts. However, the cellular components of allogenic and xenogeneic tissues, especially living cells, induce acute immune rejection reactions. Thus, the cellular components must be removed from the graft tissue. Decellularized matrix scaffolds can be produced by appropriate thermal, detergent, or NaOH (or KOH) treatment. The cell-free grafts can be used as scaffolds that guide neuronal regeneration and axonal outgrowth. These grafts, however, are not as effective as the autologous nerve grafts.

EXTRACELLULAR MATRIX COMPONENTS [13.23]. Extracellular components, including collagen, fibronectin, laminin, and fibrin, have been used to construct scaffolds for stimulating and guiding neuronal regeneration and axonal outgrowth. These molecules play an important role in the regulation of neuronal proliferation and migration as well as axonal extension and innervation during development. Some of these matrix components, such as collagen, fibronectin, and laminin, serve as substrates for cell attachment and migration, which are critical processes for the morphogenesis of the nerve system during development and regeneration of injured nerve axons. Other extracellular matrix molecules, such as fibrinogen, fibrin gels, peptide scaffolds, alginate, agarose, and chitosan, have been applied to injured nerves for enhancing axonal outgrowth in experimental models. It is important to point out that not all extracellular matrix components are neuronal growth promoters. Certain molecules of the proteoglycan family, such as chondroitin sulfate proteoglycan (CSPG) and proteoglycan phosphocan, are typical inhibitory factors for neuronal regeneration and axonal outgrowth. The inhibitory effect of these molecules may play a role in the prevention of glial scar formation, a hindrance for the regeneration and extension of nerve axons.

SYNTHETIC MATERIALS [13.24]. A number of synthetic polymeric materials have been developed and used for the regeneration of injured nerves in experimental models. These materials include poly(glycolic acid) (PGA), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(caprolactones), biodegradable poly(urethane), polyorganophosphazene, methacrylate-based hydrogels, and poly(3-hydroxybutyrate). These polymeric materials are biodegradable, relatively low in toxicity, and easy to process. Scaffolds made of these materials have been tested in animal models for nerve regeneration. Results from these investigations are promising. Nonbiodegradable synthetic materials, such as polytetrafluoroethylene (PTFE), have also been used as scaffolds for nerve regeneration with satisfactory results.

Polymeric materials can be used in combination with nerve regeneration stimulators, such as neurotrophic factors and antiapoptotic factors or their genes. A polymeric scaffold may serve not only as a guide for nerve regeneration but also as a drug delivery device. The polymeric scaffold and the nerve regeneration stimulators may synergistically enhance the regeneration of injured nerves. However, compared to autogenous nerve grafts, the effectiveness of the synthetic polymeric materials remains limited. A critical problem with

the synthetic materials is their properties of inducing host inflammatory reactions. Improving biocompatibility of polymers is a major challenge in nerve regeneration with synthetic materials.

ROLE OF GEOMETRIC FACTORS [13.25]. While the material properties are critical to the modulation of neuronal regeneration and axonal outgrowth, geometric factors, such as the shape, orientation, curvature, and dimension of matrix substrate and scaffolds, may influence the extension and direction of regenerating axons. For instance, when neurons are cultured on a polymeric matrix surface, the direction of axonal outgrowth is dependent on the matrix structure and the orientation of the matrix fibers (Fig. 13.21). The curvature of

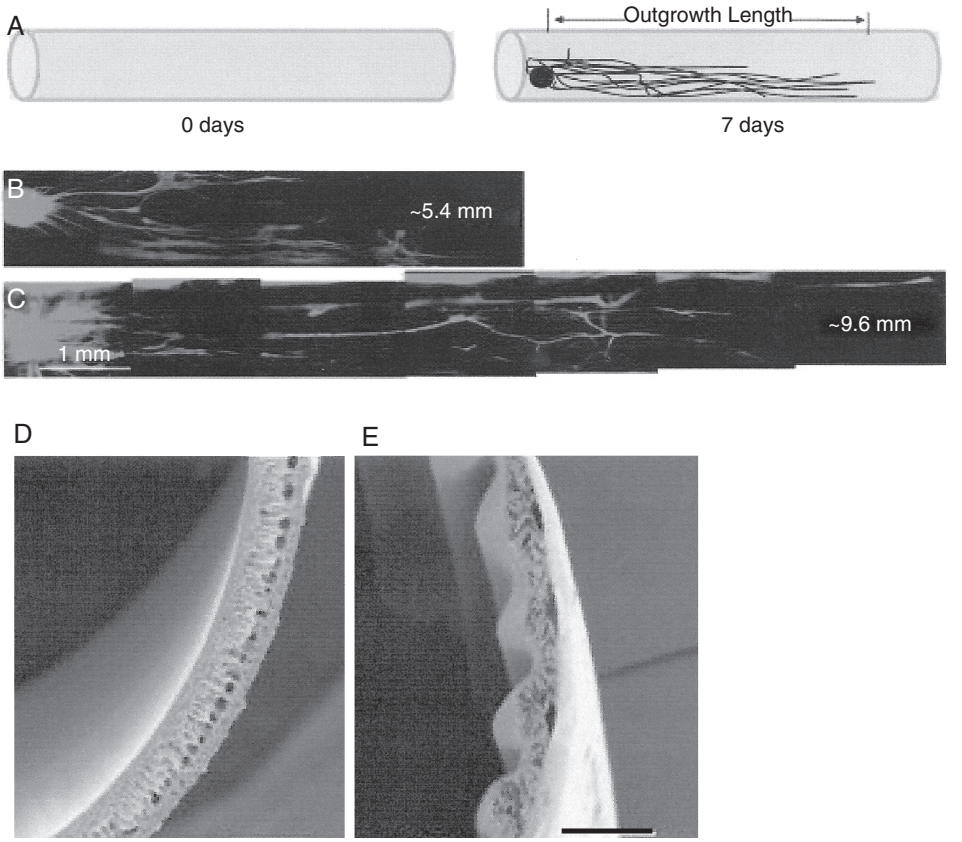


Figure 13.21. Biomaterial implantation for guiding axonal outgrowth. (A) Schematic illustration of neurite outgrowth evaluation in a dorsal root ganglion (DRG) explant model. DRG explant was seeded at one end of a cylinder of polyurethane hollow fiber membrane (HFM) and allowed to grow for 7 days. Neurite outgrowth was assessed based on neurite length measurement. (B) Neurite outgrowth on a smooth HFM surface for 7 days. (C) Neurite outgrowth on a HFM surface with aligned grooves for 7 days. (D,E) Scanning electron micrographs showing the morphology of HFM membrane with smooth and grooved surfaces, respectively. (Reprinted with permission of John Wiley & Sons, Inc. from Zhang N et al: Fabrication of semipermeable hollow fiber membranes with highly aligned texture for nervous guidance, *J Biomed Mater Rese Pt A* 75:941–9, copyright 2005.)

the matrix fibers influences the direction of axonal extension. Furthermore, the geometry of the matrix substrate regulates the rate of neuronal regeneration. As shown in several studies, magnetically aligned collagen fibers elicit a more significant effect on neuronal regeneration than randomly aligned collagen fibers. These observations demonstrate that geometric factors may serve as cues for the neuronal regeneration and axonal outgrowth.

IMPROVEMENT OF NERVE FUNCTION [13.26]. For the treatment of nerve injury, in addition to the promotion of nerve regeneration, another important strategy is to improve the function of regenerated axons. A major approach for such a purpose is to electrically stimulate impaired and regenerated neurons and axons. Several types of devices have been developed and tested. These include cochlear, retinal, spinal cord, and brain stimulators. These devices can be used to improve nerve functions such as hearing, vision, and muscular movement control. It becomes now clear that a successful treatment of nerve injury requires both anatomical nerve regeneration and functional recovery.

BIBLIOGRAPHY

13.1. Anatomy and Physiology of the Nervous System

Lai C, Peripheral glia: Schwann cells in motion, *Curr Biol* 15(9):R332–4, May 2005.

Guyton AC, Hall JE: *Textbook of Medical Physiology*, 11th ed, Saunders, Philadelphia, 2006.

McArdle WD, Katch FI, Katch VL: *Essentials of Exercise Physiology*, 3rd ed, Lippincott Williams & Wilkins, Baltimore, 2006.

Germann WJ, Stanfield CL (with contributors Niles MJ, Cannon JG): *Principles of Human Physiology*, 2nd ed, Pearson Benjamin Cummings, San Francisco, 2005.

Thibodeau GA, Patton KT: *Anatomy & Physiology*, 5th ed, Mosby, St Louis, 2003.

Boron WF, Boulpaep EL: *Medical Physiology: A Cellular and Molecular Approach*, Saunders, Philadelphia, 2003.

Ganong WF: *Review of Medical Physiology*, 21st ed, McGraw-Hill, New York, 2003.

13.2. Nerve Injury

Schneider AS, Szanto PA: *Pathology*, 3rd ed, Lippincott Williams & Wilkins, Philadelphia, 2006.

McCance KL, Huether SE: *Pathophysiology: The Biologic Basis for Disease in Adults & Children*, 5th ed, Elsevier Mosby, St Louis, 2006.

Porth CM: *Pathophysiology: Concepts of Altered Health States*, 7th ed, Lippincott Williams & Wilkins, Philadelphia, 2005.

Frazier MS, Drzymkowski JW: *Essentials of Human Diseases and Conditions*, 3rd ed, Elsevier Saunders, St Louis, 2004.

Kwon BK, Tetzlaff W: Spinal cord regeneration: From gene to transplants, *Spine* 26(Suppl):S13–22, 2001.

Feringa ER, McBride RL, Pruitt JN: Loss of neurons in the red nucleus after spinal cord transection, *Exp Neurol* 100:112–20, 1988.

Goshgarian HG, Koistinen JM, Schmidt ER: Cell death and changes in the retrograde transport of horseradish peroxidase in rubrospinal neurons following spinal cord hemisection in the adult rat, *J Compar Neurol* 214:251–7, 1983.

McDonald JW, Sadowsky C: Spinal cord injury, *Lancet* 359:417–25, 2002.

Schwab ME: Repairing the injured spinal cord, *Science* 295:1029–31, 2002.

13.3. Molecular Regenerative Engineering for the Nervous System

Koprivica V, Cho KS, Park JB, Yiu G, Atwal J et al: EGFR activation mediates inhibition of axon regeneration by myelin and chondroitin sulfate proteoglycans, *Science* 310:106–10, 2005.

Houle JD, Ye JH: Survival of chronically-injured neurons can be prolonged by treatment with neurotrophic factors, *Neuroscience* 94:929–36, 1999.

Yamada M, Natsume A, Mata M, Oligino T, Goss J et al: Herpes simplex virus vector-mediated expression of Bcl-2 protects spinal motor neurons from degeneration following root avulsion, *Exp Neurol* 168:225–30, 2001.

Chen DF, Schneider GE, Martinou JC, Tonegawa S: Bcl-2 promotes regeneration of severed axons in mammalian CNS, *Nature* 385:434–39, 1997.

Chierzi S, Strettoi E, Cenni MC, Maffei L: Optic nerve crush: axonal responses in wild-type and bcl-2 transgenic mice, *J Neurosci* 19:8367–76, 1999.

Holm K, Isacson O: Factors intrinsic to the neuron can induce and maintain its ability to promote axonal outgrowth: A role for BCL2? *Trends Neurosci* 22:269–73, 1999.

Kobayashi NR, Fan DP, Giehl KM et al: BDNF and NT-4/5 prevent atrophy of rat rubrospinal neurons after cervical axotomy, stimulate GAP-43 and Talpha1-tubulin mRNA expression, and promote axonal regeneration, *J Neurosci* 17:9583–95, 1997.

Schumacher PA, Siman RG, Fehlings MG: Pretreatment with calpain inhibitor CEP-4143 inhibits calpain I activation and cytoskeletal degradation, improves neurological function, and enhances axonal survival after traumatic spinal cord injury, *J Neurochem* 74:1646–55, 2000.

Beattie MS, Farooqui AA, Bresnahan JC: Review of current evidence for apoptosis after spinal cord injury, *J Neurotrauma* 17:915–25, 2000.

Crowe MJ, Bresnahan JC, Shuman SL et al: Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys, *Nat Med* 3:73–6, 1997.

Emery E, Aldana P, Bunge MB et al: Apoptosis after traumatic human spinal cord injury, *J Neurosurg* 89:911–20, 1998.

Martin LJ, Al Abdulla NA, Brambrink AM et al: Neurodegeneration in excitotoxicity, global cerebral ischemia, and target deprivation: A perspective on the contributions of apoptosis and necrosis, *Brain Res Bull* 46:281–309, 1998.

Liu XZ, Xu XM, Hu R et al: Neuronal and glial apoptosis after traumatic spinal cord injury, *J Neurosci* 17:5395–406, 1997.

13.4. Brain-Derived Neurotrophic Factor (BDNF)

Jones KR, Reichardt LF: Molecular cloning of a human gene that is a member of the nerve growth factor family, *Proc Natl Acad Sci USA* 87:8060–4, 1990.

Maisonpierre PC, Le Beau MM, Espinosa R III, Ip NY, Belluscio L et al: Human and rat brain-derived neurotrophic factor and neurotrophin-3: Gene structures, distributions and chromosomal localizations, *Genomics* 10:558–68, 1991.

Lee R, Kermani P, Teng KK, Hempstead BL: Regulation of cell survival by secreted proneurotrophins, *Science* 294:1945–8, 2001.

Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K et al: Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity, *Science* 306:487–91, 2004.

Chen H, Weber AJ: BDNF enhances retinal ganglion cell survival in cats with optic nerve damage, *Invest Ophthalm Vis Sci* 42:966–74, 2001.

Kawamura K, Kawamura N, Mulders SM, Sollewijn Gelpke MD et al: Ovarian brain-derived neurotrophic factor (BDNF) promotes the development of oocytes into preimplantation embryos, *Proc Natl Acad Sci USA* 102:9206–11, 2005.

Conover JC, Erickson JT, Katz DM, Bianchi LM, Poueymirou WT et al: Neuronal deficits, not involving motor neurons, in mice lacking BDNF and/or NT4, *Nature* 375:235–8, 1995.

Liu X, Ernfors P, Wu H, Jaenisch R: Sensory but not motor neuron deficits in mice lacking NT4 and BDNF, *Nature* 375:238–41, 1995.

Guillin O, Diaz J, Carroll P, Griffon N, Schwartz J-C et al: BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization, *Nature* 411:86–9, 2001.

Lyons WE, Mamounas LA, Ricaurte GA, Coppola V, Reid SW et al: Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities, *Proc Natl Acad Sci USA* 96:15239–44, 1999.

Peng S, Wuu J, Mufson EJ, Fahnestock M: Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease, *J Neurochem* 93(6):1412–21, 2005.

Jones KR, Reichardt LF: Molecular cloning of a human gene that is a member of the nerve growth factor family, *Proc Natl Acad Sci USA* 87(20):8060–4, 1990.

Baker-Herman TL, Fuller DD, Bavis RW, Zabka AG, Golder FJ et al: BDNF is necessary and sufficient for spinal respiratory plasticity following intermittent hypoxia, *Nature Neurosci* 7:48–55, 2004.

Chen H, Weber AJ: BDNF enhances retinal ganglion cell survival in cats with optic nerve damage, *Invest Ophthalm Vis Sci* 42:966–74, 2001.

Conover JC, Erickson JT, Katz DM, Bianchi LM, Poueymirou WT et al: Neuronal deficits, not involving motor neurons, in mice lacking BDNF and/or NT4, *Nature* 375:235–8, 1995.

Du J, Poo M: Rapid BDNF-induced retrograde synaptic modification in a developing retinotectal system, *Nature* 429:878–83, 2004.

Guillin O, Diaz J, Carroll P, Griffon N, Schwartz J-C et al: BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization, *Nature* 411:86–9, 2001.

Hofer MM, Barde Y-A: Brain-derived neurotrophic factor prevents neuronal death in vivo, *Nature* 331:261–2, 1988.

Huang ZJ, Kirkwood A, Pizzorusso T, Porciatti V, Morales B et al: BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex, *Cell* 98:739–55, 1999.

Jones KR, Reichardt LF: Molecular cloning of a human gene that is a member of the nerve growth factor family, *Proc Natl Acad Sci USA* 87:8060–4, 1990.

Kawamura K, Kawamura N, Mulders SM, Sollewijn Gelpke MD, Hsueh AJW: Ovarian brain-derived neurotrophic factor (BDNF) promotes the development of oocytes into preimplantation embryos, *Proc Natl Acad Sci USA* 102:9206–11, 2005.

Kernie SG, Liebl DJ, Parada LF: BDNF regulates eating behavior and locomotor activity in mice, *EMBO J* 19:1290–1300, 2000.

Kovalchuk Y, Hanse E, Kafitz KW, Konnerth A: Postsynaptic induction of BDNF-mediated long-term potentiation, *Science* 295:1729–34, 2002.

Lawrence JM, Keegan DJ, Muir EM, Coffey PJ, Rogers JH et al: Transplantation of Schwann cell line clones secreting GDNF or BDNF into the retinas of dystrophic Royal College of Surgeons rats, *Invest Ophthalm Vis Sci* 45:267–74, 2004.

Lee R, Kermani P, Teng KK, Hempstead BL: Regulation of cell survival by secreted proneurotrophins, *Science* 294:1945–8, 2001.

Li Y, Jian J-C, Cui K, Li N, Zheng Z-Y et al: Essential role of TRPC channels in the guidance of nerve growth cones by brain-derived neurotrophic factor, *Nature* 434:894–8, 2005.

Liu X, Ernfors P, Wu H, Jaenisch R: Sensory but not motor neuron deficits in mice lacking NT4 and BDNF, *Nature* 375:238–41, 1995.

Lyons WE, Mamounas LA, Ricaurte GA, Coppola V, Reid SW et al: Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities, *Proc Natl Acad Sci USA* 96:15239–44, 1999.

Ming G, Wong ST, Henley J, Yuan X, Song H et al: Adaptation in the chemotactic guidance of nerve growth cones, *Nature* 417:411–8, 2002.

Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K et al: Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity, *Science* 306:487–91, 2004.

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

13.5. Neurotrophin 3

Jones KR, Reichardt LF: Molecular cloning of a human gene that is a member of the nerve growth factor family, *Proc Natl Acad Sci USA* 87:8060–4, 1990.

Maisonpierre PC, Le Beau MM, Espinosa R III, Ip NY, Belluscio L et al: Human and rat brain-derived neurotrophic factor and neurotrophin-3: Gene structures, distributions, and chromosomal localizations, *Genomics* 10:558–68, 1991.

Kalcheim C, Carmeli C, Rosenthal A: Neurotrophin 3 is a mitogen for cultured neural crest cells, *Proc Natl Acad Sci USA* 89:1661–5, 1992.

Ramer MS, Priestley JV, McMahon SB: Functional regeneration of sensory axons into the adult spinal cord, *Nature* 403:312–6, 2000.

Cosgaya JM, Chan JR, Shooter EM: The neurotrophin receptor p75(NTR) as a positive modulator of myelination, *Science* 298:1245–8, 2002.

Kuruvilla R, Zweifel LS, Glebova NO, Lonze BE, Valdez G et al: A neurotrophin signaling cascade coordinates sympathetic neuron development through differential control of TrkA trafficking and retrograde signaling, *Cell* 118:243–55, 2004.

Ernfors P, Lee K-F, Kucera J, Jaenisch R: Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents, *Cell* 77:503–12, 1994.

Tessarollo L, Vogel KS, Palko ME, Reid SW, Parada LF: Targeted mutation in the neurotrophin-3 gene results in loss of muscle sensory neurons, *Proc Natl Acad Sci USA* 91:11844–8, 1994.

Donovan MJ, Hahn R, Tessarollo L, Hempstead BL: Identification of an essential nonneuronal function of neurotrophin 3 in mammalian cardiac development, *Nature Genet* 14:210–3, 1996.

13.6. Neurotrophin (NT)4

Ip NY, Ibanez CF, Nye SH, McClain J, Jones PF et al: Mammalian neurotrophin-4: structure, chromosomal localization, tissue distribution, and receptor specificity, *Proc Natl Acad Sci USA* 89:3060–4, 1992.

Ibanez CF: Neurotrophin-4: the odd one out in the neurotrophin family, *Neurochem Res* 21:787–93, 1996.

Robinson LLL, Townsend J, Anderson RA: The human fetal testis is a site of expression of neurotrophins and their receptors: regulation of the germ cell and peritubular cell population, *J Clin Endocr Metab* 88:3943–51, 2003.

Xie C-W, Sayah D, Chen Q-S, Wei W-Z, Smith D et al: Deficient long-term memory and long-lasting long-term potentiation in mice with a targeted deletion of neurotrophin-4 gene, *Proc Natl Acad Sci USA* 97:8116–21, 2000.

13.7. Nerve Growth Factor (NGF)

Ramer MS, Priestley JV, McMahon SB: Functional regeneration of sensory axons into the adult spinal cord, *Nature* 403:312–6, 2000.

Ieda M, Fukuda K, Hisaka Y, Kimura K, Kawaguchi H et al: Endothelin-1 regulates cardiac sympathetic innervation in the rodent heart by controlling nerve growth factor expression, *J Clin Invest* 113:876–84, 2004.

Zabel BU, Eddy RL, Scott J, Shows TB: The human nerve growth factor gene (NGF) is located on the short arm of chromosome 1 [abstract], *Cytogenet Cell Genet* 37:614, 1984.

Ullrich A, Gray A, Berman C, Dull TJ: Human beta-nerve growth factor gene sequence highly homologous to that of mouse, *Nature* 303:821–5, 1983.

Lee R, Kermani P, Teng KK, Hempstead BL: Regulation of cell survival by secreted proneurotrophins, *Science* 294:1945–8, 2001.

Harrington AW, Leiner B, Blechschmitt C, Arevalo JC, Lee R et al: Secreted proNGF is a pathophysiological death-inducing ligand after adult CNS injury, *Proc Natl Acad Sci USA* 101:6226–30, 2004.

13.8. Glial Cell-Derived Neurotrophic Factor

Lin LF, Doherty DH, Lile JD, Bektesh S, Collins F: GDNF: A glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons, *Science* 260(5111):1130–2, May 1993.

Quintero EM, Willis LM, Zaman V, Lee J, Boger HA et al: Glial cell line-derived neurotrophic factor is essential for neuronal survival in the locus coeruleus-hippocampal noradrenergic pathway, *Neuroscience* 124(1):137–46, 2004.

Gianino S, Grider JR, Cresswell J, Enomoto H, Heuckeroth RO: GDNF availability determines enteric neuron number by controlling precursor proliferation, *Development* 130(10):2187–98, May 2003.

Haase G, Dessaud E, Garces A, de Bovis B, Birling M et al: GDNF acts through PEA3 to regulate cell body positioning and muscle innervation of specific motor neuron pools, *Neuron* 35(5):893–905, Aug 2002.

Shakya R, Jho EH, Kotka P, Wu Z, Kholodilov N et al: The role of GDNF in patterning the excretory system, *Dev Biol* 283(1):70–84, July 2005.

Whitehead J, Keller-Peck C, Kucera J, Tourtellotte WG: Glial cell-line derived neurotrophic factor-dependent fusimotor neuron survival during development, *Mech Dev* 122(1):27–41, Jan 2005.

Beck KD, Valverde J, Alexi T, Poulsen K, Moffat B et al: Mesencephalic dopaminergic neurons protected by GDNF from axotomy-induced degeneration in the adult brain, *Nature* 373:339–41, 1995.

Boucher TJ, Okuse K, Bennett DLH, Munson JB, Wood JN et al: Potent analgesic effects of GDNF in neuropathic pain states, *Science* 290:124–7, 2000.

Durbec P, Marcos-Gutierrez CV, Kilkenny C, Grigoriou M, Wartiowaara K et al: GDNF signalling through the Ret receptor tyrosine kinase, *Nature* 381:789–93, 1996.

Gash DM, Zhang Z, Ovadia A, Cass WA, Yi A et al: Functional recovery in parkinsonian monkeys treated with GDNF, *Nature* 380:252–5, 1996.

Gill SS, Patel NK, Hotton GR, O'Sullivan K, McCarter R et al: Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease, *Nature Med* 9:589–95, 2003.

Kordower JH, Emborg ME, Bloch J, Ma SY, Chu Y et al: Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease, *Science* 290:767–73, 2000.

Lin L-FH, Doherty DH, Lile JD, Bektesh S, Collins F: GDNF: A glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons, *Science* 260:1130–2, 1993.

Meng X, Lindahl M, Hyvonen ME, Parvinen M, de Rooij DG et al: Regulation of cell fate decision of undifferentiated spermatogonia by GDNF, *Science* 287:1489–93, 2000.

Moore MW, Klein RD, Farinas I, Sauer H, Armanini M et al: Renal and neuronal abnormalities in mice lacking GDNF, *Nature* 382:76–9, 1996.

Nguyen QT, Parsadanian AS, Snider WD, Lichtman JW: Hyperinnervation of neuromuscular junctions caused by GDNF overexpression in muscle, *Science* 279:1725–9, 1998.

Oppenheim RW, Houenou LJ, Johnson JE, Lin L-FH, Li L et al: Developing motor neurons rescued from programmed and axotomy-induced cell death by GDNF, *Nature* 373:344–6, 1995.

Pichel JG, Shen L, Shang HZ, Granholm AC, Drago J et al: Defects in enteric innervation and kidney development in mice lacking GDNF, *Nature* 382:73–6, 1996.

Sanchez MP, Silos-Santiago I, Frisen J, He B, Lira SA et al: Renal agenesis and the absence of enteric neurons in mice lacking GDNF, *Nature* 382:70–3, 1996.

Tomac A, Lindqvist E, Lin L-FH, Ogren SO, Young D et al: Protection and repair of the nigrostriatal dopaminergic system by GDNF in vivo, *Nature* 373:335–9, 1995.

Treanor JJS, Goodman L, de Sauvage F, Stone DM, Poulsen KT et al: Characterization of a multicomponent receptor for GDNF, *Nature* 382:80–3, 1996.

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

13.9. Prevention of Ion Flux

Agrawal SK, Fehlings MG: Mechanisms of secondary injury to spinal cord axons in vitro: Role of Na+, Na(+)-K(+)-ATPase, the Na(+)-H+ exchanger, and the Na(+)-Ca2+ exchanger, *J Neurosci* 16:545–52, 1996.

Agrawal SK, Fehlings MG: Role of NMDA and non-NMDA ionotropic glutamate receptors in traumatic spinal cord axonal injury, *J Neurosci* 17:1055–63, 1997.

Agrawal SK, Fehlings MG: The effect of the sodium channel blocker QX-314 on recovery after acute spinal cord injury, *J Neurotrauma* 14:81–8, 1997.

Agrawal SK, Nashmi R, Fehlings MG: Role of L- and N-type calcium channels in the pathophysiology of traumatic spinal cord white matter injury, *Neuroscience* 99:179–88, 2000.

Li S, Jiang Q, Stys PK: Important role of reverse Na(+)-Ca(2+) exchange in spinal cord white matter injury at physiological temperature, *J Neurophysiol* 84:1116–9, 2000.

Nashmi R, Jones OT, Fehlings MG: Abnormal axonal physiology is associated with altered expression and distribution of Kv1.1 and Kv1.2 K+ channels after chronic spinal cord injury, *Eur J Neurosci* 12:491–506, 2000.

Agrawal SK, Fehlings MG: Mechanisms of secondary injury to spinal cord axons in vitro: Role of Na+, Na(+)-K(+)-ATPase, the Na(+)-H+ exchanger, and the Na(+)-Ca2+ exchanger, *J Neurosci* 16(2):545–52, Jan 1996.

Rosenberg LJ, Teng YD, Wrathall JR: 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline reduces glial loss and acute white matter pathology after experimental spinal cord contusion, *J Neurosci* 19:464–75, 1999.

Shi R, Blight AR: Differential effects of low and high concentrations of 4-aminopyridine on axonal conduction in normal and injured spinal cord, *Neuroscience* 77:553–62, 1997.

Stys PK, Steffensen I: Na(+)-Ca2+ exchange in anoxic/ischemic injury of CNS myelinated axons, *Ann NY Acad Sci* 779:366–78, 1996.

Stys PK: General mechanisms of axonal damage and its prevention, *J Neurol Sci* 233:3–13, 2005.

13.10. Prevention of Excitotoxicity

Li S, Stys PK: Mechanisms of ionotropic glutamate receptor-mediated excitotoxicity in isolated spinal cord white matter, *J Neurosci* 20:1190–8, 2000.

Wada S, Yone K, Ishidou Y et al: Apoptosis following spinal cord injury in rats and preventative effect of N-methyl-D-aspartate receptor antagonist, *J Neurosurg* 91:98–104, 1999.

Li S, Stys PK: Na(+)-K(+)-ATPase inhibition and depolarization induce glutamate release via reverse Na(+)-dependent transport in spinal cord white matter, *Neuroscience* 107(4):675–83, 2001.

Stys PK: General mechanisms of axonal damage and its prevention, *J Neurol Sci* 233:3–13, 2005.

Stys PK: White matter injury mechanisms, *Curr Mol Med* 4(2):113–30, March 2004.

13.11. Stimulation of Stem Cell Differentiation

Kobayashi NR, Fan DP, Giehl KM et al: BDNF and NT-4/5 prevent atrophy of rat rubrospinal neurons after cervical axotomy, stimulate GAP-43 and Talpha1-tubulin mRNA expression, and promote axonal regeneration, *J Neurosci* 17:9583–95, 1997.

Ramer MS, Priestley JV, McMahon SB: Functional regeneration of sensory axons into the adult spinal cord, *Nature* 403:312–6, 2000.

Houweling DA, Bar PR, Gispen WH, Joosten EA: Spinal cord injury: Bridging the lesion and the role of neurotrophic factors in repair, *Prog Brain Res* 117:455–71, 1998.

Terenghi G: Peripheral nerve regeneration and neurotrophic factors, *J Anat* 194:1–14, 1999.

Blesch A, Lu P, Tuszynski MH: Neurotrophic factors, gene therapy, and neural stem cells for spinal cord repair, *Brain Res Bull* 57:833–8, 2002.

Jones LL, Oudega M, Bunge MB, Tuszynski MH: Neurotrophic factors, cellular bridges and gene therapy for spinal cord injury, *J Physiol* 533:83–9, 2001.

Murakami Y, Furukawa S, Nitta A, Furukawa Y: Accumulation of nerve growth factor protein at both rostral and caudal stumps in the transected rat spinal cord, *J Neurol Sci* 198:63–9, 2002.

Whitworth IH, Brown RA, Dore CJ, Anand P, Green CJ et al: Nerve growth factor enhances nerve regeneration through fibronectin grafts, *J Hand Surg* 21:514–22, 1996.

Bloch J, Fine EG, Bouche N, Zurn AD, Aebischer P: Nerve growth factor- and neurotrophin-3-releasing guidance channels promote regeneration of the transected rat dorsal root, *Exp Neurol* 172:425–32, 2001.

Ramer MS, Priestley JV, McMahon SB: Functional regeneration of sensory axons into the adult spinal cord, *Nature* 403:312–16, 2000.

Sendtner M, Holtmann B, Kolbeck R, Thoenen H, Barde YA: Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section, *Nature* 360:757–9, 1992.

Yan Q, Elliott J, Snider WD: Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death, *Nature* 360:753–5, 1992.

Henderson CE, Camu W, Mettling C, Gouin A, Poulsen K et al: Neurotrophins promote motor neuron survival and are present in embryonic limb bud, *Nature* 363:266–70, 1993.

Oudega M, Hagg T: Neurotrophins promote regeneration of sensory axons in the adult rat spinal cord, *Brain Res* 818:431–8, 1999.

Bloch J, Fine EG, Bouche N, Zurn AD, Aebischer P: Nerve growth factor- and neurotrophin-3-releasing guidance channels promote regeneration of the transected rat dorsal root, *Exp Neurol* 172:425–32, 2001.

Dijkhuizen PA, Hermens WT, Teunis MA, Verhaagen J: Adenoviral vector-directed expression of neurotrophin-3 in rat dorsal root ganglion explants results in a robust neurite outgrowth response, *J Neurobiol* 33:172–84, 1997.

Schmalbruch H, Rosenthal A: Neurotrophin-4/5 postpones the death of injured spinal motoneurons in newborn rats, *Brain Res* 700:254–60, 1995.

Stucky C, Shin JB, Lewin GR: Neurotrophin-4: A survival factor for adult sensory neurons, *Curr Biol* 12:1401–4, 2002.

Sendtner M, Kreutzberg GW, Thoenen H: Ciliary neurotrophic factor prevents the degeneration of motor neurons after axotomy, *Nature* 345:440–1, 1990.

Gravel C, Gotz R, Lorrain A, Sendtner M: Adenoviral gene transfer of ciliary neurotrophic factor and brain-derived neurotrophic factor leads to long-term survival of axotomized motor neurons, *Nat Med* 3:765–70, 1997.

Siegel SG, Patton B, English AW: Ciliary neurotrophic factor is required for motoneuron sprouting, *Exp Neurol* 166:205–12, 2000.

Levison SW, Ducceschi MH, Young GM, Wood TL: Acute exposure to CNTF in vivo induces multiple components of reactive gliosis, *Exp Neurol* 141:256–68, 1996.

Henderson CE, Phillips HS, Pollock RA, Davies AM, Lemeulle C et al: GDNF: A potent survival factor for motoneurons present in peripheral nerve and muscle, *Science* 266:1062–4, 1994.

Buj-Bello A, Buchman VL, Horton A, Rosenthal A, Davies AM: GDNF is an age-specific survival factor for sensory and autonomic neurons, *Neuron* 15:821–8, 1995.

Friesel RE, Maciag T: Molecular mechanisms of angiogenesis: Fibroblast growth factor signal transduction, *FASEB J* 9:919–25, 1995.

Romero MI, Rangappa N, Li L et al: Extensive sprouting of sensory afferents and hyperalgesia induced by conditional expression of nerve growth factor in the adult spinal cord, *J Neurosci* 20:4435–45, 2000.

Zhang Y, Dijkhuizen PA, Anderson PN et al: NT-3 delivered by an adenoviral vector induces injured dorsal root axons to regenerate into the spinal cord of adult rats, *J Neurosci Res* 54:554–62, 1998.

Hastings NB, Gould E: Neurons inhibit neurogenesis, *Nat Med* 9:264–6, 2003.

Wu HH, Ivkovic S, Murray RC, Jaramillo S, Lyons KM et al: Autoregulation of neurogenesis by GDF11, *Neuron* 37:197–207, 2003.

Nakashima K, Takizawa T, Ochiai W, Yanagisawa M, Hisatsune T et al: BMP2-mediated alteration in the developmental pathway of fetal mouse brain cells from neurogenesis to astrocytogenesis, *Proc Natl Acad Sci USA* 98:5868–73, 2001.

Setoguchi T, Yone K, Matsuoka E, Takenouchi H, Nakashima K et al: Traumatic injury-induced BMP7 expression in the adult rat spinal cord, *Brain Res* 921:219–25, 2001.

13.12. Enhancement of Axonal Extension, Adhesion, and Reconnection

Morrow DR, Campbell G, Lieberman AR et al: Differential regenerative growth of CNS axons into tibial and peroneal nervous grafts in the thalamus of adult rats, *Exp Neurol* 120:60–9, 1993.

Fernandes KJ, Fan DP, Tsui BJ et al: Influence of the axotomy to cell body distance in rat rubrospinal and spinal motoneurons: Differential regulation of GAP-43, tubulins, and neurofilament-M, *J Compar Neurol* 414:495–510, 1999.

Woolhead CL, Zhang Y, Lieberman AR et al: Differential effects of autologous peripheral nervous grafts to the corpus striatum of adult rats on the regeneration of axons of striatal and nigral neurons and on the expression of GAP-43 and the cell adhesion molecules N-CAM and L1, *J Compar Neurol* 391:259–73, 1998.

Richardson PM, Issa VM: Peripheral injury enhances central regeneration of primary sensory neurones, *Nature* 309:791–3, 1984.

Herdegen T, Skene P, Bahr M: The c-Jun transcription factor: Bipotential mediator of neuronal death, survival and regeneration, *Trends Neurosci* 20:227–31, 1997.

Jenkins R, Tetzlaff W, Hunt SP: Differential expression of immediate early genes in rubrospinal neurons following axotomy in rat, *Eur J Neurosci* 5:203–9, 1993.

Fernandes KJ, Fan DP, Tsui BJ et al: Influence of the axotomy to cell body distance in rat rubrospinal and spinal motoneurons: Differential regulation of GAP-43, tubulins, and neurofilament-M, *J Compar Neurol* 414:495–510, 1999.

Becker T, Bernhardt RR, Reinhard E et al: Readiness of zebrafish brain neurons to regenerate a spinal axon correlates with differential expression of specific cell recognition molecules, *J Neurosci* 18:5789–803, 1998.

Jung M, Petrausch B, Stuermer CA: Axon-regenerating retinal ganglion cells in adult rats synthesize the cell adhesion molecule L1 but not TAG-1 or SC-1, *Mol Cell Neurosci* 9:116–31, 1997.

Woolhead CL, Zhang Y, Lieberman AR et al: Differential effects of autologous peripheral nervous grafts to the corpus striatum of adult rats on the regeneration of axons of striatal and nigral neurons and on the expression of GAP-43 and the cell adhesion molecules N-CAM and L1, *J Compar Neurol* 391:259–73, 1998.

Schreyer DJ, Skene JH: Injury-associated induction of GAP-43 expression displays axon branch specificity in rat dorsal root ganglion neurons, *J Neurobiol* 24:959–70, 1993.

Zhang Y, Bo X, Schoepfer R, Holtmaat AJ, Verhaagen J et al: Growth-associated protein GAP-43 and L1 act synergistically to promote regenerative growth of Purkinje cell axons in vivo, *Proc Natl Acad Sci USA* 102:14883–14888, 2005.

Carulli D, Buffo A, Strata P: Reparative mechanisms in the cerebellar cortex, *Prog Neurobiol* 72:373–98, 2004.

Smith GM, Romero MI: Adenoviral-mediated gene transfer to enhance neuronal survival, growth, and regeneration, *J Neurosci Res* 55:147–57, 1999.

Thanos PK, Okajima S, Terzis JK: Ultrastructure and cellular biology of nerve regeneration, *J Reconstr Microsurg* 14:423–36, 1998.

Mohajeri MH, Bartsch U, van der Putten H, Sansig G, Mucke L et al: Neurite outgrowth on non-permissive substrates in vitro is enhanced by ectopic expression of the neural adhesion molecule L1 by mouse astrocytes, *Eur J Neurosci* 8:1085–97, 1996.

Kobayashi S, Miura M, Asou H, Inoue HK, Ohye C et al: Grafts of genetically modified fibroblasts expressing neural cell adhesion molecule L1 into transected spinal cord of adult rats, *Neurosci Lett* 188:191–4, 1995.

Lin L, Rao Y, Isacson O: Netrin-1 and slit-2 regulate and direct neurite growth of ventral midbrain dopaminergic neurons, *Mol Cell Neurosci* 28:547–55, 2005.

13.13. Prevention of Fibrous Scar Formation

Beggah AT, Dours-Zimmermann MT, Barras FM, Brosius A, Zimmermann DR et al: Lesion-induced differential expression and cell association of Neurocan, Brevican, Versican V1 and V2 in the mouse dorsal root entry zone, *Neuroscience* 133(3):749–62, 2005.

Moon LD, Brecknell JE, Franklin RJ et al: Robust regeneration of CNS axons through a track depleted of CNS glia, *Exp Neurol* 161:49–66, 2000.

Brambilla R, Bracchi-Ricard V, Hu WH, Frydel B, Bramwell A et al: Inhibition of astroglial nuclear factor kappaB reduces inflammation and improves functional recovery after spinal cord injury, *J Exp Med* 202(1):145–56, July 2005.

Caroni P, Schwab ME: Two membrane protein fractions from rat central myelin with inhibitory properties for neurite growth and fibroblast spreading, *J Cell Biol* 106:1281–8, 1988.

Schwab ME, Caroni P: Oligodendrocytes and CNS myelin are nonpermissive substrates for neurite growth and fibroblast spreading in vitro, *J Neurosci* 8:2381–93, 1988.

Cai D, Shen Y, De Bellard M et al: Prior exposure to neurotrophins blocks inhibition of axonal regeneration by MAG and myelin via a cAMP-dependent mechanism, *Neuron* 22:89–101, 1999.

McKerracher L, David S, Jackson DL et al: Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth, *Neuron* 13:805–11, 1994.

Mukhopadhyay G, Doherty P, Walsh FS et al: A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration, *Neuron* 13:757–67, 1994.

Brosamle C, Huber AB, Fiedler M et al: Regeneration of lesioned corticospinal tract fibers in the adult rat induced by a recombinant, humanized IN-1 antibody fragment, *J Neurosci* 20:8061–8, 2000.

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

13.14. Enhancement of Synaptic Formation

Mendell LM, Munson JB, Arvanian VL: Neurotrophins and synaptic plasticity in the mammalian spinal cord, *J Physiol* 533:91–7, 2001.

Jones LL, Oudega M, Bunge MB, Tuszynski MH: Neurotrophic factors, cellular bridges and gene therapy for spinal cord injury, *J Physiol* 533:83–9, 2001.

Ruegg MA: Agrin, laminin beta 2 (s-laminin) and ARIA: Their role in neuromuscular development, *Curr Opin Neurobiol* 6:97–103, 1996.

13.15. Embryonic and Fetal Stem Cells

McDonald JW, Liu XZ, Qu Y et al: Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord, *Nat Med* 5:1410–2, 1999.

Lakatos A, Franklin RJ: Transplant mediated repair of the central nervous system: An imminent solution? *Curr Opin Neurol* 15:701–5, 2002.

Kwon BK, Tetzlaff W: Spinal cord regeneration: From gene to transplants, *Spine* 26(Suppl 24): S13–22, 2001.

Akesson E, Kjaeldgaard A, Seiger A: Human embryonic spinal cord grafts in adult rat spinal cord cavities: Survival, growth, and interactions with the host, *Exp Neurol* 149:262–76, 1998.

Bernstein-Goral H, Bregman BS: Axotomized rubrospinal neurons rescued by fetal spinal cord transplants maintain axon collaterals to rostral CNS targets, *Exp Neurol* 148:13–25, 1997.

Bregman BS, Broude E, McAtee M et al: Transplants and neurotrophic factors prevent atrophy of mature CNS neurons after spinal cord injury, *Exp Neurol* 149:13–27, 1998.

Mori F, Himes BT, Kowada M et al: Fetal spinal cord transplants rescue some axotomized rubrospinal neurons from retrograde cell death in adult rats, *Exp Neurol* 143:45–60, 1997.

Gokhan S, Mehler MF: Basic and clinical neuroscience applications of embryonic stem cells, *Anat Rec* 265:142–56, 2001.

13.16. Adult Nerve Stem Cells

Song HJ, Stevens CF, Gage FH: Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons, *Nat Neurosci* 5:438–45, 2002.

Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O: Neuronal replacement from endogenous precursors in the adult brain after stroke, *Nat Med* 8:963–70, 2002.

Bauer S, Hay M, Amilhon B, Jean A, Moyse E: In vivo neurogenesis in the dorsal vagal complex of the adult rat brainstem, *Neuroscience* 130:75–90, 2005.

Magnus T, Rao MS: Neural stem cells in inflammatory CNS diseases: mechanisms and therapy, *J Cell Mol Med* 9:303–19, 2005.

Palmer TD, Willhoite AR, Gage FH: Vascular niche for adult hippocampal neurogenesis, *J Compar Neurol* 425:479–94, 2000.

Shihabuddin LS, Horner PJ, Ray J, Gage FH: Adult spinal cord stem cells generate neurons after transplantation in the adult dentate gyrus, *J Neurosci* 20:8727–35, 2000.

Tropepe V, Coles BL, Chiasson BJ, Horsford DJ, Elia AJ et al: Retinal stem cells in the adult mammalian eye, *Science* 287:2032–6, 2000.

Lie DC, Dziewczapolski G, Willhoite AR, Kaspar BK, Shults CW et al: The adult substantia nigra contains progenitor cells with neurogenic potential, *J Neurosci* 22:6639–49, 2002.

Lie DC, Song H, Colamarino SA, Ming GL, Gage FH: Neurogenesis in the adult brain: New strategies for central nervous system diseases, *Annu Rev Pharmacol Toxicol* 44:399–421, 2004.

Reynolds BA, Weiss S: Generation of neurons and astrocytes from isolated cells of the adult mammalian central nerve system, *Science* 255:1707–10, 1992.

Richards LJ, Kilpatrick TJ, Bartlett PF: De novo generation of neuronal cells from the adult mouse brain, *Proc Natl Acad Sci USA* 89:8591–5, 2002.

Park KI, Ourednik J, Ourednik V, Taylor RM, Aboody KS et al: Global gene and cell replacement strategies via stem cells, *Gene Ther* 9:613–24, 2002.

13.17. Bone Marrow Cells

Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stedeford T et al: Adult bone marrow stromal cells differentiate into neural cells in vitro, *Exp Neurol* 164:247–56, 2000.

Rice CM, Scolding NJ: Adult stem cells—reprogramming neurological repair? *Lancet* 364:193–9, 2004.

Brazelton TR, Rossi FMV, Keshet GI, Blau HM: From Marrow to brain: Expression of neuronal phenotypes in adult mice, *Science* 290:1775–9, 2000.

Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR: Turning blood into brain: Cells bearing neuronal antigens generated in vivo from bone marrow, *Science* 290:1779–82, 2000.

Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stedeford T, Willing A et al: Adult bone marrow stromal cells differentiate into neural cells in vitro, *Exp Neurol* 164:247–56, 2000.

Weimann JM, Charlton CA, Brazelton TR, Hackman RC, Blau HM: Contribution of transplanted bone marrow cells to Purkinje neurons in human adult brains, *Proc Natl Acad Sci USA* 100:2088–93, 2003.

Cogle CR, Yachnis AT, Laywell ED, Zander DS, Wingard JR et al: Bone marrow transdifferentiation in brain after transplantation: A retrospective study, *Lancet* 363:1432–7, 2004.

Sigurjonsson OE, Perreault MC, Egeland T, Glover JC: Adult human hematopoietic stem cells produce neurons efficiently in the regenerating chicken embryo spinal cord, *Proc Natl Acad Sci USA* 102:5227–32, 2005.

Wagers AJ, Sherwood RI, Christensen JL, Weissman IL: Little evidence for developmental plasticity of adult hematopoietic stem cells, *Science* 297:2256–9, 2002.

13.18. Neuronal Supporting Cells

Imaizumi T, Lankford KL, Kocsis JD: Transplantation of olfactory ensheathing cells or Schwann cells restores rapid and secure conduction across the transected spinal cord, *Brain Res* 854:70–8, 2000.

Cheng H, Cao Y, Olson L: Spinal cord repair in adult paraplegic rats: Partial restoration of hind limb function, *Science* 273:510–3, 1996.

Xu XM, Guenard V, Kleitman N et al: A combination of BDNF and NT-3 promotes supraspinal axonal regeneration into Schwann cell grafts in adult rat thoracic spinal cord, *Exp Neurol* 134:261–72, 1995.

Jones LL, Oudega M, Bunge MB, Tuszynski MH: Neurotrophic factors, cellular bridges and gene therapy for spinal cord injury, *J Physiol* 533:83–9, 2001.

Devon R, Doucette R: Olfactory ensheathing cells myelinate dorsal root ganglion neurites, *Brain Res* 589:175–9, 1992.

Ramon-Cueto A, Valverde F: Olfactory bulb ensheathing glia: A unique cell type with axonal growth-promoting properties, *Glia* 14:163–73, 1995.

Navarro X, Valero A, Gudino G et al: Ensheathing glia transplants promote dorsal root regeneration and spinal reflex restitution after multiple lumbar rhizotomy, *Ann Neurol* 45:207–15, 1999.

Ramon-Cueto A, Cordero MI, Santos-Benito FF et al: Functional recovery of paraplegic rats and motor axon regeneration in their spinal cords by olfactory ensheathing glia, *Neuron* 25:425–35, 2000.

Li Y, Field PM, Raisman G: Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells, *Science* 277:2000–2, 1997.

Ramon-Cueto A, Nieto-Sampedro M: Regeneration into the spinal cord of transected dorsal root axons is promoted by ensheathing glia transplants, *Exp Neurol* 127:232–44, 1994.

13.19. Transgenic Cell Lines

Blesch A, Tuszynski MH: Robust growth of chronically injured spinal cord axons induced by grafts of genetically modified NGF-secreting cells, *Exp Neurol* 148:444–52, 1997.

Grill R, Murai K, Blesch A et al: Cellular delivery of neurotrophin-3 promotes corticospinal axonal growth and partial functional recovery after spinal cord injury, *J Neurosci* 17:5560–72, 1997.

Liu Y, Kim D, Himes BT et al: Transplants of fibroblasts genetically modified to express BDNF promote regeneration of adult rat rubrospinal axons and recovery of forelimb function, *J Neurosci* 19:4370–87, 1999.

Tuszynski MH, Peterson DA, Ray J et al: Fibroblasts genetically modified to produce nervous growth factor induce robust neuritic ingrowth after grafting to the spinal cord, *Exp Neurol* 126:1–14, 1994.

Menei P, Montero-Menei C, Whittemore SR et al: Schwann cells genetically modified to secrete human BDNF promote enhanced axonal regrowth across transected adult rat spinal cord, *Eur J Neurosci* 10:607–21, 1998.

Tuszynski MH, Weidner N, McCormack M et al: Grafts of genetically modified Schwann cells to the spinal cord: Survival, axon growth, and myelination, *Cell Transplant* 197:187–96, 1998.

Weidner N, Blesch A, Grill RJ et al: Nerve growth factor-hypersecreting Schwann cell grafts augment and guide spinal cord axonal growth and remyelinate central nervous system axons in a phenotypically appropriate manner that correlates with expression of L1, *J Compar Neurol* 413:495–506, 1999.

Hendriks WT, Ruitenberg MJ, Blits B, Boer GJ, Verhaagen J: Viral vector-mediated gene transfer of neurotrophins to promote regeneration of the injured spinal cord, *Prog Brain Res* 146:451–76, 2004.

Blits B, Boer GJ, Verhaagen J: Pharmacological, cell, and gene therapy strategies to promote spinal cord regeneration, *Cell Transplant* 11:593–613, 2002.

Kwon BK, Tetzlaff W: Spinal cord regeneration: From gene to transplants, *Spine* 26(Suppl 24): S13–22, 2001.

Cheng H, Cao Y, Olson L: Spinal cord repair in adult paraplegic rats: Partial restoration of hind limb function, *Science* 273:510–13, 1996.

Davies SJ, Fitch MT, Memberg SP, Hall AK, Raisman G et al: Regeneration of adult axons in white matter tracts of the central nervous system, *Nature* 390:680–3, 1997.

Lakatos A, Franklin RJ: Transplant mediated repair of the central nervous system: An imminent solution? *Curr Opin Neurol* 15:701–5, 2002.

13.20. Stimulation of Neuronal Regeneration and Guidance of Axonal Outgrowth by Graft-Based Assistance

Schmidt CE, Leach JB: Neural tissue engineering: strategies for repair and regeneration, *Annu Rev Biomed Eng* 5:293–347, 2003.

Ide C, Tohyama K, Tajima K, Endoh K, Sano K et al: Long acellular nervous transplants for allogeneic grafting and the effects of basic fibroblast growth factor on the growth of regenerating axons in dogs: A preliminary report, *Exp Neurol* 154:99–112, 1998.

Sondell M, Lundborg G, Kanje M: Regeneration of the rat sciatic nerve into allografts made acellular through chemical extraction, *Brain Res* 795:44–54, 1998.

Sondell M, Lundborg G, Kanje M: Vascular endothelial growth factor stimulates Schwann cell invasion and neovascularization of acellular nerve grafts, *Brain Res* 846:219–28, 1999.

Kauppila T, Jyvasjarvi E, Huopaniemi T, Hujanen E, Liesi P: A laminin graft replaces neurorrhaphy in the restorative surgery of the rat sciatic nerve, *Exp Neurol* 123:181–91, 1993.

Tong XJ, Hirai K, Shimada H, Mizutani Y, Izumi T et al: Sciatic nervous regeneration navigated by laminin-fibronectin double coated biodegradable collagen grafts in rats, *Brain Res* 663:155–62, 1994.

Chen YS, Hsieh CL, Tsai CC, Chen TH, Cheng WC et al: Peripheral nerve regeneration using silicone rubber chambers filled with collagen, laminin and fibronectin, *Biomaterials* 21:1541–7, 2000.

Yoshii S, Oka M: Peripheral nerve regeneration along collagen filaments, *Brain Res* 888:158–62, 2001.

Itoh S, Takakuda K, Kawabata S, Aso Y, Kasai K et al: Evaluation of cross-linking procedures of collagen tubes used in peripheral nerve repair, *Biomaterials* 23:4475–81, 2002.

Yoshii S, Oka M, Shima M, Taniguchi A, Akagi M: 30 mm regeneration of rat sciatic nerve along collagen filaments, *Brain Res* 949:202–8, 2002.

Evans GR, Brandt K, Katz S, Chauvin P, Otto L et al: Bioactive poly(L-lactic acid) conduits seeded with Schwann cells for peripheral nervous regeneration, *Biomaterials* 23:841–48, 2002.

Valero-Cabre A, Tsironis K, Skouras E, Perego G, Navarro X et al: Superior muscle reinnervation after autologous nerve graft or poly-L-lactide-epsilon-caprolactone (PLC) tube implantation in comparison to silicone tube repair, *J Neurosci Res* 63:214–23, 2001.

Soldani G, Varelli G, Minnocci A, Dario P: Manufacturing and microscopical characterisation of polyurethane nerve guidance channel featuring a highly smooth internal surface, *Biomaterials* 19:1919–24, 1998.

Dalton PD, Flynn L, Shoichet MS: Manufacture of poly(2-hydroxyethyl methacrylate-co-methyl methacrylate) hydrogel tubes for use as nerve guidance channels, *Biomaterials* 23:3843–51, 2002.

Schmidt CE, Shastri VR, Vacanti JP, Langer R: Stimulation of neurite outgrowth using an electrically conducting polymer, *Proc Natl Acad Sci USA* 94:8948–53, 1997.

Collier JH, Camp JP, Hudson TW, Schmidt CE: Synthesis and characterization of polypyrrole-hyaluronic acid composite biomaterials for tissue engineering applications, *J Biomed Mater Res* 50:574–84, 2000.

Lore AB, Hubbell JA, Bobb DS Jr, Ballinger ML, Loftin KL et al: Rapid induction of functional and morphological continuity between severed ends of mammalian or earthworm myelinated axons, *J Neurosci* 19:2442–54, 1999.

Novikova LN, Novikov LN, Kellerth JO: Biopolymers and biodegradable smart implants for tissue regeneration after spinal cord injury, *Curr Opin Neurol* 16:711–15, 2003.

Bellamkonda R, Aebischer P: Review: Tissue engineering in the nervous system, *Biotechnol Bioeng* 43:543–54, 1994.

Valentini RF: Nerve guidance channels, in *The Biomedical Engineering Handbook*, 2nd ed, Bronzino JD, ed. CRC Press, Boca Raton, FL, 2000, pp 2:135-1–12.

Furnish EJ, Schmidt CE: Tissue engineering of the peripheral nervous system, in *Frontiers in Tissue Engineering*, Patrick CW Jr, Mikos AG, McIntire LV, eds, Elsevier, New York, 1998, pp 514–35.

Thompson DM, Buettner HM: Neurite outgrowth is directed by Schwann cell alignment in the absence of other guidance cues, *Ann Biomed Eng* (in press).

13.21. Autologous Nerve Tissue Grafts

Sunderland S: *Nervous Injuries and Their Repair*, Churchill Livingston, London, 1991.

Chiu DT, Janecka I, Krizek TJ, Wolff M, Lovelace RE: Autogenous vein graft as a conduit for nervous regeneration, *Surgery* 91:226–33, 1982.

Walton RL, Brown RE, Matory WE Jr, Borah GL, Dolph JL: Autogenous vein graft repair of digital nervous defects in the finger: a retrospective clinical study, *Plast Reconstr Surg* 84:944–9, 1989.

Risitano G, Cavallaro G, Merrino T, Coppolino S, Ruggeri F: Clinical results and thoughts on sensory nerve repair by autologous vein graft in emergency hand reconstruction, *Chir Main* 21:194–7, 2002.

Battiston B, Tos P, Cushway TR, Geuna S: Nerve repair by means of vein filled with muscle grafts I. Clinical results, *Microsurgery* 20:32–6, 2000.

Meek MF, Varejao AS, Geuna S: Muscle grafts and alternatives for nerve repair, *J Oral Maxillofac Surg* 60:1095–96, 2002.

Brandt J, Dahlin LB, Lundborg G: Autologous tendons used as grafts for bridging peripheral nerve defects, *J Hand Surg* 24:284–90, 1999.

Hudson TW, Zawko S, Deister C, Lundy S, Hu CY et al: Optimized acellular nerve graft is immunologically tolerated and supports regeneration, *Tissue Eng* 10:1641–51, 2004.

13.22. Allogenic and Xenogeneic Tissue Grafts

Hadlock TA, Sundback CA, Hunter DA, Vacanti JP, Cheney ML: A new artificial nerve graft containing rolled Schwann cell monolayers, *Microsurgery* 21:96–101, 2001.

Mohammad JA, Warnke PH, Pan YC, Shenaq S: Increased axonal regeneration through a biodegradable amnionic tube nerve conduit: Effect of local delivery and incorporation of nerve growth factor/hyaluronic acid media, *Ann Plast Surg* 44:59–64, 2000.

Meek MF, Coert JH, Nicolai JP: Amnion tube for nerv regeneration, *Plast Reconstr Surg* 107:622–23, 2001.

Mligiliche N, Endo K, Okamoto K, Fujimoto E, Ide C: Extracellular matrix of human amnion manufactured into tubes as conduits for peripheral nerve regeneration, *J Biomed Mater Res* 63:591–600, 2002.

13.23. Extracellular Matrix Components

Grimpe B, Silver J: The extracellular matrix in axon regeneration, *Prog Brain Res* 137:333–49, 2002.

Bovolenta P, Fernaud-Espinosa I: Nervous system proteoglycans as modulators of neurite outgrowth, *Prog Neurobiol* 61:113–32, 2000.

Asher RA, Morgenstern DA, Moon LD, Fawcett JW: Chondroitin sulphate proteoglycans: Inhibitory components of the glial scar, *Prog Brain Res* 132:611–19, 2001.

Chamberlain LJ, Yannas IV, Hsu HP, Strichartz GR, Spector M: Near-terminus axonal structure and function following rat sciatic nerve regeneration through a collagen-GAG matrix in a ten-millimeter gap, *J Neurosci Res* 60:666–77, 2000.

Herbert CB, Nagaswami C, Bittner GD, Hubbell JA, Weisel JW: Effects of fibrin micromorphology on neurite growth from dorsal root ganglia cultured in three-dimensional fibrin gels, *J Biomed Mater Res* 40:551–9, 1998.

Holmes TC, de Lacalle S, Su X, Liu G, Rich A et al: Extensive neurite outgrowth and active synapse formation on self-assembling peptide scaffolds, *Proc Natl Acad Sci USA* 97:6728–33, 2000.

Hashimoto T, Suzuki Y, Kitada M, Kataoka K, Wu S et al: Peripheral nerve regeneration through alginate gel: Analysis of early outgrowth and late increase in diameter of regenerating axons, *Exp Brain Res* 146:356–68, 2002.

Balgude AP, Yu X, Szymanski A, Bellamkonda RV: Agarose gel stiffness determines rate of DRG neurite extension in 3D cultures, *Biomaterials* 22:1077–84, 2001.

Mosahebi A, Simon M, Wiberg M, Terenghi G: A novel use of alginate hydrogel as Schwann cell matrix, *Tissue Eng* 7:525–34, 2001.

Haipeng G, Yinghui Z, Jianchun L, Yandao G, Nanming Z et al: Studies on nerve cell affinity of chitosan-derived materials, *J Biomed Mater Res* 52:285–95, 2000.

Rosner BI, Siegel RA, Grosberg A, Tranquillo RT: Rational design of contact guiding, neurotrophic matrices for peripheral nerve regeneration, *Ann Biomed Eng* 31:1383–401, 2003.

13.24. Synthetic Materials

Molander H, Olsson Y, Engkvist O, Bowald S, Eriksson I: Regeneration of peripheral nerve through a polyglactin tube, *Muscle Nervous* 5:54–7, 1982.

Nyilas E, Chiu TH, Sidman RL, Henry EW, Brushart TM et al: Peripheral nerve repair with bioresorbable prosthesis, *Trans Am Soc Artif Intern Organs* 29:307–13, 1983.

13.25. Role of Geometric Factors

Smeal RM, Rabbitt R, Biran R, Tresco PA: Substrate curvature influences the direction of nerve outgrowth, *Ann Biomed Eng* 33:376–82, 2005.

Dubey N, Letourneau PC, Tranquillo RT: Guided neurite elongation and Schwann cell invasion into magnetically aligned collagen in simulated peripheral nerve regeneration, *Exp Neurol* 158:338–50, 1999.

Ceballos D, Navarro X, Dubey N, Wendelschafer-Crabb G, Kennedy WR et al: Magnetically aligned collagen gel filling a collagen nerve guide improves peripheral nerve regeneration, *Exp Neurol* 158:290–300, 1999.

13.26. Improvement of Nerve Function

Prochazka A, Mushahwar VK, McCreery DB: Neural prostheses, *J Physiol* 533:99–109, 2001.

Grill WM, McDonald JW, Peckham PH, Heetderks W, Kocsis J et al: At the interface: Convergence of neural regeneration and neural prostheses for restoration of function, *J Rehab Res Dev* 38:633–9, 2001.

Condic ML, Lemons ML: Extracellular matrix in spinal cord regeneration: Getting beyond attraction and inhibition, *NeuroReport* 13:A37–48, 2002.

Degenerative Neural Diseases

Degenerative neural diseases are a group of disorders characterized by progressive atrophic changes in the brain and spinal cord, resulting in the disorder of intellectual ability, motor control, and/or sensation, depending on the types of neuron involved. While there are numbers of degenerative neural disorders, we will focus on several common types, including Alzheimer's disease (progressive dementia), Huntington's chorea (progressive dementia complicated by other neurological disorders), Parkinson's disease (disorder of the motor control system), and muscular atrophy (progressive spinal muscular atrophy). The clinical symptoms and signs, etiology, pathology, pathogenesis, and treatment of these disorders are discussed in the following sections.

Alzheimer's Disease

Etiology, Pathology, and Clinical Manifestations [13.27]. Alzheimer's disease, also known as *presenile dementia*, is a neural degenerative disorder characterized by progressive loss of intellectual capability involving the impairment of memory, judgment, and logical thinking as well as changes in personality within a course of 5–10 years. The primary cause of Alzheimer's disease is apoptosis of the cholinergic neurons in the basal forebrain. The disorder may occur at any age, but is traditionally diagnosed as Alzheimer's disease when it is initiated before age 65. Similar neurological changes after 65 are considered consequences of aging, known as *senile dementia*. About 1% of patients with Alzheimer's disease have a family history of the disease, suggesting genetic influence on the occurrence of the disease.

Alzheimer's disease is a progressive disorder associated with sequential clinical features. The onset of the disease is insidious and difficult to notice. There may be a long asymptomatic, preclinical period (7 years or more) before the disease is diagnosed. It is usually found at a certain level of severity. There are several loosely defined neurological deficits, which may occur in sequence. These include amnesia, dysnomia, disorientation, and paranoia. Amnesia may occur in the early stage of Alzheimer's disease. The patient may exhibit gradual loss of short- or long-term retentive memory. The disorders may be intermittent. With the progression of the disease, the patient may show dysnomia. Major signs include inappropriate use of names, forgetting common words, and impairment of speech. Eventually, the patient may be unable to read and understand. Visuospatial disorientation is another common sign of Alzheimer's disease. The patient may find it difficult

to park a car, put arms into sleeves, put books in order, or find the way home. Paranoia or changes in personality may occur in a large fraction of patients. Eventually the patient completely loses intellectual abilities, including memory and judgment.

Alzheimer's disease is associated with a number of pathological changes. These include diffuse atrophy of the cerebral cortex with an apparent decrease in brain size, especially the size of the hippocampus, and an increase in the volume of cerebral ventricles (Fig. 13.22). At the microscopic level, the loss of neurons or neuronal apoptosis is noticeable in the hippocampus and cerebral cortex (Fig. 13.23). Neuronal degeneration is associated with proliferation of astrocytes as a compensatory mechanism. There exist scattered pathological lesions in the extracellular space known as *senile plaques*, which are composed of fragmented axons and dendrites surrounding an amyloid core structure. In the cytoplasm of the neurons, fiber-like structures, such as coils and knots, known as neurofibrillary *tangles* or *clumps*, are often observed. The neurofibrillary tangles are composed of a highly phosphorylated form of a microtubule-associated protein τ. These changes are associated with progressive degeneration of neurons primarily in the cerebral cortex.

Alzheimer's disease is characterized by the death of cholinergic neurons (Fig. 13.24). A number of factors contribute to the pathogenesis of cholinergic neuron death. These include the accumulation of amyloid plaques in the extracellular matrix, the accumulation of neurofibrillary tangles in the neuronal cytoplasm (Fig. 13.25), and progressive loss of choline acetyltransferase and acetylcholine in the cerebral cortex. In particular, the formation of amyloid plaques and neurofibrillary tangles is considered a major pathological change that contributes to the death of cholinergic neurons and the progression of Alzheimer's disease. However, the mechanisms remain poorly understood.

Formation of amyloid plaques is due to the accumulation of proteins, inclduing a small protein known as *amyloid* β (Aβ) *peptide*. This peptide is a product of the cleavage of the amyloid β protein precursor (APP), a protein normally bound to the membrane of the neurons. The amyloid β protein precursor can be cleaved by β- and γ-secretases and metalloproteases, generating 40- and 42-amino acid amyloid β peptides. The 42-amino acid peptide is shed from the neuronal membrane, and aggregates into Alzheimer plaques (Fig. 13.26).

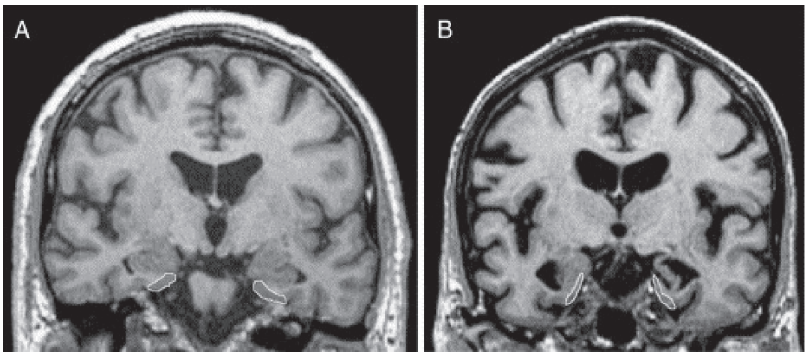


Figure 13.22. Coronal MRI at the level of the mammillary bodies. (A) A normal control; (B) a person with mild cognitive impairment. (Reprinted from Jose C, Masdeu JC et al: Neuroimaging as a marker of the onset and progression of Alzheimer's disease, *J Neurol Sci* 236:55–64, copyright 2005, with permission from Elsevier.)

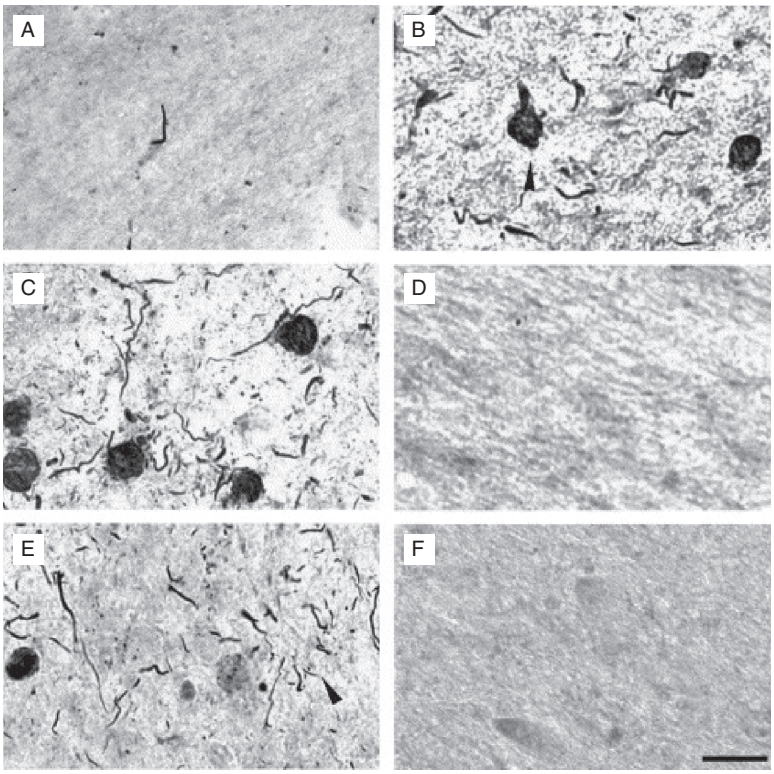


Figure 13.23. In the nucleus basalis of Meynert (NBM) of normal control individuals, rare Fas-associated death-domain (FADD)-positive neurites were observed (A). However, many FADD-positive tangle-like structures (arrowhead in panel B) and neurites (arrowhead in panel E) were observed within the NBM of all the Alzheimer's cases. The Alzheimer's brains with either Braak stage V (B) or Braak stage VI (C) displayed the same pattern of FADD-positive structures within the NBM. Preabsorption of the antibody with excess FADD peptide resulted in complete absence of FADD immunoreactivity (D). Immunoreactivity for caspase-3 (F) was completely absent from the NBM of AD. Scale bar: 100 μm for all panels. (Reprinted from Wu CK et al: Apoptotic signals within the basal forebrain cholinergic neurons in Alzheimer's disease, *Exp Neurol* 195:484–96, copyright 2005, with permission from Elsevier.)

Figure 13.25. Formation of amyloid plaques and neurofibrillary tangles in Alzheimer's disease. Amyloid plaques contain the Aβ peptide, which is produced from the amyloid precursor protein (APP). Cleavage of APP at one point (not shown) generates a carboxy-terminal fragment. This is then severed by the γ-secretase complex (which consists of at least four proteins: presenilin-1, APH1, PEN2, and nicastrin), producing Aβ. Tangles are produced from hyperphosphorylated τ protein, possibly by means of the enzyme glycogen synthase kinase-3 (GSK3). GSK3 might also regulate the cleavage of APP carboxy-terminal fragments. Lithium and kenpaullone, two GSK3 inhibitors, as well as small interfering RNAs that knock down GSK3 expression, inhibit Aβ production. The GSK3 inhibitors might interfere with the generation of both amyloid plaques and tangles in Alzheimer's disease. The precise roles of the two forms of GSK3, α and β, are unknown. (Reprinted by perission from Macmillan Publishers Ltd.: De Strooper B, Woodgett J: Alzheimer's disease: Mental plaque removal, *Nature* 423:392–3, copyright 2003.)

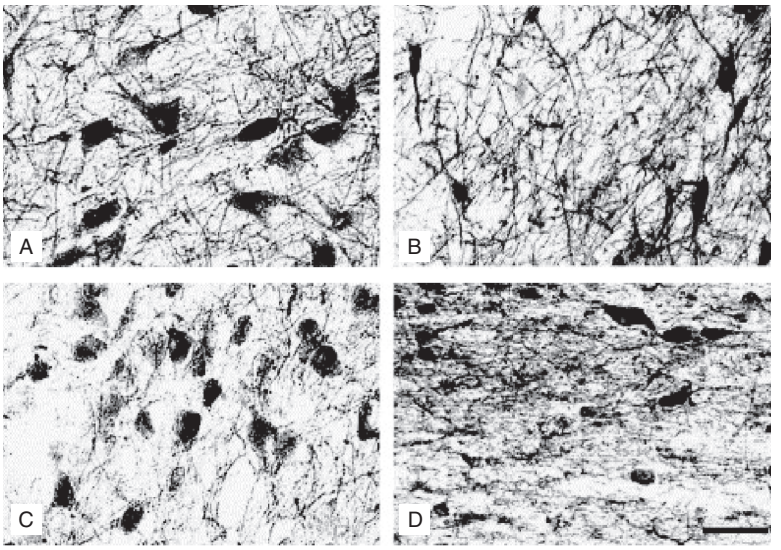
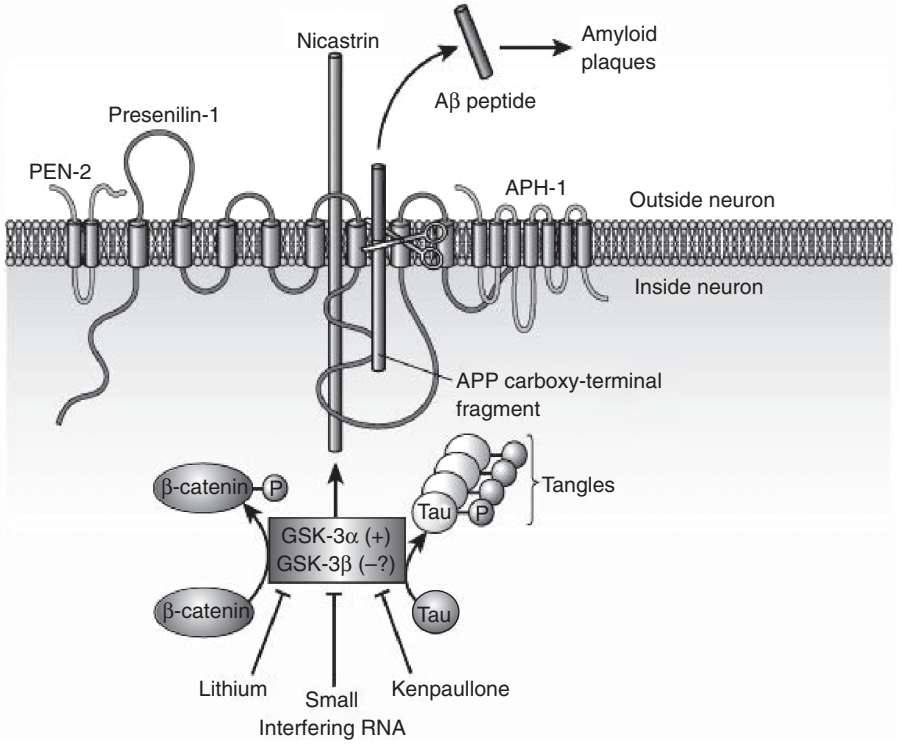


Figure 13.24. Choline acetyltransferase (ChAT) neurons in normal and Alzheimer's brains. In the basal forebrain of normal cases, many ChAT- (A) or p75-positive (C) cholinergic neurons were present. In contrast, very few ChAT- (B) or p75-positive (D) cholinergic neurons were found within the nucleus basalis of Meynert (NBM) of Alzheimer's cases. Scale bar: 100 μm, in all panels. (Reprinted from Wu CK et al: Apoptotic signals within the basal forebrain cholinergic neurons in Alzheimer's disease, *Exp Neurol* 195:484–96, copyright 2005, with permission from Elsevier.)



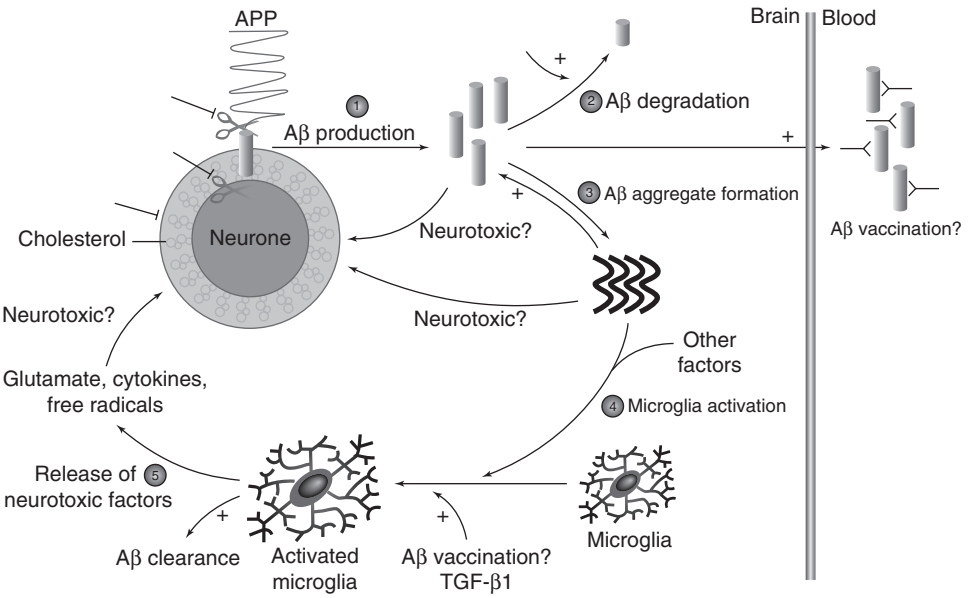


Figure 13.26. The amyloid hypothesis in Alzheimer's disease (AD) and candidate targets for therapeutic intervention. AD is characterized by two types of protein aggregates, neurofibrillary tangles and β-amyloid (Aβ) plaques, distributed in regions of the central nervous system involved in learning and memory. Soluble Aβ is formed following the cleavage of Aβ precursor protein (APP) by enzymatic activities known as β-secretase and γ-secretase (1). Aβ formed is then degraded by enzymes (2). The balance between Aβ production and degradation can be disrupted, leading to Aβ accumulation beyond pathological levels and, in turn, increased levels of Aβ aggregates (3) and deposits in the brain. Aggregates and Aβ fibrils could themselves be neurotoxic or could activate microglia (4), which can release neurotoxic factors as part of an inflammatory response (5). Several steps could be targeted pharmacologically to treat AD. For example, inhibitors of β-secretase and γ-secretase or cholesterol-lowering drugs (blunt arrows) could be used to decrease the production of Aβ. It is possible that activators of Aβ-degrading enzymes could be developed to reduce Aβ levels and metal chelators could be used to dissolve amyloid plaques. Furthermore, Aβ vaccination is proposed to sequester Aβ in the blood, which in turn would induce a rapid efflux of Aβ from the brain. Microglia can also be activated by Aβ vaccination or by transforming growth factor β1 (TGFβ1), leading to increased Aβ clearance and neuroprotection. (Reprinted from Dominguez DI, De Strooper B: Novel therapeutic strategies provide the real test for the amyloid hypothesis of Alzheimer's disease, *Trends Pharmacol Sci* 23:324–30, copyright 2002 with permission from Elsevier.)

The formation of the neurofibrillary tangles may be related to alterations in the phosphoralation of the τ protein. This protein may undergo several pathological changes, including hyperphosphorylation, enzymatic cleavage, and conformational alterations, which contribute to the formation of Alzheimer's tangles and the pathogenesis of Alzheimer's disease. The role of the protein τ in initiating Alzheimer's disease has been established with the observation that hyperphosphorylated τ is present in the neuronal tangles in Alzheimer's disease (chapter-opening figure). However, the exact mechanisms of τ-involved pathogenesis remains poorly understood. A serine/threonine kinase that induces τ hyperphosphorylation is the enzyme glycogen synthase kinase 3 (GSK3) (Fig.

13.25). This enzyme may also mediate the cleavage of the amyloid β protein precursor, contributing to the formation of the amyloid plaques. The inhibition of the GSK3 by specific pharmarcological inhibitors, such as lithium and kenpaullone, and small interfering RNA prevents the formation of neurofibrillary tangles and amyloid plaques.

Another factor that contributes to the initiation and development of Alzheimer's disease is the progressive loss of choline acetyltransferase. This enzyme catalyzes the transfer of an acetyl group to choline to form acetylcholine, which is the primary neurotransmitter of the cholinergic neurons. The loss of this enzyme reduces the production of acetylcholine, thus inducing the impairment of the function and survival of cholinergic neurons. The defect of the choline acetyltransferase has been considered a major pathogenic factor for Alzheimer's disease.

Conventional Treatment of Alzheimer's Disease [13.28]. Alzheimer's disease has been traditionally treated with several approaches, including: (1) improvement of the cerebral bloodflow, (2) supplementation of cholinergic neurotransmitter precursors, (3) reduction of the degradation of the neurotransmitter acetylcholine, and (4) treatment of abnormal behaviors. The cerebral bloodflow can be enhanced by administrating vasodilators. The improvement of blood supply to the brain may reduce the degeneration of neurons, thus slowing down the progression of Alzheimer's disease. Cholinergic neurotransmitter precursors, such as choline, lecithin, and acetyl-L-carnitine, are often used to enhance the production of acetylcholine, the neurotransmitter of cholinergic neurons. However, the concentration of a precursor may not be the rate-limiting factor for the production of acetylcholine. Many studies in which these substances were used failed to show substantial beneficial effects. An alternative approach for reducing the degradation of acetylcholine is to suppress the enzyme acetylcholinesterase, which breaks down acetylcholine in synapses. Several pharmacological inhibitors, including tacrine, physostigmine, donepezil, and rivastigmine, have been used to suppress acetylcholinesterase, thus reducing the degradation of acetylcholine. The use of acetylcholinesterase inhibitors has shown mild beneficial effects on the progression of Alzheimer's disease. When patients express significant signs of depression and hallucinations, tranquilizers or antidepressants can be administrated to control the patients.

A new approach has been established and used to treat Alzheimer's disease by modulating the activity of the nicotinic and muscarinic receptors in the neuron. These receptors, which can interact with acetylcholine and participate in the regulation of cognitive function, are decreased in the brain of Alzheimer's patients. A potential treatment is to administrate muscarinic receptor agonists, such as AF102B [(+/−)-cis-2-methyl-spiro (1,3-oxathiolane-5,3′)quinuclidine] and AF150(S) [1-methylpiperidine-4-spiro-(2′-methylthiazoline)], which are cholinomimetics or analogs of acetylcholine. These substances can enhance the activity of the muscarinic receptor, act synergistically with neurotrophic factors to stimulate neuronal regeneration, protect neurons from oxidative stress-induced apoptosis, and reduce the level of β-amyloid, which contributes to the development of Alzheimer's disease. Preliminary investigations have shown that these substances exert a beneficial effect on the cognitive function of patients with Alzheimer's disease.

Similarly, agonists to the nicotinic acetylcholine receptor, such as SIB-1553A and GTS-21, have been used to enhance the function of the receptor in animal models of Alzheimer's disease. SIB-1553A is a nicotinic acetylcholine receptor ligand, also known as (+/−)-4-[[2-(1-methyl-2-pyrrolidinyl)ethyl]thio]phenol hydrochloride. This substance has been tested in aged rodents and nonhuman primates for its effect on the cognitive

function of these animals. A treatment with SIB-1553A (subcutaneous, intramuscular, or oral administration) improves choice accuracy as well as spatial and nonspatial working memory in aged rhesus monkeys. Furthermore, a treatment with SIB-1553A induces the release of acetylcholine in the hippocampus of aged rats, suggesting that the SIB-1553A-induced cognitive improvement may be dependent on the release acetylcholine. Thus SIB-1553A may serve as a therapeutic substance for Alzheimer's disease.

GTS-21, also known as *DMXBA*, is a benzylidene anabaseine derivative [3-(2,4-dimet hoxybenzylidene)anabaseine] based on a marine worm toxin named *anabaseine*. This substance nonselectively activates both muscle-type and neuronal nicotinic acetylcholine receptors. GTS-21 has been shown to exert a therapeutic effect on Alzheimer's disease. GTS-21 exhibits neuroprotective activity in cultured neurons deprived of nervous growth factors or exposed to β-amyloid. A treatment with this substance enhances cognitive function in animal models such as the mouse, monkey, rat, and rabbit. This substance exhibits lower toxicity than nicotine. At a dose that enhances the cognitive behavior of animals, it does not significantly affect the activities of the nervous and skeletal muscle systems. Clinical trials have demonstrated promising results for the treatment of Alzheimer's disease. Oral administration of GTS-21 improves the cognitive behavior of patients with Alzheimer's disease.

Molecular Regenerative Engineering for Alzheimer's Disease. Based on the pathogenic mechanisms as discussed on page 554, several strategies have been established for the molecular treatment of Alzheimer's disease, including prevention of neuronal degeneration and inhibition of glycogen synthase kinase 3. These strategies are briefly discussed as follows.

PREVENTION OF NEURONAL DEGENERATION BY DELIVERING NEUROTROPHIC FACTORS [13.29]. As discussed above, Alzheimer's disease is caused at least in part by the degeneration of cerebral cholinergic neurons resulting from the reduction in the neurotransmitter acetylcholine. Thus, the principle of molecular treatment for Alzheimer's disease is to reduce the degradation of acetylcholine and promote the generation of this neurotransmitter. A common molecular approach for the treatment of Alzheimer's disease is to deliver locally neurotrophic factors, including brain-derived neurotrophic factor, neurotrophins, and nervous growth factor, or the genes encoding these growth factors, into target sites such as the forebrain cortex and the hippocampus, which are involved in Alzheimer's disease. These growth factors play a critical role in controlling neuronal survival (see page 521 for the characteristics of neurotrophic factors). In particular, neurotrophins and nervous growth factor can stimulate neuronal release of acetylcholine. These growth factors or their genes can be delivered by using methods described on page 436.

Experimental investigations have produced promising results for the treatment of Alzheimer's disease with neurotrphins. For instance, the transfection of the forebrain neurons with the nervous growth factor and brain-derived neurotrophic factor genes can significantly boost the production of acetylcholine and enhances the survival of impaired cholinergic neurons in animal models. It should be noted that the selection of the delivery route for cerebral gene transfer is an important issue. The bloodstream delivery of growth factors or their genes is a relatively simple method, but these molecules may not be able to reach the target sites because of the blockade of the blood–brain barrier. The direct injection of nervous growth factors or their genes into the brain has been shown to be an effective method. However, it is difficult to deliver the therapeutic factors to a precise site.

Furthermore, this method causes neural injury. Spinal cavity delivery of growth factors or their genes is a relatively safe and effective approach. Delivered proteins or genes can be transported to the central nervous system via the cerebrospinal fluid. However, a large amount of protein or gene is required to reach an effective level of therapy.

Another approach for neuronal survival is to promote the production and release of nervous growth-related factors by using pharmacological substances. The synthetic purine AIT-082 or Neotrofin {4-[[3-(1,6 dihydro-6-oxo-9-purin-9-yl)-1-oxypropyl] amino] benzoic acid} has been shown to stimulate neurotrophin secretion from astrocytes. This substance also enhances several other neural activities, such as neuronal regeneration, axonal outgrowth, cognitive activity of animals and humans, and the restoration of age-induced memory deficits in animals. AIT-082 has been applied to patients with mild to moderate Alzheimer's disease. A treatment with AIT-082 induces an increase in the glucose metabolic rate, indicative of enhanced bloodflow, in the cerebellum and the prefrontal cortical area as detected by PET scanning. Furthermore, AIT-082 improves the memory, executive functioning, and attention of patients with Alzheimer's disease. Several other pharmacological substances, such as propentofylline (a synthetic xanthine derivative that inhibits phosphodiesterase), idebenone (a quinone derivative that serves as an antioxidant), and pyrroloquinoline quinone (a vitamin and redox cofactor of quinoprotein dehydrogenases), have been shown to stimulate the production and release of nervous growth factor and exert indirectly a therapeutic effect on Alzheimer's disease.

INHIBITION OF GLYCOGEN SYNTHASE KINASE 3 [13.30]. As discussed on page 556, the enzyme glycogen synthase kinase 3 induces hyperphosphorylation of the protein tau, contributing to the formation of the neurofibrillary tangles, the production of amyloid β peptides, and the development of Alzheimer's disease. Thus, one of the strategies for treating Alzheimer's disease is to suppress the activity of glycogen synthase kinase 3 (GSK3). An effective means of inhibiting GSK3 is to construct and deliver small interfering RNA (siRNA) specific to the GSK3 mRNA. This approach has been shown to significantly suppress the expression of GSK3 and reduce the formation of the neurofibrillary tangles and amyloid plaques. In addition, there are two pharmarcological inhibitors, lithium and kenpaullone, specific to the enzyme glycogen synthase kinase-3. These inhibitors can be used to suppress the activity of GSK3 and are effective for the treatment of Alzheimer's disease. However, lithium is toxic to the kidney at a high blood concentration. It should be used with caution.

Cell Regenerative Engineering for Alzheimer's Disease [13.31]. For the treatment of Alzheimer's disease, several cell types have been used for transplantation into the brain to improve the production and release of the cholinergic neurotransmitter in the basal forebrain area. These cells include fetal basal forebrain cells, embryonic acetylcholine-enriched cells, and gene-transfected stem cells. The transplantation of embryonic and fetal stem cells into the brain has long been known to enhance the production of acetylcholine and the survival of cholinergic neurons. The transplanted stem cells may differentiate into cholinergic neurons, release acetylcholine, and prevent apoptosis of neurons. Furthermore, genetic modulation of the stem cells can greatly enhance the cell's ability to stimulate neuronal cell survival. For example, the transfection of stem or progenitor cells with the nervous growth factor gene significantly promotes the cell's ability to secrete nervous growth factor. Such a cell line, when transplanted into a target tissue, can release excessive nervous growth factor, thus enhancing cell survival. In addition to embryonic and fetal

stem cells, other cell types, such as bone marrow cells and umbilical cord blood cells, have been used and tested in animal Alzheimer's models. The transplantation of these cell types has been shown to exert a beneficial effect on the treatment of Alzheimer's disease. For cell transplantation into the brain, direct injection is the method of choice (see page 460 for method).

Huntington's Disease

Etiology, Pathology, and Clinical Features [13.32]. Huntington's disease, also known as *Huntington's chorea*, is a hereditary autosomal dominant neural disorder characterized by chronic ceaseless occurrence of rapid, involuntary, complex jerky movements, mental deterioration, and dementia. This is one of the most frequently observed hereditary diseases in the United States. The average occurrence is about 4–5 per million. The disease is often found at the age of 40–50, but also occurs in children and young adults.

There are several distinct clinical features for Huntington's disease. In the early phase, patients may express annoying behaviors, such as constant complaining and reduced self-control. With the progression of the disease, patients may show mood disturbance and depression signs, reduced communication and fine manual skills, reduced work performance and responsibility, difficulties in maintaining attention, changes in personality, and deteriorating intellectual abilities. These mental changes are associated with abnormalities in muscular movement. Early motor-related signs include reduced movement of fingers and hands and increased rate of blinking. In the advanced stage, patients exhibit constant and rapid involuntary jerky movements and are not able to speak well because of inadequate control of the tongue muscles. The speed of voluntary movements is significantly reduced.

Huntington's disease is associated with a number of pathological changes. These include atrophy of the central nervous system, loss of neuron dendrites, disappearance of myelinated axons, and compensatory astrocyte proliferation. The pathogenesis of Huntington's disease is thought to relate to several biochemical factors. An increase in the production of dopamine and/or in the sensitivity of striatal dopamine receptors may promote the dopamine effect, inducing involuntary movement. Other neurotransmitters, such as acetylcholine and norepinephrine, are disturbed in Huntington's disease, contributing to the pathogenesis of the disease. However, the exact mechanisms remain poorly understood.

The pathogenesis of Huntington's disease is related to several genetic mechanisms, including the mutation of the CAG-repeating sequence, mutation of the huntingtin gene, and the impairment of histone acetylation. The CAG-repeating sequence is found in genes that encode multiple glutamine or polyglutamine residues. The number of the CAG groups ranges from 3 to 34 in a normal gene. In Huntington's disease, however, the number of the CAG groups can increase significantly, ranging from 36 to 121, resulting in a significant increase in the length of the polyglutamine sequence in involved proteins. Proteins with expanded polyglutamine sequences exhibit reduced solubility and form aggregates with amyloid or other polyglutamine proteins. These protein aggregates exert a toxic effect on the cerebral neurons by abnormal interaction with essential cellular signaling proteins. Such toxicity may influence a number of cellular processes, including protein folding, proteasomal degradation, mitochondrial energy metabolism, and gene transcription. These abnormalities may contribute to the initiation and development of Huntington's disease. A typical example is the polyglutamine expansion in the TATA-binding protein (TBP), which binds the TATA box of genes and regulates gene transcription. Polyglutamine

expansion in this protein disturbs the binding of the protein to the TATA box of target genes involved in the regulation of neuronal activities, thus contributing to the development of the symptoms of Huntington's disease.

The mutation of the huntingtin gene is another cause of Huntington's disease. *Huntingtin*, also known as *Huntington's disease protein*, is a 348-kDa protein expressed ubiquitously and plays a critical role in the regulation of development. Huntingtin exerts several functions, including the protection of neural and nonneural cells from apoptosis and excitotoxicity, enhancement of brain-derived neurotrophic factor gene expression, and regulation of fast axonal trasport and synaptic transmission. Huntingtin mutation induces a decrease in the transcription of the brain-derived neurotrophic factor gene, alterations in axonal trafficking and synaptic transmission, and impairment of neuronal functions. Experimental knockout of the huntingtin gene is assocaited with pathological changes seen in Huntington's disease. Overexpression of the huntington gene improves the function of neurons. These observations suggest a role for huntingtin in the pathogenesis of Huntington's disease.

Another potential pathogenic mechanism for Huntington's chorea is the impairment of histone acetylation and gene transcription regulated by cAMP responsive element binding protein (CREB)-binding protein (CBP). CBP is a 250-kDa transcriptional coactivator that binds to and mediates the activity of phosphorylated CREB, which is a transcriptional factor activated by protein kinase A and responsible for activating genes involved in learning and memory in the central nervous system. The CREB coactivator CBP is a histone acetyltransferase that catalyzes histone acetylation. This protein has been found to aggregate with proteins containing expanded polyglutamines in cultured cells. In transgenic mouse models with polyglutamine-expanded huntingtin, CBP can form aggregates with huntingtin, resulting in a reduction in the level of CBP. Such a change ultimately impairs the function of CREB and possibly contributes to the pathogenesis of Huntington's disease.

In the brain of Huntington's patients, aggregates of CBP with polyglutamine-expanded proteins can be found by electron microscopy. The expression of glutamine-expanded huntingtin can be observed by immunohistochemistry. These changes are often associated with a decrease in the level of soluble CBP, which affects CREB-mediated transcription activities, resulting in cell death in tissue culture models of Huntington disease. These changes can be mitigated by overexpression of CBP. Furthermore, the aggregates of CBP with polyglutamine-expanded proteins are greatly reduced when the polyglutamine-containing domain is deleted. These observations suggest that polyglutamine-related sequestration of CBP may contribute to the impairment of histone acetylation and disorder of gene transcription, thus leading to the development of Huntington's disease. However, how these alterations influence the structure and function of cerebral neurons remains poorly understood.

Conventional Treatment of Huntington's Disease [13.33]. There are few methods available for the treatment of Huntington's disease. Dopamine antagonists are used to suppress movement disorders and behavioral abnormalities. One of such antagonists is haloperidol, which is also used as a tranquilizer for the management of psychoses. Although this drug is effective, it often causes dyskinesia or impairment of voluntary movements. Substances that enhance the degradation of dopamine or block the dopamine receptor are also used for treating the symptoms of Huntington's disease. These substances include reserpine, clozapine, and tetrabenazine, which are effective for the control of Huntington's symp-

toms. However, these drugs often cause severe side effects such as drowsiness, dyskinesia, and akathisia. It is important to note that these drugs are used only to treat the symptoms of the disease without influencing the progression of the disease. Unfortunately, drugs that prevent and suppress Huntington's disease are not available.

Molecular Regenerative Engineering for Huntington's Disease [13.34]. Since the mechanisms of Huntington's disease are not well understood, there are few therapeutic approaches that can be used for the treatment of the disease. Recent investigations have begun to reveal the genetics of Huntington's disease, which suggest potential therapeutic approaches. As discussed on page 562, the sequestration of CREB-binding protein by polyglutamine-expanded proteins induces a significant decrease in the level of the CREB-binding protein and may contribute to the pathogenesis of Huntington's disease. CREB-binding protein is a signaling molecule that participates in the regulation of a variety of cellular processes such as cell survival and proliferation. A potential therapeutic approach is to enhance the expression of the CREB-binding protein. Investigations with cell culture models have demonstrated that overexpression of the CREB-binding protein gene can reduce the influence of polyglutamine-expanded proteins. The CREB-binding protein gene can be potentially used for the treatment of Huntington's disease.

The impairment of histone acetylation contributes to the pathogenesis of Huntington's disease. A typical example is histone deacetylation, which is catalyzed by histone deacetylase. The prevention of histone deacetylation may be a potential therapeutic approach for treating Huntington's disease. Histone deacetylase inhibitors, such as butyrate and suberoylanilide hydroxamic acid (SAHA), have been used and tested in models of neuronal degeneration induced by polyglutamine-expanded proteins. In a transgenic *Drosophila* model of neurodegeneration induced by expression of expanded polyglutamines, histone deacetylase inhibitors (suberoylanilide hydroxamic acid) significantly reduced the progression of neurodegeneration. In another study with a cell culture model, polyglutamine expansion in the androgen receptor caused cell death, which was reversed by a treatment with the histone deacetylase inhibitor suberoylanilide hydroxamic acid. These investigations demonstrate that histone deacetylase inhibitors may be potentially used as therapeutic agents for Huntington's disease. To date, histone deacetylase inhibitors have only been tested in cell culture and *Drosophila* models. Further experimental tests are needed in mammalian models.

Another potential therapeutic approach is to modulate the activity of huntingtin (Fig. 13.27). Huntingtin is a soluble cytoplasmic protein expressed widely in the central nervous system and found in somatodendritic regions and axons. Huntingtin can interact with numbers of signaling molecules and plays a critical role in regulating cellular processes, such as channel control, protein processing, and pre-mRNA splicing. The mutation of huntingtin may disrupt these cellular processes and contribute to the development of Huntington's disease. Other functions of huntingtin are to stimulate the expression of brain-derived neurotrophic factor in the cortical area and facilitate vesicular transport of brain-derived neurotrophic factor along microtubules in neurons. Huntingtin mutation is associated with a reduction in brain-derived neurotrophic factor, resulting in insufficient neurotrophic support and apoptosis of cortical neurons. The expression and release of brain-derived neurotrophic factor are attenuated when the level of wildtype huntingtin is reduced. The restoration of wildtype huntingtin via gene transfer enhances the expression of brain-derived neurotrophic factor and the survival of cortical neurons. This may be a potential approach for the treatment of Huntington's disease.

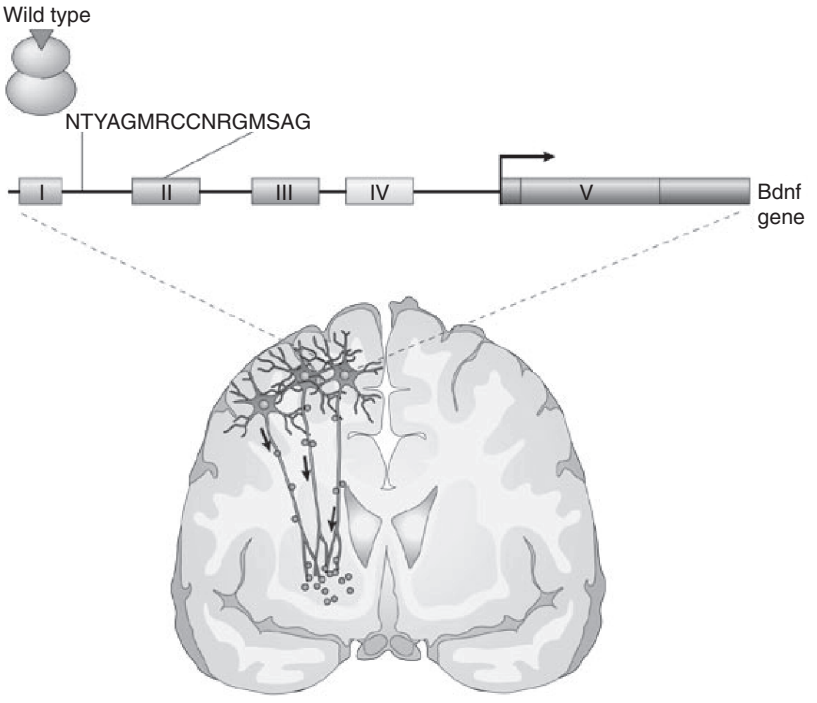


Figure 13.27. Treatment of Huntington's disease with huntingtin. Wildtype but not mutant huntingtin facilitates production of brain-derived neurotrophic factor (BDNF) in the cortical neurons, which project to the striatum, by inhibiting the repressor element 1/neuron-restrictive silencer element (RE1/NRSE) that is located in BDNF promoter exon II. I–IV indicate BDNF promoter exons in the rodent BDNF gene; V indicates the coding region. The RE1/NRSE consensus sequence is shown. Inactivation of the RE1/NRSE in the BDNF gene leads to increased mRNA transcription and protein production in the cortex. BDNF, which is also produced through translation from exons III and IV is then made available to the striatal targets via the corticostriatal afferents. Wildtype huntingtin might also facilitate vesicular BDNF transport from the cortex to the striatum. (Reprinted by permission from Macmillan Publishers Ltd.: Cattaneo E, Zuccato C, Tartari M: Normal huntingtin function: an alternative approach to Huntington's disease, *Nat Rev Neurosci* 6:919–30, copyright 2005.)

Parkinson's disease

Etiology, Pathology, and Clinical Manifestation [13.35]. *Parkinson's disease* is a neural disorder that is characterized by tremor of resting muscles, slow voluntary movements, festinating gait, peculiar posture, and muscle weakness. Patients also exhibit excessive sweating and feeling of heat. This disease often occurs at the age of 40–70. The incidence of Parkinson's disease is very low in the population under 30 years of age, but is fairly high (about 1%) in the elder population (over 65) in the United States. Several factors such as cranial injury, overwork, emotional shock, extreme stress, and exposure to unusual environments may facilitate the deterioration of the disease. Indeed, the disease may often be found after such incidents.

Parkinson's disease is associated with several distinct pathological changes. These include the progressive loss of pigmented neurons in the substantia nigra and dorsal motor nucleus, the presence of Lewy bodies (eosinophilic cytoplasmic contents with halos) (Fig. 13.28), and reduction in nonpigmented neurons. The pathogenesis of the disease may be related to the disorder of the dopamine metabolic system, resulting in a reduction in the production of dopamine. It has been observed that the level of tyrosine hydroxylase, a key enzyme for the production of dopamine, is reduced significantly in patients with Parkinson's disease. This is consistent with the reduction in the level of dopamine. Thus, reduced dopamine in the central motor control system may contribute to the degeneration of the pigmented neurons. Although it is not certain whether hereditary factors play a role, there are patients with a family history of Parkinson's disease.

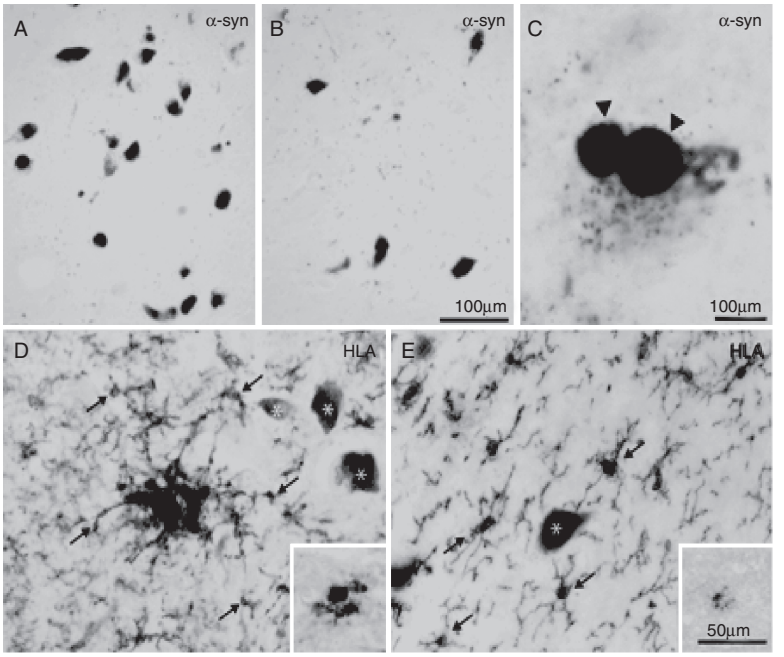


Figure 13.28. Neuron loss and Lewy body formation in Parkinson's disease. (A) Control. (B–E) Parkinson's disease. (A–C) Neuromelanin pigmented neurons immunohistochemically stained with an anti-α synuclein antibody. Scale in B is equivalent to that for A. There is an obvious loss of pigmented dopamine neurons in Parkinson's disease (B) compared with the control (A). Some remaining pigmented dopamine neurons in Parkinson's disease contain α-synuclein-immunoreactive Lewy bodies (arrowheads in C). (D,E) Specimens immunohistochemically stained with antibodies to HLA-DP/DQ/DR (HLA), a marker for the major histocompatibility complex class II protein. Scale in E is equivalent to that for D. HLA-immunoreactive upregulated microglia (arrows) near nonimmunoreactive pigmented neurons (asterisks) in thick (D) and thin (E) midbrain sections from patients with Parkinson's disease (D and E). The inserts in D and E were from Parkinson's disease patients with α-synuclein and parkin gene mutation, respectively. (Reprinted from Orr CF et al: A possible role for humoral immunity in the pathogenesis of Parkinson's disease, *Brain* 128:2665–74, copyright 2005 by permission of Oxford University Press.)

Conventional Treatment [13.35]. Parkinson's disease is induced predominantly by reduction or depletion of cerebral dopamine, the neurotransmitter of dopaminergic neurons. Thus, the principle of treating Parkinson's disease is to maintain or restore the level of dopamine in the central nervous system. Several types of pharmacological substances have bee used to achieve such a goal. These include dopamine precursors, such as L-dihydroxyphenylalanine (L-dopa; also known as *levodapa*), dopamine agonists, such as bromocriptine, pergolide, lisuride, ropinirole, and pramipexole, and substances that inhibit the degradation of dopamine, such as selegiline. L-Dopa is the most effective drug for the treatment of Parkinson's disease. L-Dopa can be used alone or in combination with other agents. One type of such agents is decarboxylase inhibitors such as carbidopa and benserizide. Decarboxylase is an enzyme that catalyzes L-dopa breakdown and the conversion of L-dopa to dopamine. Carbidopa reduced the activity of decarboxylase. Because carbidopa cannot pass through the blood–brain barrier, the administration of carbidopa together with L-dopa reduces the rate of L-dopa breakdown in the peripheral system, allowing a large fraction of L-dopa to reach the brain. This strategy also reduces the side effect of dopamine, such as nausea and hypotension, in the peripheral systems.

Dopamine agonists, such as bromocriptine, pergolide, lisuride, ropinirole, and pramipexole, can interact with the dopamine receptor, mimicking the activity of dopamine. These agents can be administrated before L-dopa is used or as a supplement to L-dopa treatment. Selegiline is an inhibitor for the monoamine oxidase, which degrades dopamine, and thus can be used to reduce the degradation of dopamine in the brain. It should be noted that treatment with all these agents may only be effective for treating the symptoms of Parkinson's disease, but not be effective for preventing the progression of the disease.

Molecular Regenerative Engineering for Parkinson's Disease [13.36]. Given the pathogenic mechanisms of Parkinson's disease as discussed on page 565, strategies for molecular engineering treatment of Parkinson's disease include the promotion of the survival of dopaminergic neurons, maintenance of the dopamine level in the brain, and protection of dopaminergic neurons from apoptosis. These strategies are tested primarily in rodent and nonhuman primate models of Parkinson's disease. These models are induced by administration of chemical toxins, such as 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which reduce the production of dopamine in neurons and promote neuronal apoptosis. It is important to note that these animal models may not completely assemble the human Parkinson's disease. Results form these models should be interpreted with caution.

PROMOTION OF THE SURVIVAL OF DOPAMINERGIC NEURONS. For promoting the survival of dopaminergic neurons, neurotrophic factors, such as nervous growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), and platelet-derived growth factor (PDGF) or their genes can be delivered to the brain by using approaches of direct injection or spinal cavity injection (see page 521 of this chapter for these neurotrophic factors) (Fig. 13.29). In animal models of Parkinson's disease, the overexpression of neurotrophic factors can exert several effects that benefit the treatment of the disease, including an increase in the synthesis of dopamine, protection of dopaminergic neurons from toxic injury and apoptosis, and enhancement of the sprouting of dopaminergic neurons. A large number of investigations have demonstrated the effectiveness of neurotrophic factor gene transfer in mitigating the symptoms of Parkinson's

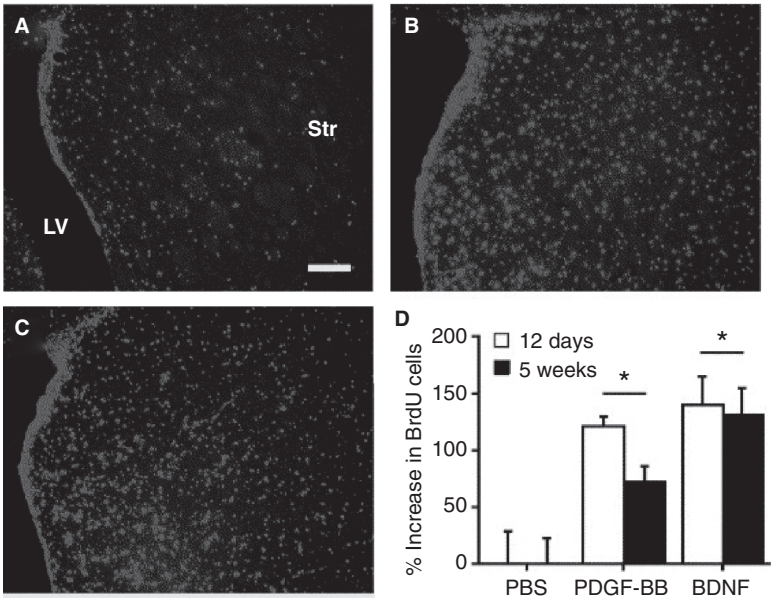


Figure 13.29. Infusion of platelet-derived growth factor BB (PDGF-BB) and brain-derived neurotrophic factor (BDNF) into the right lateral ventricle (LV) enhances the density of BrdU-positive cells in the denervated striatum (Str) of the rat brain with unilateral 6-OHDA lesions. Red BrdU-labeled cells in PBS- (A), PDGF-BB- (B), and BDNF-treated (C) rats at 12 days after growth factor infusion (5 weeks after 6-OHDA lesions). (D) Measurements of BrdU-positive cells in the Str at 12 days and 5 weeks after growth factor infusion. Both growth factors enhanced BrdU incorporation compared to the PBS control, with no differences between PDGF-BB and BDNF (* $P < 0.05$). Scale bar: 100 μm. (Reprinted from Mohapel P et al: Platelet-derived growth factor (PDGF-BB) and brain-derived neurotrophic factor (BDNF) induce striatal neurogenesis in adult rats with 6-hydroxydopamine lesions, *Neuroscience* 132:767–76, copyright 2005, with permission from Elsevier.)

disease. For instance, in the rodent model of 6-OHDA-induced Parkinson's disease, the transfer of the glial cell line-derived neurotrophic factor gene into the striatum results in a significant increase in the dopamine level and a reduction in the animal rotary behavior, a typical sign of Parkinson's animals. In the nonhuman primate model of Parkinson's disease, striatal transfer of the glial cell line-derived neurotrophic factor gene induces increased gene expression of tyrosine hydroxylase, an enzyme participating in the synthesis of dopamine.

MAINTENANCE OF THE DOPAMINE LEVEL IN THE BRAIN. A critical issue for promoting the survival of dopaminergic neurons is to maintain the level of dopamine in the central nervous system. Since tyrosine hydroxylase catalyzes the synthesis of L-dopa, one of the approaches for achieving such a goal is to transfer the tyrosine hydroxylase gene into the striatum. A number of investigations have shown that such an approach results in a significant increase in the dopamine level, and a decrease in the symptoms of Parkinson's disease, such as rotational movements. There are other enzymes that regulate the synthesis of dopamine. One of such enzymes is the amino acid decarboxylase (AADC), which

converts L-dopa to dopamine. The deficiency of this enzyme may contribute to the reduction in the dopamine level and the pathogenesis of Parkinson's disease. The transfer of the amino acid decarboxylase gene into the striatum of nonhuman primates with induced Parkinson's disease results in increased conversion of L-dopa to dopamine and reduction in Parkinson's symptoms. The cotransfer of the genes encoding multiple enzymes for the synthesis of dopamine has been demonstrated to provide a synergistic effect and exhibit significant improvement in the dopamine level and motor control in Parkinson's animal models. Similar results have also been found in studies with rodent models. The tyrosine hydroxylase and amino acid decarboxylase genes are considered potential candidate genes for the treatment of human Parkinson's disease.

PROTECTION OF DOPAMINERGIC NEURONS FROM APOPTOSIS. Another molecular approach for treating Parkinson's disease is to protect neurons from apoptosis. Parkinson's disease is associated with an increase in neuronal apoptosis, which is thought to contribute to the pathogenesis of Parkinson's disease. Several factors, including oxidative stress and free radicals resulting from dopamine metabolism and ion toxicity, may induce neuronal apoptosis. An enzyme, known as Cu/Zn superoxide dismutase, plays a critical role in detoxifying superoxide, which forms toxic peroxynitrite with nitric oxide. The overexpression of the Cu/Zn superoxide dismutase gene in cultured neurons by gene transfection has been shown to protect the cells from 6-OHDA-induced toxic effects. Animals with overexpressed Cu/Zn superoxide dismutase gene exhibit increased resistance to oxidative stress and apoptosis. These observations suggest that the Cu/Zn superoxide dismutase gene can potentially serve as a therapeutic gene for the treatment of Parkinson's disease.

Apoptosis is regulated by the Bcl2 family of proteins. While some of these proteins are proapoptotic, the Bcl2 protein exerts an antiapoptotic effect (see page 304 for signaling mechanisms of apoptosis). Experimental investigations have shown that overexpression of the Bcl2 protein in the striatum by gene transfection protects neurons from apoptosis and induces an increase in the number of tyrosine hydroxylse-positive neurons. In transgenic mice with overexpressed Bcl2 gene, neurons in the central nervous system are protected from the toxic effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which induces a reduction in dopamine and degeneration of dopaminergic neurons. Cotransfer of the glial cell line-derived neurotrophic factor gene with the Bcl2 gene significantly augments the beneficial effect of glial cell line-derived neurotrophic factor gene. Both factors synergistically prevent neurons from apoptosis. These investigations suggest that the antiapoptosis protein genes can be used to as therapeutic genes for enhancing the survival of dopaminergic neurons.

Cell apoptosis is regulated by a protein kinase known as C-Jun *N*-terminal kinase (JNK). The phosphorylation of C-Jun *N*-terminal kinase has been shown to activate c-Jun and promote cell apoptosis. A protein called *JNK-interacting protein 1* (JIP1) can inhibit the phosphorylation of C-Jun *N*-terminal kinase. The transfer of the JIP1 gene into the striatum induces the overexpression of the gene, which is associated with reduced phosphorylation of C-Jun *N*-terminal *kinase and* reduced activity of caspase 3, a proteinase that induces cell apoptosis. Furthermore, the transfer of a dominant negative c-jun gene protects neurons from the toxic effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and promotes cell survival. Thus, the negative regulators of JNK may serve as therapeutic factors for the molecular treatment of Parkinson's disease.

Caspase 3 is a critical downstream proteinase that cleaves a variety of intracellular proteins, such as actin filaments, signaling protein kinases, and nuclear proteins, and

induces cell apoptosis. Caspase inhibitor genes can be used and transferred into the brain to suppress cell apoptosis. Such inhibitor genes include the p35 gene and the family of the inhibitor of apoptosis protein genes. The transfer of these genes into the striatum in the model of 6-OHDA-induced Parkinson's disease significantly inhibits the activity of caspases and delays the occurrence of cell apoptosis. Transgenic mice with p35 overexpression exhibit increased neuronal tolerance to the toxic effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Caspase inhibitors also augment the mitogenic effect of neurotrophic factors. In general, all caspase inhibitor genes can be used for molecular treatment of Parkinson's disease.

Cell Regenerative Engineering for Parkinson's Disease [13.37]. In cell regenerative engineering for Parkinson's disease, the primary goal is to transplant stem or other types of cell into the brain to restore the structure and function of impaired dopaminergic neurons. Typical cell types for such a purpose include embryonic stem cells, fetal neuronal stem and progenitor cells, adult neuronal stem cells, and cell lines with transferred genes that enhance desired functions. To date, cell regenerative engineering approaches have been tested primarily in animal models. Since these models may not completely assemble the human Parkinson's disease, results from animal models should be interpreted with caution.

As discussed on page 381, embryonic stem cells from the blastocyst are capable of differentiating into all specified cell types, including neurons, under appropriate conditions. Fetal neuronal precursor cells can differentiate into dopamine-synthesizing neurons. These stem cells can be used and transplanted into the central nervous system for neuronal regeneration. It is expected that the embryonic and fetal stem cells can differentiate into dopaminergic neurons, thus improving the motor control capability. A number of investigations have demonstrated that dopamine-synthesizing neurons from embryonic and fetal stem cells can be used for transplantation into the central nervous system. The transplanted cells can be integrated into the host brain (Fig. 13.30). Such a treatment significantly improves the level of dopamine production and the survival of dopaminergic neurons in association with reduced Parkinson's symptoms. However, the application of embryonic or fetal stem cells to human patients remains an issue of ethical debate. To date, most investigations in this area are conducted by using animal models.

Given the controversial issues for the use of embryonic and fetal stem cells, many investigators have searched for neuronal stem cells in the central nervous system. Indeed, the adult brain contains cells that exhibit stem cell characteristics such as self-renewing and differentiation. These cells are considered adult neural stem cells and can differentiate into neurons, astrocytes, and oligodendrocytes. The adult neural stem cells can be found in the wall of the ventricular system, the olfactory system, and the hippocampus of the brain (see page 395 for details). These cells can be potentially used for the treatment of Parkinson's disease.

Although the transplantation of stem and progenitor cells demonstrates beneficial effects on the treatment of Parkinson's disease, natural wildtype cells may not be able to sufficiently boost the production and release of the neurotransmitter dopamine and neurotrophic factors necessary for the survival of neurons. To resolve such an issue, stem and progenitor cells have been transfected with genes encoding proteins that promote the differentiation of stem and progenitor cells to dopaminergic neurons. Two types of gene, including the Nurr1 and von Hippel–Lindau (VHL) protein genes, have been used for such a purpose.

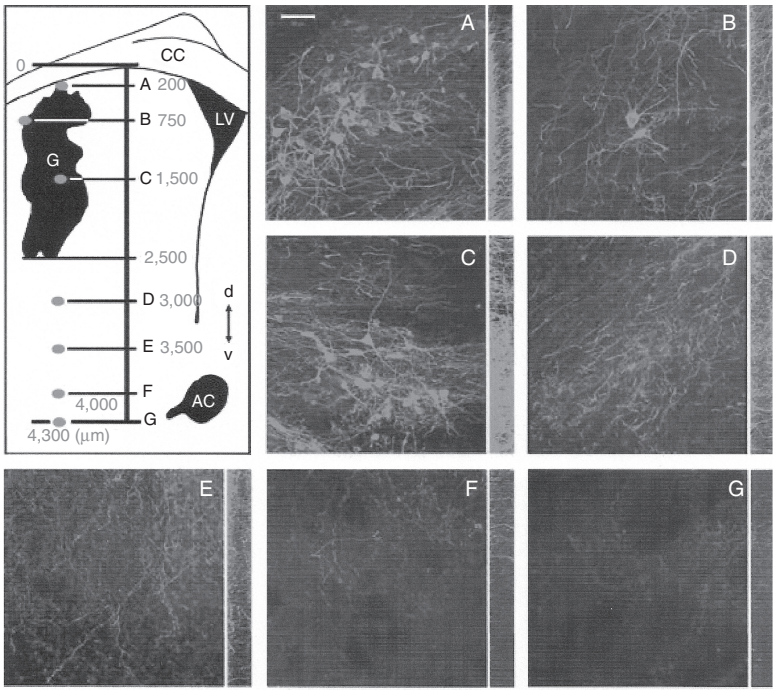


Figure 13.30. Integration of Nurr1 embryonic stem cells into the striatum of hemiparkinsonian rats. The diagram shows a drawing of a single section through a graft (G) in the striatum (LV, lateral ventricle; AC, anterior commissure). Single confocal images after immunohistochemistry for tyrosine hydroxylase (TH) are shown (A–G) from regions marked by red dots in the diagram. The distribution of cells and processes through the thickness of the section (35 μm) is shown by the *z* series displayed in green on the right. Note the many TH^+ processes that extend away from the graft into the parenchyma of the host striatum (D–F). Scale bar: 50 μm. (Reprinted by permission from Macmillan Publishers Ltd.: Kim JH et al: Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease, *Nature* 418:50–6, copyright 2002.)

Nurr1 is a protein that belongs to the nuclear receptor superfamily of transcription factors. This protein is expressed predominantly in the developing and mature dopaminergic neurons of the central nervous system, and is essential for the differentiation of the mesencephalic precursors to dopaminergic neurons and for the survival of mature dopaminergic neurons. The von Hippel–Lindau protein is a tumor suppressor that downregulates the transcriptional activity of mitogenic genes. This protein is primarily expressed in the central nervous system and plays a role in regulating the differentiation of the mesencephalic precursors to dopaminergic neurons. Thus, genes encoding the Nurr1 and von Hippel–Lindau proteins can be potentially used to boost the differentiation of stem and progenitor cells into dopamine-synthesizing neurons. Several investigations have demonstrated that the transfer of these genes into stem or progenitor cells enhances the synthesis of dopamine, increases the density of tyrosine hydroxylase-positive neurons, improves the survival of dopaminergic cells, and reestablishes dopamine-dependent motor behaviors in animal models of Parkinson's disease. Other potential genes that can be used

to boost the function of transplanted neuronal stem cells include neurotrophic factor genes, dopamine synthesis-promoting genes, and Cu/Zn superoxide dismutase genes.

Nonneuronal cell types have also been used to create dopamine-synthesizing cell lines by gene transfer. These cells include fibroblasts, astrocytes, Schwann cells, myoblasts, and marrow stromal cells. These cell types are readily available and easy to collect in comparison to embryonic and fetal stem cells. Although these cell types may not be able to differentiate into neurons, they can carry and deliver necessary proteins such as neurotrophic factors and dopamine synthesis enzymes for enhancing the survival of neurons.

Multiple Sclerosis

Etiology, Pathogenesis, and Clinical Manifestations [13.38]. *Multiple sclerosis* is a neural disorder that is characterized by myelin degradation, demyelination, and the generation of insoluble precipitates or plaques in various locations, in association with dysfunction of multiple cerebral regions and the spinal cord. Clinical signs include motor weakness, partial paralysis, abnormal sensation, abnormal vision, tremor, and dysarthria. The insoluble plaques can be detected by X-ray and magnetic resonance imaging. The disorder progresses slowly with a latent period of 1–10 years starting with a minor symptom. One of the distinct features of the disorder is the intermittent occurrence of the symptoms and signs. Multiple sclerosis is found in about 0.001% of the population. The disorder is often detected in patients at the age of 20–40. Hereditary factors may influence the occurrence of the disease. About 15% of the patients with multiple sclerosis have relatives with the same disorder. Siblings of patients with multiple sclerosis have a risk factor significantly greater than that of the general population.

Multiple sclerosis is associated with the destruction of myelin sheaths. However, this process does not apparently affect the structure of neurons and axons. A pathological examination often reveals an uneven surface of the spinal cord, while the brain surface may appear normal. Scattered lesions or insoluble plaques may be found in the nervous system with a size ranging from millimeters to centimeters with pink/gray-colored white matter due to demyelination. The lesion is found primarily in the white matter near the cerebral ventricles, in the brainstem, and in the spinal cord, but not in peripheral nerves. The optic nerves are often affected by the disorder. Structural alterations are dependent on the progression of the disorder. Fresh lesions exhibit partial destruction of myelin sheaths and infiltration of lymphocytes and mononuclear cells. With the progression of the disorder, there appears increased infiltration of microglial cells (macrophages) and increased size of sclerotic lesions. In the late stage, few myelin sheaths can be found. Affected regions often exhibit increased fibrous tissue with reduced lymphocytes and macrophages.

A number of factors may contribute to the pathogenesis of multiple sclerosis. These factors include viral infection, autoimmune reactions, and synergistic effects of both viral and autoimmune factors. Patients with multiple sclerosis exhibit immune reactions against viral products. Herpes viruses have been implicated in the initiation and development of multiple sclerosis, since DNA from these viruses is found in multiple sclerosis plaques. However, little direct evidence has been obtained for the role of viruses. Another factor that potentially contributes to the induction and development is autoimmune reaction. In such a case, the host immune cells may not recognize myelin proteins as the body's own components and may initiate immune reactions to attack and destroy the myelin structure

and sometimes the nerve axon. A major line of supporting evidence for the autoimmune mechanism is the existence of antibodies against the myelin components in the serum of patients with multiple sclerosis. Viral infection and autoimmune reactions may coordinately influence the process of demyelination. Several types of virus, such as rubella and rubeola, may contain protein components that are similar to some proteins in the myelin sheath of nerve axons. The exposure of T and B lymphocytes to these viruses may induce lymphocyte immunization. Immunized lymphocytes in turn recognize and attack the host myelin.

Further investigations have demonstrated that multiple sclerosis is associated with the infiltration of mononuclear cells in the area of demyelination, axonal loss, and glial fibrosis. Given the involvement of T lymphocytes, it has been hypothesized that activated antigen-specific T cells initiate specific immune reactions and induce the infiltration of non-antigen-specific mononuclear cells into the brain. The mononuclear cells in turn interact with and destroy oligodendrocytes by releasing toxic substances in association with the degradation of myelin sheaths. In the early stage of the disease, oligodendrocytes are capable of surviving and remyelinating axons. However, in the late stage, these cells are gradually committed to apoptosis.

Multiple sclerosis causes changes in the physiological function of nervous axons. A major change is delayed transmission of action potentials. This is due to the destruction of the axon myelin sheath. Under physiological conditions, the transmission of action potentials in the myelinated axons (as high as 100 m/s) is much faster than that in unmyelinated axons (as low as 0.25 m/s). The destruction of the myelin sheath influences the electrical conduction of the axons. In severe cases, the transmission of electrical signals can be completely blocked. The impairment and blockade of electrical signals ultimately influence the function of peripheral tissues and organs.

Conventional Treatment [13.39]. Multiple sclerosis is a disease possibly induced by viral infection and autoimmune reactions. The principle of treating multiple sclerosis is administration of antiinflammatory and immunosuppressor agents. Corticosteroids are commonly used as antiinflammatory agents. These agents usually give noticeable results within about 2 weeks. Immunosuppressor agents, such as azathioprine and cyclophosphamide, have been used for the treatment of multiple sclerosis with some positive results. However, these substances compromise with the normal immune function and impose toxic effects. Such harmful influences may preclude the widespread use of the immunosuppressor agents. In addition, appropriate physical exercise is necessary to stimulate impaired motor control systems. Bacterial infection should be prevented and treated promptly, if any. Other disorders associated with multiple sclerosis should be treated properly.

Molecular Regenerative Engineering for Multiple Sclerosis [13.40]. As the pathogenesis of multiple sclerosis is attributed to the destruction of the myelin sheath and oligodendrocytes by inflammatory reactions involving activated mononuclear cells, a potential approach for this disease is to suppress inflammatory reactions and inhibit the infiltration of bloodborne mononuclear cells into the brain. The enhancement of oligodendrocyte proliferation and migration into the area of demyelinated axons may also provide therapeutic effects. Gene therapeutic approaches have been developed to achieve these goals. These approaches have been tested primarily in the animal model of multiple sclerosis: autoimmune encephalomyelitis (EAE). It should be noted that, since the animal model

may not completely assemble the human disease, information from experimental observations may not be directly applied to the human disease.

Several therapeutic strategies have been developed and used to suppress inflammatory reactions. These include the enhancement of antiinflammatory cytokines and induction of B-lymphocyte tolerance to myelin-related antigens. Since mononuclear cells and B-lymphocytes are bloodborne cells, genes encoding antiinflammatory proteins can be delivered into the bloodstream. Potential genes for antiinflammatory purposes include the interleukin (IL)1β, IL2, IL4, IL6, IL10, tumor necrosis factor (TNF)α, and transforming growth factor (TGF)β1 genes. The transfer of these genes into animals with autoimmune encephalomyelitis reduces pathological signs and clinical symptoms of the disorder, although controversial results are observed for some IL molecules, such as IL4 and IL10. These antiinflammatory protein genes can be delivered to target tissues by three approaches: injection into the bloodstream, directly into the brain, and into the cerebrospinal fluid cavities.

The enhancement of B-lymphocyte tolerance to myelin-related antigens is another potential approach for the treatment of multiple sclerosis. Certain viruses may contain components that partially assemble the structure of myelin proteins. Viral infection may expose B lymphocytes to the myelin-like viral components and induce immunization of the lymphocytes, producing antimyelin protein antibodies. These antibodies may interact with host myelin proteins and contribute to autoimmune processes, potentially inducing multiple sclerosis. In an experimental study, a recombinant IgG–myelin basic protein (MBP) gene is inserted into a retroviral vector and transferred into B lymphocytes. These B cells were introduced into the bloodstream of mice with autoimmune encephalomyelitis-induced multiple sclerosis. Compared to control mice without B cell transplantation, the B cell-transplanted mice exhibit reduced pathological signs and clinical symptoms of multiple sclerosis. It is thought that the presence of myelin basic protein in the B cells increases the tolerance of these cells to the myelin basic protein, thus reducing the production and secretion of antimyelin protein antibodies and mitigating autoimmune reactions.

Gene therapeutic approaches have also been developed to enhance the proliferation and migration of oligodendrocytes and to promote the remyelination of impaired axons, as demyelination results in axonal loss and neurological impairment. Candidate genes for such a purpose include neurotrophic factor and nerve growth factor genes. Since the therapeutic targets of these genes are the oligodendrocytes in the central nervous system, direct brain gene delivery usually gives satisfactory results. Although neurotrophic factors and their genes can be injected into the bloodstream, the therapeutic efficiency is usually low, as it is difficult for protein and DNA molecules to pass through the blood–brain barrier. Another delivery route is the cerebrospinal fluid. In an experimental model of autoimmune encephalomyelitis-induced multiple sclerosis, a herpes virus-derived vector containing the fibroblast growth factor gene was transferred into the cerebrospinal fluid. Fibroblast growth factor is known to promote the proliferation and differentiation of oligodendrocytes. The introduction of this growth factor to the cerebrospinal fluid enhances remyelination of impaired axons and reduces pathological signs and clinical symptoms of multiple sclerosis.

Cell Regenerative Engineering [13.41]. Cellular engineering approaches have been developed for the treatment of experimental multiple sclerosis induced by autoimmune encephalomyelitis. These include the transplantation of oligodendrocytes, oligodendrocyte

precursors, or genetically modified memory T lymphocytes with enhanced secretion of growth factors or antiinflammatory factors. Oligodendrocytes or their precursors can be directly delivered to the lesion sites of multiple sclerosis. A fraction of these cells can integrate into the native system and generate myelin proteins and sheaths. These cells can also be transfected with growth factor genes to enhance their capability of proliferation and migration. T lymphocytes can be transfected with antiinflammatory protein genes, such as the interleukin (IL)1β, IL2, IL4, IL6, IL10, tumor necrosis factor (TNF)α genes, enhancing the production and secretion of antiinflammatory factors. These cells can be transplanted to the bloodstream, from where they can migrate into the lesion sites of the brain. Alternatively, T cells can be directly delivered to the central nervous system. T lymphocytes can also be transfected with growth factor genes to promote their capability of producing growth factors.

BIBLIOGRAPHY

13.27. Etiology, Pathology, and Clinical Manifestations for Alzheimer's Disease

Wilquet V, De Strooper B: Amyloid-beta precursor protein processing in neurodegeneration, *Curr Opin Neurobiol* 14:582–8, 2004.

Reinhard C, Hebert SS, De Strooper B: The amyloid-beta precursor protein: Integrating structure with biological function, *EMBO J* 24:3996–4006, 2005.

Annaert W, De Strooper B: A cell biological perspective on Alzheimer's disease, *Annu Rev Cell Dev Biol* 18:25–51, 2002.

Dominguez DI, De Strooper B: Novel therapeutic strategies provide the real test for the amyloid hypothesis of Alzheimer's disease, *Trends Pharmacol Sci* 23:324–30, 2002.

Lu PJ, Wulf G, Zhou XZ, Davies P, Lu KP: The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein, *Nature* 399:784–8, 1999.

Panda D, Samuel JC, Massie M, Feinstein SC, Wilson L: Differential regulation of microtubule dynamics by three- and four-repeat tau: Implications for the onset of neurodegenerative disease, *Proc Natl Acad Sci USA* 100:9548–53, 2003.

Rapoport M, Dawson HN, Binder LI, Vitek MP, Ferreira A: Tau is essential to beta-amyloid-induced neurotoxicity, *Proc Natl Acad Sci USA* 99:6364–9, 2002.

Ros R, Thobois S, Streichenberger N, Kopp N, Sanchez MP et al: A new mutation of the tau gene, G303V, in early-onset familial progressive supranuclear palsy, *Arch Neurol* 62:1444–50, 2005.

SantaCruz K, Lewis J, Spires T, Paulson J, Kotilinek L et al: Tau suppression in a neurodegenerative mouse model improves memory function, *Science* 309:476–81, 2005.

Skipper L, Wilkes K, Toft M, Baker M, Lincoln S et al: Linkage disequilibrium and association of MAPT H1 in Parkinson disease, *Am J Hum Genet* 75:669–77, 2004.

Spillantini MG, Murrell JR, Goedert M, Farlow MR, Klug A et al: Mutation in the tau gene in familial multiple system tauopathy with presenile dementia, *Proc Natl Acad Sci USA* 95:7737–41, 1998.

Spittaels K, van den Haute C, van Dorpe J, Geerts H, Mercken M et al: Glycogen synthase kinase-3-beta phosphorylates protein tau and rescues the axonopathy in the central nervous system of human four-repeat tau transgenic mice, *J Biol Chem* 275:41340–9, 2000.

Stamer K, Vogel R, Thies E, Mandelkow E, Mandelkow EM: Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress, *J Cell Biol* 156:1051–63, 2002.

Tatebayashi Y, Miyasaka T, Chui DH, Akagi T, Mishima K et al: Tau filament formation and associative memory deficit in aged mice expressing mutant (R406W) human tau, *Proc Natl Acad Sci USA* 99:13896–901, 2002.

Tesseur I, van Dorpe J, Spittaels K, van den Haute C, Moechars D et al: Expression of human apolipoprotein E4 in neurons causes hyperphosphorylation of protein tau in the brains of transgenic mice, *Am J Pathol* 156:951–64, 2000.

13.28. Conventional Treatment of Alzheimer's Disease

Victor M, Ropper AH: *Principles of Neurology*, 7th ed, McGraw-Hill, New York, 2001 pp 1106–74.

Etienne P, Robitaille Y, Wood P, Gauthier S: Nair NP et al: Nucleus basalis neuronal loss, neuritic plaques and choline acetyltransferase activity in advanced Alzheimer's disease, *Neuroscience* 19:1279–91, 1986.

Tucek S: Short-term control of the synthesis of acetylcholine, *Prog Biophys Mol Biol* 60:59–69, 1993.

Bontempi B, Whelan KT, Risbrough VB, Rao TS, Buccafusco JJ et al: SIB-1553A, (+/−)-4-[[2-(1-methyl-2-pyrrolidinyl)ethyl]thio]phenol hydrochloride, a subtype-selective ligand for nicotinic acetylcholine receptors with putative cognitive-enhancing properties: effects on working and reference memory performances in aged rodents and nonhuman primates, *J Pharmacol Exp Ther* 299:297–306, 2001.

Woodruff-Pak DS, Santos IS: Nicotinic modulation in an animal model of a form of associative learning impaired in Alzheimer's disease, *Behav Brain Res* 113:11–9, 2000.

Fisher A, Pittel Z, Haring R, Bar-Ner N, Kliger-Spatz M et al: Ml muscarinic agonists can modulate some of the hallmarks in Alzheimer's disease: implications in future therapy, *J Mol Neurosci* 20:349–56, 2003.

Auld DS, Kornecook TJ, Bastianetto S, Quirion R: Alzheimer's disease and the basal forebrain cholinergic system: Relations to beta-amyloid peptides, cognition, and treatment strategies, *Prog Neurobiol* 68:209–45, 2002.

Ruske AC, White KG: Facilitation of memory performance by a novel muscarinic agonist in young and old rats, *Pharmacol Biochem Behav* 63:663–7, 1999.

Papke RL, Meyer EM, Lavieri S, Bollampally SR, Papke TA et al: Effects at a distance in alpha 7 nAChR selective agonists: Benzylidene substitutions that regulate potency and efficacy, *Neuropharmacology* 46:1023–38, 2004.

Kem WR: The brain alpha7 nicotinic receptor may be an important therapeutic target for the treatment of Alzheimer's disease: Studies with DMXBA (GTS-21), *Behav Brain Res* 113:169–81, 2000.

13.29. Prevention of Neuronal Degeneration by Delivering Neurotrophic Factors

Auld DS, Kornecook TJ, Bastianetto S, Quirion R: Alzheimer's disease and the basal forebrain cholinergic system: Relations to beta-amyloid peptides, cognition, and treatment strategies, *Prog Neurobiol* 68:209–45, 2002.

Gustilo MC, Markowska AL, Breckler SJ, Fleischman CA, Price DL et al: Evidence that nerve growth factor influences recent memory through structural changes in septohippocampal cholinergic neurons, *J Comp Neurol* 405:491–507, 1999.

Fischer W, Bjorklund A, Chen K, Gage FH: NGF improves spatial memory in aged rodents as a function of age, *J Neurosci* 11:1889–906, 1991.

Markowska AL, Koliatsos VE, Breckler SJ, Price DL, Olton DS: Human nervous growth factor improves spatial memory in aged but not in young rats, *J Neurosci* 14:4815–24, 1994.

Scali C, Casamenti F, Pazzagli M, Bartolini L, Pepeu G: Nerve growth factor increases extracellular acetylcholine levels in the parietal cortex and hippocampus of aged rats and restores object recognition, *Neurosci Lett* 170:117–20, 1994.

Auld DS, Mennicken F, Day JC, Quirion R: Neurotrophins differentially enhance acetylcholine release, acetylcholine content and choline acetyltransferase activity in basal forebrain neurons, *J Neurochem* 77:253–62, 2001.

Auld DS, Mennicken F, Quirion R: Nervous growth factor rapidly induces prolonged acetylcholine release from cultured basal forebrain neurons: Differentiation between neuromodulatory and neurotrophic influences, *J Neurosci* 21:3375–82, 2001.

Klein RL, Muir D, King MA, Peel AL, Zolotukhin S et al: Long-term actions of vector-derived nervous growth factor or brain-derived neurotrophic factor on choline acetyltransferase and Trk receptor levels in the adult rat basal forebrain, *Neuroscience* 90:815–21, 1999.

Mandel RJ, Gage FH, Clevenger DG, Spratt SK, Snyder RO et al: Nerve growth factor expressed in the medial septum following in vivo gene delivery using a recombinant adeno-associated viral vector protects cholinergic neurons from fimbria–fornix lesion-induced degeneration, *Exp Neurol* 155:59–64, 1999.

Mahoney MJ, Saltzman WM: Millimeter-scale positioning of a nervous-growth-factor source and biological activity in the brain, *Proc Natl Acad Sci USA* 96:4536–9, 1999.

Lahiri DK, Ge YW, Farlow MR: Effect of a memory-enhancing drug, AIT-082, on the level of synaptophysin, *Ann NY Acad Sci* 903:387–93, 2000.

Keator D, Carreon D, Fleming K, Fallon JH: Brain metabolic effects of Neotrofin in patients with Alzheimer's disease, *Brain Res* 951:87–95, 2002.

Arenas E, Persson H: Neurotrophin-3 prevents the death of adult central noradrenergic neurons in vivo, *Nature* 367:368–71, 1994.

13.30. Inhibition of Glycogen Synthase Kinase 3 (GSK3)

Phiel CJ, Wilson CA, Lee VM, Klein PS: GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides, *Nature* 423:435–9, 2003.

Dominguez DI, De Strooper B: Novel therapeutic strategies provide the real test for the amyloid hypothesis of Alzheimer's disease, *Trends Pharmacol Sci* 23:324–30, 2002.

De Strooper B, Woodgett J: Alzheimer's disease: Mental plaque removal, *Nature* 423:392–3, 2003.

13.31. Cell Regenerative Engineering for Alzheimer's Disease

Fine A, Dunnett SB, Bjorklund A, Iversen SD: Cholinergic ventral forebrain grafts into the neocortex improve passive avoidance memory in a rat model of Alzheimer's disease, *Proc Natl Acad Sci USA* 82:5227–30, 1985.

Dunnett SB: Neural transplants as a treatment for Alzheimer's disease, *Psychol Med* 21:825–30, 1991.

Turnpenny L, Cameron IT, Spalluto CM, Hanley KP, Wilson DI et al: Human embryonic germ cells for future neuronal replacement therapy, *Brain Res Bull* 68:76–82, 2005.

Holden C: Neuroscience. Versatile cells against intractable diseases, *Science* 297:500–2, 2002.

Stahl T, Goldammer A, Luschekina E, Beck M, Schliebs R, Bigl V: Long-term basal forebrain cholinergic-rich grafts derived from trisomy 16 mice do not develop beta-amyloid pathology and neurodegeneration but demonstrate neuroinflammatory responses, *Int J Dev Neurosci* 16:763–75, 1998.

Baekelandt V, De Strooper B, Nuttin B, Debyser Z: Gene therapeutic strategies for neurodegenerative diseases, *Curr Opin Mol Ther* 2:540–54, 2000.

Barkats M, Bilang-Bleuel A, Buc-Caron MH, Castel-Barthe MN, Corti O et al: Adenovirus in the brain: recent advances of gene therapy for neurodegenerative diseases, *Prog Neurobiol* 55:333–41, 1998.

Fischer MSW, Bjorklund A: Reversal of age-dependent cognitive impairments and cholinergic neuron atrophy by NGF-secreting neural progenitors grafted to the basal forebrain, *Neuron* 15:473–84, 1995.

Wyman T, Rohrer D, Kirigiti P, Nichols H, Pilcher K et al: Promoter-activated expression of nerve growth factor for treatment of neurodegenerative diseases, *Gene Ther* 6:1648–60, 1999.

Ende N, Chen R, Ende-Harris D: Human umbilical cord blood cells ameliorate Alzheimer's disease in transgenic mice, *J Med* 32:241–7, 2001.

13.32. Etiology, Pathology, and Clinical Manifestations of Huntington's Disease

Shiwach RS, Norbury CG: A controlled psychiatric study of individuals at risk for Huntington's disease, *Br J Psychiat* 165:500–5, 1994.

Lovestone S, Hodgson S, Sham P, Differ AM, Levy R: Familial psychiatric presentation of Huntington's disease, *J Med Genet* 33:128–31, 1996.

Giordani B, Berent S, Boivin MJ, Penney JB, Lehtinen S et al: Longitudinal neuropsychological and genetic linkage analysis of persons at risk for Huntington's disease, *Arch Neurol* 52:59–64, 1995.

Zoghbi HY, Orr HT: Glutamine repeats and neurodegeneration, *Annu Rev Neurosci* 23:217–47, 2000.

Hughes RE: Polyglutamine disease: Acetyltransferases awry, *Curr Biol* 12:R141–3, 2002.

Perez MK, Paulson HL, Pendse SJ, Saionz SJ, Bonini NM, Pittman RN: Recruitment and the role of nuclear localization in polyglutamine-mediated aggregation, *J Cell Biol* 143:1457–70, 1998.

Fujigasaki H, Martin JJ, De Deyn PP, Camuzat A, Deffond D et al: CAG repeat expansion in the TATA box-binding protein gene causes autosomal dominant cerebellar ataxia, *Brain* 124:1939–47, 2001.

Carlezon WA Jr, Duman RS, Nestler EJ: The many faces of CREB, *Trends Neurosci* 28:436–45, 2005.

Steffan JS, Kazantsev A, Spasic-Boskovic O, Greenwald M, Zhu YZ et al: The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription, *Proc Natl Acad Sci USA* 97:6763–8, 2000.

Steffan JS, Bodal L, Pallos J, Poelman M, McCampbell A et al: Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila, *Nature* 413:739–43, 2001.

Kazantsev A, Preisinger E, Dranovsky A, Goldgaber D, Housman D: Insoluble detergent-resistant aggregates form between pathological and nonpathological lengths of polyglutamine in mammalian cells, *Proc Natl Acad Sci USA* 96:11404–9, 1999.

Nucifora FC Jr, Sasaki M, Peters MF, Huang H, Cooper JK et al: Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity, *Science* 291:2423–8, 2001.

Cattaneo E, Zuccato C, Tartari M: Normal huntingtin function: An alternative approach to Huntington's disease, *Nat Rev Neurosci* 6:919–30, 2005.

13.33. Conventional Treatment for Huntington's Disease

Victor M, Ropper AH: *Principles of Nerology*, 7th ed, McGraw-Hill, New York, 2001, pp 1106–74.

13.34. Molecular Regenerative Engineering for Huntington's Disease

Nucifora FC Jr, Sasaki M, Peters MF, Huang H, Cooper JK et al: Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity, *Science* 29:2423–8, 2001.

Steffan JS, Bodai L, Pallos J, Poelman M, McCampbell A et al: Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila, *Nature* 413:739–43, 2001.

McCampbell AA, Taye L, Whitty EP, Steffan JS, Fischbeck KH: Histone deacetylase inhibitors reduce polyglutamine toxicity, *Proc Natl Acad Sci USA* 98:15179–84, 2001.

Zoghbi HY, Orr HT: Glutamine repeats and neurodegeneration, *Annu Rev Neurosci* 23:217–47, 2000.

Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D et al: Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease, *Science* 293:493–8, 2001.

Gauthier LR, Charrin BC, Borrell-Pages M, Dompierre JP, Rangone H et al: Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules, *Cell* 118:127–38, 2004.

Cattaneo E, Zuccato C, Tartari M: Normal huntingtin function: An alternative approach to Huntington's disease, *Nat Rev Neurosci* 6:919–30, 2005.

13.35. Etiology, Pathology, Clinical Manifestation, and Conventional Treatment of Parkinson's Disease

Victor M, Ropper AH: *Principles of Nerology*, 7th ed, McGraw-Hill, New York, 2001, pp 1106–74.

Anderson KE, Weiner WL, Lang AE: *Behavioral Neurology of Movement Disorders*, 2nd ed, Lippincott Williams & Wilkins, Philadelphia, 2005.

Schapira AHV, Warren Olanow C: *Principles of Treatment in Parkinson's Disease*, Butterworth-Heinemann/Elsevier, Philadelphia, 2005.

Silverstein A, Silverstein V, Silverstein Nunn L: *Parkinson's Disease*, Enslow Publishers, Berkeley Heights, NJ, 2002.

13.36. Molecular Regenerative Engineering for Parkinson's Disease

Mamah CE, Lesnick TG, Lincoln SJ, Strain KJ, de Andrade M et al: Interaction of alpha-synuclein and tau genotypes in Parkinson's disease, *Ann Neurol* 57:439–43, 2005.

Martin ER, Scott WK, Nance MA, Watts RL, Hubble JP et al: Association of single-nucleotide polymorphisms of the Tau gene with late onset Parkinson disease, *JAMA* 286:2245–50, 2001.

Burton EA, Glorioso JC, Fink DJ: Gene therapy progress and prospects: Parkinson's disease, *Gene Ther* 10:1721–7, 2003.

Dunnett SB, Björklund A: Prospects for new restorative and neuroprotective treatments in Parkinson's disease, *Nature* 399(Suppl):A32–9, 1999.

Kordower JH: In vivo gene delivery of glial cell line–derived neurotrophic factor for Parkinson's disease, *Ann Neurol* 53(Suppl 3):S120–32, 2003.

Choi-Lundberg DL, Lin Q, Chang YN, Chiang YL, Hay CM et al: Dopaminergic neurons protected from degereration by GDNF gene therapy, *Science* 275:838–41, 1997.

During MJ, Naegele JR, O'Malley KL, Geller AI: Long-term behavioral recovery in parkinsonian rats by an HSV vector expressing tyrosine hydroxylase, *Science* 266:1399–403, 1994.

Horellou P, Vigne E, Castel MN, Barneoud P, Colin P et al: Direct intracerebral gene transfer of an adenoviral vector expressing tyrosine hydroxylase in a rat model of Parkinson's disease, *Neuroreport* 6:49–53, 1994.

Bankiewicz KS, Eberling JL, Kohutnicka M et al: Convection-enhanced delivery of AAV vector in parkinsonian monkeys; in vivo detection of gene expression and restoration of dopaminergic function using pro-drug approach, *Exp Neurol* 164:2–14, 2000.

Lee WY, Chang JW, Nemeth NL, Kang UJ: Vesicular monoamine transporter-2 and aromatic L-amino acid decarboxylase enhance dopamine delivery after L-3, 4-dihydroxyphenylalanine administration in parkinsonian rats, *J Neurosci* 19:3266–74, 1999.

Leff SE, Spratt SK, Snyder RO, Mandel RJ: Long-term restoration of striatal l-aromatic amino acid decarboxylase activity using recombinant adeno-associated viral vector gene transfer in a rodent model of Parkinson's disease, *Neuroscience* 92:185–96, 1999.

Azzouz M, Martin-Rendon E, Barber RD, Mitrophanous KA, Carter EE et al: Multicistronic lentiviral vector-mediated striatal gene transfer of aromatic L-amino acid decarboxylase, tyrosine hydroxylase, and GTP cyclohydrolase I induces sustained transgene expression, dopamine production, and functional improvement in a rat model of Parkinson's disease, *J Neurosci* 22:10302–12, 2002.

Shen Y, Muramatsu SI, Ikeguchi K, Fujimoto KI, Fan DS et al: Triple transduction with adeno-associated virus vectors expressing tyrosine hydroxylase, aromatic-L-amino-acid decarboxylase, and GTP cyclohydrolase I for gene therapy of Parkinson's disease, *Hum Gene Ther* 11:1509–19, 2000.

Muramatsu S, Fujimoto K, Ikeguchi K, Shizuma N, Kawasaki K et al: Behavioral recovery in a primate model of Parkinson's disease by triple transduction of striatal cells with adeno-associated viral vectors expressing dopamine-synthesizing enzymes, *Hum Gene Ther* 13:345–54, 2002.

Sun M, Zhang GR, Kong L, Holmes C, Wang X et al: Correction of a rat model of Parkinson's disease by coexpression of tyrosine hydroxylase and aromatic amino acid decarboxylase from a helper virus-free Herpes simplex virus type 1 vector, *Hum Gene Ther* 14:415–24, 2003.

Asanuma M, Hirata H, Cadet JL: Attenuation of 6-hydroxydopamine-induced dopaminergic nigrostriatal lesions in superoxide dismutase transgenic mice, *Neuroscience* 85:907–17, 1998.

Yamada M, Oligino T, Mata M, Goss JR, Glorioso JC et al: Herpes simplex virus vector-mediated expression of Bcl-2 prevents 6-hydroxydopamine-induced degeneration of neurons in the substantia nigra in vivo, *Proc Natl Acad Sci USA* 96:4078–83, 1999.

Natsume A, Mata M, Goss J, Huang S, Wolfe D et al: Bcl-2 and GDNF delivered by HSV-mediated gene transfer act additively to protect dopaminergic neurons from 6-OHDA-induced degeneration, *Exp Neurol* 169:231–8, 2001.

Xia XG, Harding T, Weller M, Bieneman A, Uney JB et al: Gene transfer of the JNK interacting protein-1 protects dopaminergic neurons in the MPTP model of Parkinson's disease, *Proc Natl Acad Sci USA* 98:10433–8, 2001.

Hayley S, Crocker SJ, Smith PD, Shree T, Jackson-Lewis V et al: Regulation of dopaminergic loss by Fas in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease, *J Neurosci* 24:2045–53, 2004.

13.37. Cellular Regenerative Engineering for Parkinson's Disease

Dunnett SB, Björklund A: Prospects for new restorative and neuroprotective treatments in Parkinson's disease, *Nature* 399 (Suppl):A32–9, 1999.

Kim JH, Auerbach JM, Rodriguez-Gomez JA, Velasco I, Gavin D et al: Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease, *Nature* 418:50–6, 2002.

Lee SH, Lumelsky N, Auerbach JM, McKay RD: Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells, *Nature Biotechnol* 18:675–9, 2000.

Studer L, Tabar V, McKay RD: Transplantation of expanded mesencephalic precursors leads to recovery in parkinsonian rats, *Nature Neurosci* 1:290–5, 1998.

McKay R: Stem cells in the central nervous system, *Science* 276:66–71, 1997.

Sanchez-Pernaute R, Studer L, Bankiewicz KS, Major EO, McKay RD: In vitro generation and transplantation of precursor-derived human dopamine neurons, *J Neurosci Res* 65:284–8, 2001.

Thomson JA et al: Embryonic stem cell lines derived from human blastocysts, *Science* 282:1145–7, 1998.

Lee SH, Lumelsky N, Auerbach JM, McKay RD: Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells, *Nature Biotechnol* 18:675–9, 2000.

Okabe S, Forsberg-Nilsson K, Spiro AC, Segal M, McKay RD: Development of neuronal precursor cells and functional postmitotic neurons from embryonic stem cells in vitro, *Mech Dev* 59:89–102, 1996.

Lumelsky N et al: Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets, *Science* 292:1389–94, 2001.

Studer L, Tabar V, McKay RD: Transplantation of expanded mesencephalic precursors leads to recovery in parkinsonian rats, *Nature Neurosci* 1:290–5, 1998.

Kim JH, Auerbach JM, Rodriguez-Gomez JA, Velasco I, Gavin D et al: Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease, *Nature* 418:50–6, 2002.

Reynolds BA, Weiss S: Clonal and population analyses demonstrate that an EGF-responsive mammalian embryonic CNS precursor is a stem cell, *Dev Biol* 175:1–13, 1996.

Lois C, Alvarez-Buylla A: Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia, *Proc Natl Acad Sci USA* 90:2074–7, 1993.

Morshead CM, Reynolds BA, Craig CG et al: Neural stem cells in the adult mammalian forebrain: A relatively quiescent subpopulation of subependymal cells, *Neuron* 13:1071–82, 1994.

Weiss S, Dunne C, Hewson J et al: Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis, *J Neurosci* 16:7599–609, 1996.

Palmer TD, Takahashi J, Gage H: The adult rat hippocampus contains primordial neural stem cells, *Mol Cell Neurosci* 8:389–404, 1997.

Sabate O, Horellou P, Vigne E, Colin P, Perricaudet M et al: Transplantation to the rat brain of human neural progenitors that were genetically modified using adenoviruses, *Nat Genet* 9:256–60, 1995.

Yamada H, Dezawa M, Shimazu S, Baba M, Sawada H et al: Transfer of the von Hippel–Lindau gene to neuronal progenitor cells in treatment for Parkinson's disease, *Ann Neurol* 54:352–9, 2003.

Saucedo-Cardenas O et al: Nurr1 is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons, *Proc Natl Acad Sci USA* 95:4013–8, 1998.

Le W et al: Selective agenesis of mesencephalic dopaminergic neurons in Nurr1-deficient mice, *Exp Neurol* 159:451–8, 1999.

Kanno H, Saljooque F, Yamamoto I et al: Role of the von Hippel–Lindau tumor suppressor protein during neuronal differentiation, *Cancer Res* 60:2820–4, 2000.

Shingo T, Date I, Yoshida H, Ohmoto T: Neuroprotective and restorative effects of intrastriatal grafting of encapsulated GDNF-producing cells in a rat model of Parkinson's disease, *J Neurosci Res* 69:946–54, 2002.

Barkats M, Nakao N, Grasbon-Frodl EM, Bilang-Bleuel A, Revah F et al: Intrastriatal grafts of embryonic mesencephalic rat neurons genetically modified using an adenovirus encoding human Cu/Zn superoxide dismutase, *Neuroscience* 78:703–13, 1997.

Wyman T, Rohrer D, Kirigiti P, Nichols H, Pilcher K et al: Promoter-activated expression of nervous growth factor for treatment of neurodegenerative diseases, *Gene Ther* 6:1648–60, 1999.

Hermens WT, Verhaagen J: Viral vectors, tools for gene transfer in the nervous system, *Prog Neurobiol* 55:399–432, 1998.

Ericson C, Wictorin K, Lundberg C: Ex vivo and in vitro studies of transgene expression in rat astrocytes transduced with lentiviral vectors, *Exp Neurol* 173:22–30, 2002.

Cortez N, Trejo F, Vergara P, Segovia J: Primary astrocytes retrovirally transduced with a tyrosine hydroxylase transgene driven by a glial-specific promoter elicit behavioral recovery in experimental parkinsonism, *J Neurosci Res* 59:39–46, 2000.

Cunningham LA, Su C: Astrocyte delivery of glial cell line-derived neurotrophic factor in a mouse model of Parkinson's disease, *Exp Neurol* 174:230–42, 2002.

Wang ZH, Ji Y, Shan W, Zeng B, Raksadawan N et al: Therapeutic effects of astrocytes expressing both tyrosine hydroxylase and brain-derived neurotrophic factor on a rat model of Parkinson's disease, *Neuroscience* 113:629–40, 2002.

Cao L, Zhao YC, Jiang ZH, Xu DH, Liu ZG et al: Long-term phenotypic correction of rodent hemiparkinsonism by gene therapy using genetically modified myoblasts, *Gene Ther* 7:445–9, 2000.

Schwarz EJ, Alexander GM, Prockop DJ, Azizi SA: Multipotential marrow stromal cells transduced to produce L-DOPA: Engraftment in a rat model of Parkinson disease, *Hum Gene Ther* 10:2539–49, 1999.

13.38. Etiology, Pathogenesis, and Clinical Features of Multiple Sclerosis

Prat A, Antel J: Pathogenesis of multiple sclerosis, *Curr Opin Neurol* 18:225–30, 2005.

Franklin RJ: Why does remyelination fail in multiple sclerosis? *Nat Rev Neurosci* 3:705–14, 2002.

Furlan R, Pluchino S, Martino G: The therapeutic use of gene therapy in inflammatory demyelinating diseases of the central nervous system, *Curr Opin Neurol* 16:385–92, 2003.

Martino G, Hartung HP: Immunopathogenesis of multiple sclerosis: The role of T cells, *Curr Opin Neurol* 12:309–21, 1999.

Kieseier BC, Storch MK, Archelos JJ et al: Effector pathways in immune mediated central nervous system demyelination, *Curr Opin Neurol* 12:323–36, 1999.

Martino G, Adorini L, Rieckmann P et al: Inflammation in multiple sclerosis: The good, the bad, and the complex, *Lancet Neurol* 1:499–509, 2002.

Franklin RJ: Why does remyelination fail in multiple sclerosis? *Nat Rev Neurosci* 3:705–14, 2002.

13.39. Molecular Regenerative Engineering for Multiple Sclerosis

Shaw MK, Lorens JB, Dhawan A, DalCanto R, Tse HY et al: Local delivery of interleukin 4 by retrovirus-transduced T lymphocytes ameliorates experimental autoimmune encephalomyelitis, *J Exp Med* 185:1711–4, 1997.

Mathisen PM, Yu M, Johnson JM et al: Treatment of experimental autoimmune encephalomyelitis with genetically modified memory T cells, *J Exp Med* 186:159–64, 1997.

Chen LZ, Hochwald GM, Huang C et al: Gene therapy in allergic encephalomyelitis using myelin basic protein-specific T cells engineered to express latent transforming growth factor-beta1, *Proc Natl Acad Sci USA* 95:12516–21, 1998.

Piccirillo CA, Prud'homme GJ: Prevention of experimental allergic encephalomyelitis by intramuscular gene transfer with cytokine-encoding plasmid vectors, *Hum Gene Ther* 10:1915–22, 1999.

Garren H, Ruiz PJ, Watkins TA et al: Combination of gene delivery and DNA vaccination to protect from and reverse Th1 autoimmune disease via deviation to the Th2 pathway, *Immunity* 15:15–22, 2001.

Croxford JL, Triantaphyllopoulos K, Podhajcer OL et al: Cytokine gene therapy in experimental allergic encephalomyelitis by injection of plasmid DNA-cationic liposome complex into the central nervous system, *J Immunol* 160:5181–7, 1998.

Croxford JL, Triantaphyllopoulos KA, Neve RM et al: Gene therapy for chronic relapsing experimental allergic encephalomyelitis using cells expressing a novel soluble p75 dimeric TNF receptor, *J Immunol* 164:2776–81, 2000.

Triantaphyllopoulos K, Croxford J, Baker D, Chernajovsky Y: Cloning and expression of murine IFN beta and a TNF antagonist for gene therapy of experimental allergic encephalomyelitis, *Gene Ther* 5:253–63, 1998.

Weiner HL: Immunosuppressive treatment in multiple sclerosis, *J Neurol Sci* 223:1–11, 2004.

Melo ME, Qian J, El-Amine M et al: Gene transfer of Ig-fusion proteins into B cells prevents and treats autoimmune diseases, *J Immunol* 168:4788–95, 2002.

Ruffini F, Furlan R, Poliani PL et al: Fibroblast growth factor-II gene therapy reverts the clinical course and the pathological signs of chronic experimental autoimmune encephalomyelitis in C57BL/6 mice, *Gene Ther* 8:1207–13, 2001.

13.40. Cellular Regenerative Engineering or Multiple Sclerosis

Franklin RJ: Why does remyelination fail in multiple sclerosis? *Nat Rev Neurosci* 3:705–14, 2002.

Croxford JL, Triantaphyllopoulos KA, Neve RM et al: Gene therapy for chronic relapsing experimental allergic encephalomyelitis using cells expressing a novel soluble p75 dimeric TNF receptor, *J Immunol* 164:2776–81, 2000.

Ohashi T, Watabe K, Uehara K et al: Adenovirus-mediated gene transfer and expression of human [beta]-glucuronidase gene in the liver, spleen, and central nervous system in mucopolysaccharidosis type VII mice, *Proc Natl Acad Sci USA* 94:1287–92, 1997.

Furlan R, Pluchino S, Martino G: The therapeutic use of gene therapy in inflammatory demyelinating diseases of the central nervous system, *Curr Opin Neurol* 16:385–92, 2003.

Shaw MK, Lorens JB, Dhawan A et al: Local delivery of interleukin 4 by retrovirus-transduced T lymphocytes ameliorates experimental autoimmune encephalomyelitis, *J Exp Med* 185:1711–4, 1997.

Dal Canto RA, Shaw MK, Nolan GP et al: Local delivery of TNF by retrovirus-transduced T lymphocytes exacerbates experimental autoimmune encephalomyelitis, *Clin Immunol* 90:10–14, 1999.

Mathisen PM, Yu M, Johnson JM et al: Treatment of experimental autoimmune encephalomyelitis with genetically modified memory T cells, *J Exp Med* 186:159–64, 1997.

Chen LZ, Hochwald GM, Huang C et al: Gene therapy in allergic encephalomyelitis using myelin basic protein-specific T cells engineered to express latent transforming growth factor-beta1, *Proc Natl Acad Sci USA* 95:12516–21, 1998.