

# CHAPTER 11

# Brain Disorders

In this chapter, we examine how nervous system dysfunction causes neurological and psychiatric disorders. Brain disorders are greater causes of disability than any other class of diseases in our modern society. An important and obvious goal of studying brain disorders is to identify therapeutic strategies that will decrease disability and alleviate human suffering. In addition, research that focuses on specific diseases offers unique perspectives on normal brain development and function in the same way that studying genetic mutants can reveal the normal function of genes and the biological processes they control. Conversely, some of the most important progress made in understanding brain disorders has come from basic research seemingly unrelated to disease, as numerous disease examples introduced in previous chapters have shown. Thus, basic and disease-focused investigations mutually enhance each other to help us understand the function and dysfunction of the nervous system. While focusing on specific brain disorders, this chapter also seeks to integrate and extend the knowledge and principles presented in all previous chapters.

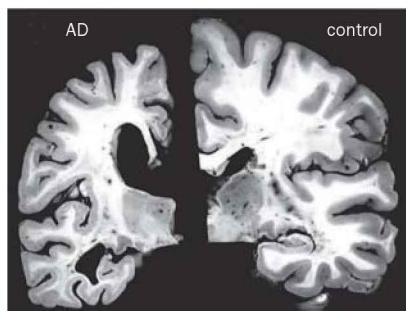
Rather than comprehensively addressing the vast array of brain disorders, we will focus primarily on select disorders, the principles of which can be applied broadly to other disorders not named here. Some disorders are selected because they have a large impact on human society; others are selected because their pathogenic mechanisms are better understood. We group these disorders as neurodegenerative, psychiatric, or neurodevelopmental. Although generally useful, these groupings also reflect our ignorance of the underlying disease mechanisms. For instance, as we will see, some of the classic psychiatric disorders were thought to arise in adulthood, but in fact have a developmental origin. We start with Alzheimer's disease, the most common neurodegenerative disorder.

## ALZHEIMER'S DISEASE AND OTHER NEURODEGENERATIVE DISEASES

**Alzheimer's disease (AD)** is well known because of its prevalence: in the United States, it affects 1 in 20 people by age 65 and about half of the population above age 85. Thus, AD is a disease of aging. With the dramatic global increase in life-span over the past century, AD has become an escalating burden on society. As in all **neurodegenerative disorders**, the progression of AD causes an increasing number of neurons to become dysfunctional: synaptic connections are lost, dendrites and axons deteriorate, and neurons eventually die. As a result, the brain undergoes significant atrophy (**Figure 11-1**). Memory loss, an early and characteristic symptom of AD, is followed by loss of other cognitive and intellectual capabilities such as reasoning and language. Patients may also become depressed early in the disease when they are aware of their deterioration, and may exhibit personality changes and behavioral problems as the disease progresses. Gradually, patients lose their ability to cope with daily life and often require round-the-clock care for years before they succumb to death.

And men ought to know that from nothing else but the brain come joys, delights, laughter and sports, and sorrows, griefs, despondency, and lamentations. And by this, in an especial manner, we acquire wisdom and knowledge, and see and hear, and know what are foul and what are fair, what are bad and what are good, what are sweet, and what unsavory... And by the same organ we become mad and delirious, and fears and terrors assail us... All these things we endure from the brain, when it is not healthy....

Hippocrates (~400 BC)



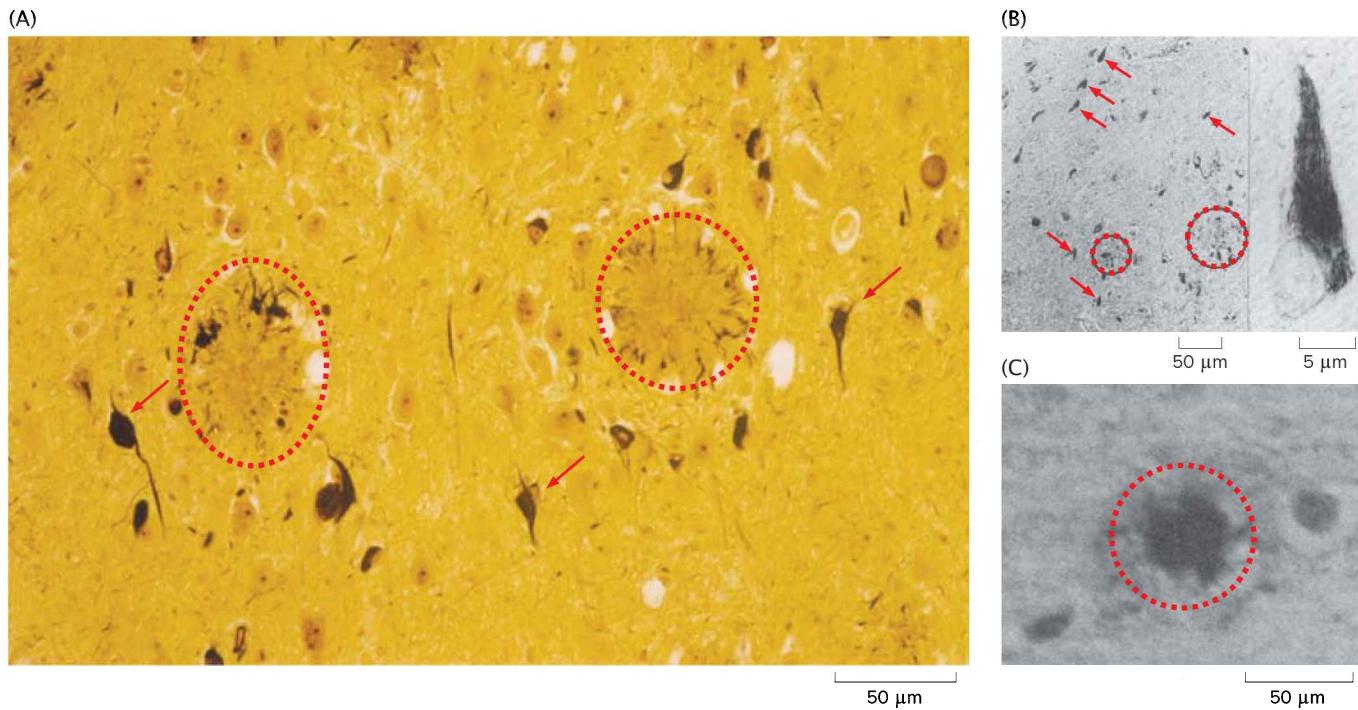
**Figure 11-1 Brain atrophy in Alzheimer's disease (AD).** Postmortem brain sections from an AD patient (left) and an age-matched, cognitively normal subject (right) show severe brain atrophy in the AD case. (Courtesy of Nigel Cairns, Washington University, Department of Neurology.)

Alzheimer's disease is one of the best-understood brain disorders in terms of its neuropathological underpinnings, thanks to biochemical and molecular-genetic studies since the 1980s. AD research has also offered valuable lessons that can be applied to other brain disorders. However, many key questions remain unanswered, and there is no effective treatment or prevention.

### 11.1 Alzheimer's disease is defined by brain deposition of numerous amyloid plaques and neurofibrillary tangles

In 1907, a German psychiatrist, Alois Alzheimer, reported a case of a patient who suffered from numerous psychiatric symptoms and severe memory loss, and who passed away four-and-a-half years after being admitted to an insane asylum. Using a newly invented silver-staining procedure to visualize postmortem brain specimens, Alzheimer described two major pathological features in the patient's cerebral cortex: numerous abnormal intracellular fibrils (now termed **neurofibrillary tangles**) in one-quarter to one-third of all neurons, and extracellular plaques (now termed **amyloid plaques**) distributed throughout the cerebral cortex. Today, although the clinical diagnosis based on history and examination of symptoms is usually correct, the combined presence in brain sections taken at autopsy of abundant neurofibrillary tangles and amyloid plaques remains the standard for a definitive pathological diagnosis of the disease that bears Alzheimer's name (**Figure 11-2A**). Both tangles and plaques are also found in the brains of non-symptomatic aged subjects, but their prevalence throughout the cerebral cortex, hippocampus, and amygdala increases drastically in AD brains.

What is the molecular nature of the neurofibrillary tangles and amyloid plaques? Could these pathological features offer clues to understanding this devastating disease? With these questions in mind, researchers biochemically characterized these structures and identified their molecular nature in the



**Figure 11-2** Neurofibrillary tangles and amyloid plaques in Alzheimer's disease. **(A)** Silver staining of a postmortem cortical section of an AD patient. Amyloid plaques (circles) and neurofibrillary tangles (arrows) are prevalent. **(B)** An antibody against the microtubule-associated protein tau strongly stains the neurofibrillary tangles (arrows; high magnification on the right), but does not stain the core of the amyloid plaques (circles). **(C)** An antibody against a peptide

derived from amyloid  $\beta$  protein stains the core of an amyloid plaque intensely (circle). (A, from Selkoe DJ [1999] *Nature* 399:A23–A31. With permission from Macmillan Publishers Ltd.; B, from Grundke-Iqbali I, Iqbal K, Quinlan M et al. [1986] *J Biol Chem* 261:6084–6089. With permission from ASBMB; C, from Wong CW, Quaranta V & Glenner GG [1985] *Proc Natl Acad Sci USA* 82:8729–8732.)

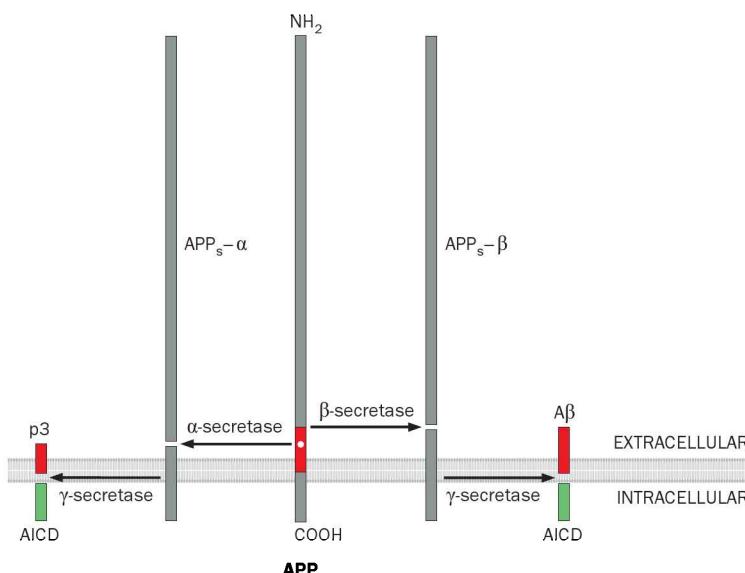
mid-1980s. The neurofibrillary tangles consist of abnormal aggregates of hyperphosphorylated microtubule-binding protein **tau** (Figure 11–2B). Amyloid plaques are composed mostly of a 39- to 43-amino-acid peptide named **amyloid  $\beta$  protein (A $\beta$ )** for its strong tendency to form aggregates of  $\beta$ -pleated sheets (Figure 11–2C). While neurofibrillary tangles have also been found in several other neurodegenerative diseases, which are collectively called **tauopathies**, amyloid plaques are most characteristic of AD. Research focused on both A $\beta$  and tau has provided important insights in our understanding of Alzheimer's disease. We start our story with A $\beta$ .

## 11.2 Amyloid plaques mainly consist of aggregates of proteolytic fragments of the amyloid precursor protein (APP)

The peptide sequence of A $\beta$  was used to isolate its gene from cDNA libraries, leading to the discovery that A $\beta$  is part of a transmembrane protein called **amyloid precursor protein**, or **APP**. The predicted protein sequence indicated that APP has a large extracellular domain, a single transmembrane domain, and a small cytoplasmic domain (Figure 11–3). APP homologs have been found in the fly and worm, indicating that the proteins are evolutionarily conserved, although their exact cellular function is still the subject of investigation. The sequence of the A $\beta$  peptide itself is not conserved in the fly or worm, or in two other APP paralogs in humans that share similar overall structure and sequence. These data suggest that the normal function of APP does not depend on A $\beta$ ; rather, A $\beta$ 's amino acid sequence renders it particularly prone to aggregation after it is excised from APP.

The location of A $\beta$  within APP is peculiar: two-thirds of the peptide is at the C-terminal end of the extracellular domain and one-third is part of the predicted transmembrane domain (Figure 11–3). This implies that to produce A $\beta$ , APP must be cleaved by two different proteases, one of which must cut APP in the middle of the transmembrane domain. The existence of proteases capable of cleaving within the membrane was unknown when APP was first discovered. Indeed, the study of APP processing has enriched our understanding of the cell biology of regulated proteolysis.

The first protease identified as being able to process APP, named  **$\alpha$ -secretase**, cuts APP in the middle of the A $\beta$  peptide and therefore prevents the production of the pathology-associated A $\beta$  (Figure 11–3, left). This proteolysis is likely related to the physiological function of APP, as the fly homolog of APP also produces a secreted form of APP by proteolysis near the end of the extracellular domain. Subsequently, the proteases that cut APP at the N- and C-termini of A $\beta$ , which produce the intact A $\beta$  peptide, were identified and named  **$\beta$ -secretase** and



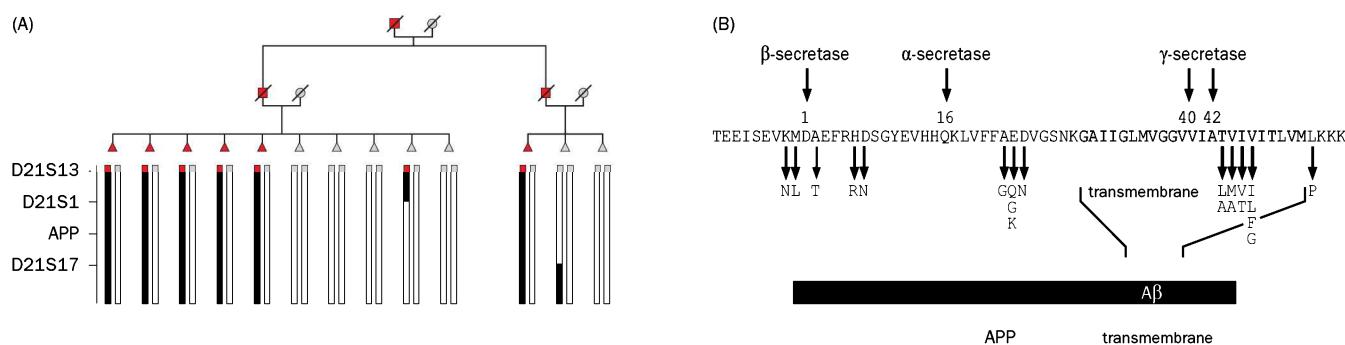
**Figure 11–3 Proteolytic processing of amyloid precursor protein (APP) produces A $\beta$ .** APP is synthesized as a transmembrane protein (middle), with an N-terminal large extracellular domain, a single transmembrane domain, and a short C-terminal cytoplasmic domain. A $\beta$  (red) spans the junction of the extracellular and transmembrane domains. APP is usually cleaved by  $\alpha$ -secretase (left; dot indicates the  $\alpha$ -secretase cleavage site) or  $\beta$ -secretase (right) to produce a secreted form (APP<sub>s</sub>- $\alpha$  or APP<sub>s</sub>- $\beta$ , respectively). The remaining portion is further processed by the  $\gamma$ -secretase, to yield an intracellular fragment (AICD). Whereas the combined actions of  $\alpha$ - and  $\gamma$ -secretase produce a protein of 3 kilodalton (p3), the combined actions of the  $\beta$ - and  $\gamma$ -secretases produce intact A $\beta$ .

$\gamma$ -secretase, respectively (Figure 11–3, right). The cleavage site of the  $\gamma$ -secretase is not fixed: it can produce A $\beta$  of different lengths, ranging from 39 to 43 amino acids. The predominant forms have 40 or 42 amino acids and are called A $\beta_{40}$  and A $\beta_{42}$ , respectively.  $\gamma$ -Secretase was subsequently found to be a general intramembrane protease complex important for many signaling events. Studies of APP and other  $\gamma$ -secretase substrates indicate that a major trigger for  $\gamma$ -secretase activation is an extracellular cleavage that produces a transmembrane protein with a short extracellular stub (such as those produced by  $\alpha$ - or  $\beta$ -secretase). APP is normally expressed in many cell types, and is proteolytically processed by the  $\alpha$ - or  $\beta$ -secretases followed by the  $\gamma$ -secretase. So what goes wrong in Alzheimer's disease?

### 11.3 Mutations in human APP and $\gamma$ -secretase cause early-onset familial Alzheimer's disease

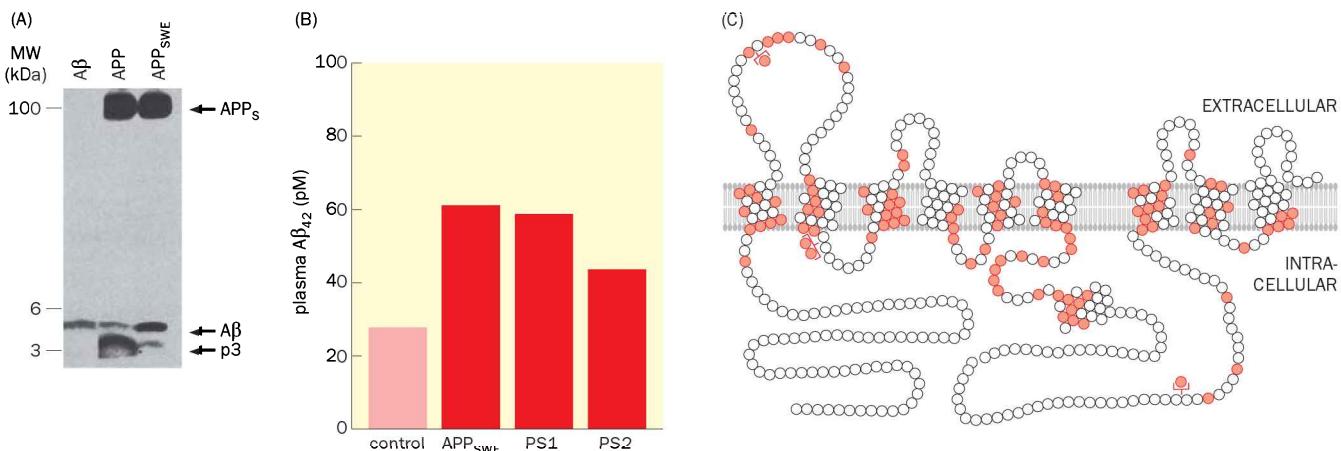
At this point you may ask: are APP and A $\beta$  related to the cause of AD, or is A $\beta$  plaque formation simply the consequence of a disease whose causes lie elsewhere? Genetic studies of early-onset **familial Alzheimer's disease (FAD)** have shed light on this important issue. Most AD cases are late onset (age 65 or older) and are **sporadic** (from the Latin word for scattered) because patients do not have an identifiable family history of the disease; however, as with many sporadic illnesses, genetic risk factors of smaller effect play an important role in AD, as we discuss below. Patients with early-onset FAD usually develop AD symptoms in their 40s or 50s, and have a clear inheritance pattern. Most types of FAD follow a Mendelian autosomal dominant inheritance pattern: an AD patient is heterozygous for the disease allele, and imparts the disease allele to 50% of his/her progeny (we will discuss genetics of human disease in more detail in Box 11–3). Those who inherit the disease allele invariably develop AD if they live long enough (Figure 11–4A). In these cases, genetic mapping has helped to pinpoint mutations in specific genes that cause AD.

The first FAD mutation was mapped onto the *App* gene itself (Figure 11–4A): a missense mutation that changed a valine to an isoleucine in the middle of the transmembrane domain near the C-terminus of A $\beta_{42}$  (Figure 11–4B). Subsequently, about 20 FAD mutations have been mapped onto the *App* gene. Interestingly, most mutations are clustered near the  $\gamma$ - or  $\beta$ -secretase cleavage sites, with some in the middle of the A $\beta$  peptide (Figure 11–4B). Biochemical studies indicate that mutations near the  $\gamma$ -secretase site increase the ratio of A $\beta_{42}$  over



**Figure 11–4 Mutations in the *App* gene cause familial Alzheimer's disease (FAD).** (A) Pedigree of an early-onset FAD family (average onset  $57 \pm 5$  years). Square, male; circle, female; triangle, either sex to preserve anonymity. Oblique lines indicate deceased individuals. Beneath the pedigree are maps of Chromosome 21 in which segments of the chromosomes were mapped according to the markers on the left. The linkage data suggest that chromosome segments in red were inherited from the disease-causing chromosome of the affected fathers (red squares). Inheriting from the father the red chromosome segment that includes mutant *APP* correlates perfectly with having AD (red triangles). (B) Summary

of FAD mutations in the APP protein, most of which are located within or near A $\beta$ . The cleavage sites for the three secretases are also indicated. Numbers indicate amino acid residues starting from the beginning of the A $\beta$  peptide. The green arrow points to an AD-protective A  $\rightarrow$  T (alanine to threonine) mutation that reduces A $\beta$  production. (A, adapted from Goate A, Chartier-Harlin MC, Mullan M et al. [1991] *Nature* 349:704–706. With permission from Macmillan Publishers Ltd; B, adapted from Holtzman DM, John CM & Goate A [2011] *Sci Transl Med* 3:77sr1. See also Jonsson T, Atwal JK, Steinberg S et al. [2012] *Nature* 488:96–99.)



**Figure 11–5 Mutations In APP and presenilin Increase A $\beta$  production.**

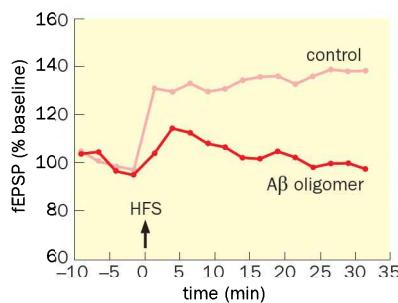
**(A)** Left lane: synthetic A $\beta$  as a control. Middle lane: transfecting cDNA expressing wild-type APP into cultured cells produced a major 3-kilodalton (kDa) band (p3) corresponding to the cleavage product of  $\alpha$ - and  $\gamma$ -secretase, and a minor 4-kDa band corresponding to A $\beta$ . Right lane: cells transfected with cDNA expressing APP<sub>SWE</sub> produced more A $\beta$  than p3. MW, molecular weight in kilodalton. In these experiments, culture media containing radioactively labeled proteins from transfected cells were immunoprecipitated with an antibody against A $\beta$ ; immunoprecipitated proteins were run on a gel. **(B)** Compared with controls, the plasma A $\beta$ <sub>42</sub> concentration (in picomoles per liter) was increased in AD patients with the APP<sub>SWE</sub> mutation or with pathogenic

mutations in presenilin-1 (PS1) or presenilin-2 (PS2). **(C)** Structure of presenilin-1 and locations of FAD mutations. PS1 spans the membrane nine times; PS2, not shown here, has a similar structure. Mutations in PS1 that result in FAD were first reported by Sherrington et al. in 1995 (*Nature* 375:754); by 2010, more than 170 mutations that cause FAD had been identified in PS1 (red amino acid residues in the figure; red brackets denote insertions). (A, from Citron M, Oltersdorf T, Haass C et al. [1992] *Nature* 360:672–674. With permission from Macmillan Publishers Ltd.; B, adapted from Scheuner D, Eckman C, Jensen M et al. [1996] *Nat Med* 2:864–870. With permission from Macmillan Publishers Ltd.; C, adapted from De Strooper B & Annaert W [2010] *Ann Rev Cell Dev Biol* 26:235–260.)

A $\beta$ <sub>40</sub>. Although A $\beta$ <sub>40</sub> is the dominant form produced by  $\gamma$ -secretase cleavage, A $\beta$ <sub>42</sub> has a higher tendency to form aggregates. FAD mutations in the middle of A $\beta$  may also increase the aggregation tendency. The FAD mutation near the  $\beta$  cleavage site (KM → NL amino acid changes; also called the Swedish mutation, or APP<sub>SWE</sub>) leads to an increase of A $\beta$  production (Figure 11–5A, B). Most of these mutations cause early-onset AD. Interestingly, an A → T amino acid change recently found near the  $\beta$ -secretase cleavage site (green arrow in Figure 11–4B), which reduces  $\beta$ -secretase cleavage and A $\beta$  production *in vitro*, confers *protection* against late-onset AD and age-related cognitive decline. Thus, these genetic data strongly suggest that an increase in A $\beta$  aggregation or production is causally linked to at least some forms of AD.

Another piece of evidence supporting the causal link between increased A $\beta$  production and AD came from **Down syndrome**, which is caused by having an extra copy of Chromosome 21 in every cell. Down syndrome patients invariably have high levels of amyloid deposits in their 30s and 40s and exhibit Alzheimer's-like dementia in their 50s. The *App* gene is located on Chromosome 21, so Down syndrome patients have an extra copy of *App*. Indeed, people with smaller duplications of Chromosome 21 that cover the *App* gene also have early-onset AD symptoms, suggesting that increasing the *App* gene dose is sufficient to cause AD.

Genetic mapping studies have also identified two other loci, on human Chromosomes 14 and 1, respectively, that harbor autosomal dominant FAD mutations. The causal genes encode similar transmembrane proteins named **presenilin-1** (Figure 11–5C) and **presenilin-2**. Subsequent biochemical and genetic studies showed that the presenilins, together with three associated proteins, constitute the  $\gamma$ -secretase protein complex responsible for cleaving APP near the C-terminus of A $\beta$  within the transmembrane domain. AD patients with presenilin mutations have increased A $\beta$ <sub>42</sub> levels compared to control subjects (Figure 11–5B). Thus, mutations in APP and its processing enzymes point to increases in A $\beta$  production or aggregation as a common cause that underlies AD pathogenesis, at least in early-onset FAD cases. This **A $\beta$  hypothesis** does not exclude the possibility that other mechanisms can additionally contribute to



**Figure 11–6 Oligomeric A $\beta_{42}$  disrupts hippocampal long-term potentiation (LTP).** Application of 500-nM oligomeric A $\beta_{42}$  caused a marked reduction of LTP at the perforant path → dentate gyrus synapse in rat hippocampal slices, induced by high-frequency stimulation (HFS) at  $t = 0$ . A $\beta_{42}$  oligomers were applied to the experimental group at  $-45$  min (not shown). See Figure 10–8 for a schematic of the LTP assay. (Adapted from Lambert MP, Barlow AK, Chromy BA et al. [1998] Proc Natl Acad Sci USA 95:6448–6453.)

AD; for example, disruption of presenilin function might interfere with cellular processes unrelated to A $\beta$  production that also contribute to AD pathogenesis.

How might excessive A $\beta$  production and aggregation cause AD? While studies have suggested that amyloid deposits are toxic to neurons, the severity of AD symptoms does not always correlate with the density of amyloid plaques. This indicates that amyloid plaques may not be the only pathological factor. Indeed, as we will see in the next section, amyloid plaques and tau-enriched neurofibrillary tangles exhibit a synergistic relationship. Furthermore, diffuse oligomeric forms of A $\beta$  have been found to be potent toxins to neurons. For example, an early study showed that long-term potentiation in a hippocampal slice was markedly impaired within 45 min of applying nanomolar concentrations of A $\beta_{42}$  oligomers synthesized *in vitro* (Figure 11–6), and significant neuronal death occurred within 24 hours of A $\beta_{42}$  oligomer application. Subsequent investigations revealed that oligomeric A $\beta$  induces synaptic depression, spine loss, and abnormal synaptic plasticity, suggesting that non-aggregated A $\beta$  oligomers may be potent AD-promoting agents. Future studies must further elucidate the relative contributions made by A $\beta$  aggregates and different forms of oligomers to disease phenotypes *in vivo*.

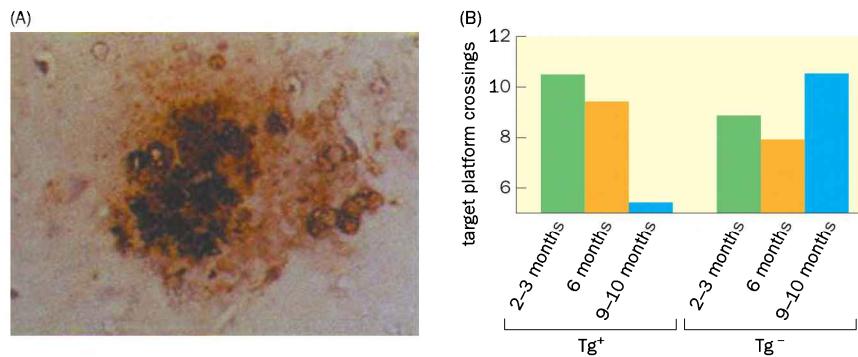
## 11.4 Animal models offer crucial tools to investigate pathogenic mechanisms

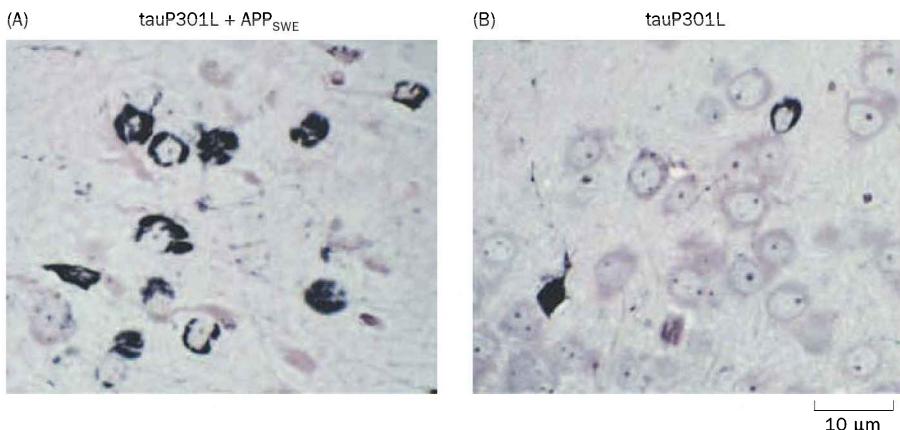
Animal models are instrumental in human disease research. Appropriate animal models can validate causality between suspected pathogenic processes and disease outcomes. They can be used to trace disease progression, investigate disease mechanisms, and test the effects of therapeutic agents. The development of AD animal models offers excellent illustrations of the utility of this approach. The technical feasibility of performing precise genetic manipulations in mice (see Sections 13.7 and 13.10) has made this species the dominant mammalian model for AD and many other brain disorders. However, as will be discussed in more detail later, there are many differences between the mouse brain and human brain, such that even very useful mouse models do not tend to produce identical pathology or treatment responses in humans.

Mice normally do not exhibit AD pathology such as amyloid plaques and neurofibrillary tangles. This is probably because the mouse's lifespan of about 2 years is not long enough for abnormal protein aggregates to cause sufficient insult to the nervous system, a process that usually takes decades in humans. However, this protein pathology can be accelerated by overexpressing wild-type or FAD-mutation alleles of the human *App* gene in transgenic mice (Figure 11–7A). Moreover, some transgenic mice also develop age-dependent cognitive decline, such as deficits in spatial memory in the Morris water maze (Figure 11–7B). These experiments support the A $\beta$  hypothesis: overproduction of human APP with FAD mutations is sufficient to produce pathology and cognitive defects consistent with AD.

Transgenic mice have been also used to investigate the relationship between APP and presenilin. Mice expressing a mutant form of presenilin by itself did not develop AD pathology. However, mice co-expressing transgenes with FAD mutations in human APP and human presenilin exhibited earlier onset of amyloid

**Figure 11–7 Transgenic mice overexpressing human APP exhibit amyloid plaques and cognitive deficits.** (A) Amyloid plaque stained with antibody against A $\beta$  found in a 354-day-old transgenic mouse overexpressing APP with the Swedish mutation (APP<sub>SWE</sub>). (B) As they age, mice transgenic for APP<sub>SWE</sub> ( $Tg^+$ ) exhibit learning defects in the Morris water maze. Mice were trained to locate a hidden platform using spatial cues. When the hidden platform was removed, control mice ( $Tg^-$ ) aged 9–10 months logged more target platform crossings (that is, spent more time near the platform's previous location, an indication of spatial memory) than did APP<sub>SWE</sub> transgenic mice of the same age. See Figure 10–32 for a schematic of this assay. (Adapted from Hsiao K, Chapman P, Nilsen S et al. [1996] Science 274:99–103.)





**Figure 11-8 Enhanced neurofibrillary tangle pathology in transgenic mice expressing mutant APP and tau.** In humans, a leucine → proline mutation in tau (tauP301L) causes frontotemporal dementia with parkinsonism. Neurofibrillary tangles, as seen by intense silver staining (dark blue), are markedly increased in mice that were doubly transgenic for the Swedish allele of APP (APP<sub>SWE</sub>) and tauP301L (**A**), compared with transgenic mice that expressed tauP301L alone (**B**). (From Lewis J, Dickson DW, Lin WL et al. [2001] *Science* 293:1487–1491. With permission from AAAS.)

plaque deposition and memory deficits compared with transgenic mice expressing mutant APP alone, indicating that mutations in presenilin and APP synergize to accelerate AD-like pathological changes.

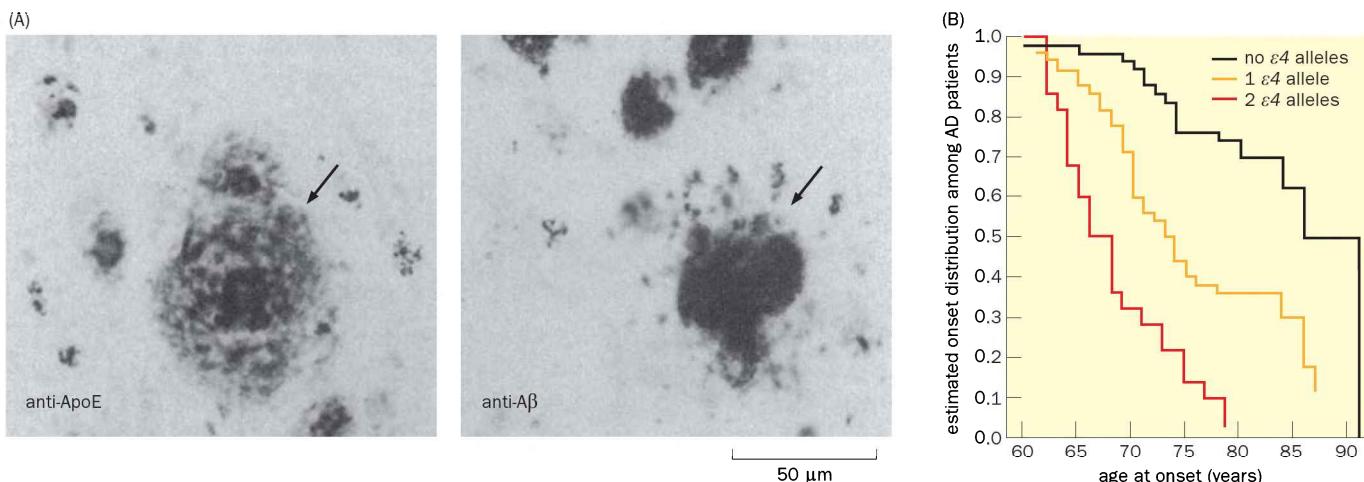
Transgenic mice have also been used to investigate the relationship between amyloid plaques and neurofibrillary tangles, the two major pathological features of AD. As noted in Section 11.1, the major component of neurofibrillary tangles is the microtubule-associated protein tau, and abnormal accumulation of tau is a feature of several neurodegenerative tauopathies. Although mutations in human tau have not been found in AD patients, they have been found in patients with a tauopathy called frontotemporal dementia with parkinsonism (FTDP), which exhibits neurofibrillary tangle pathology. Transgenic mice that express human tau with a dominant FTDP mutation at high levels can recapitulate tau aggregation similar to the neurofibrillary tangles observed in human AD and FTDP patients, without amyloid plaques. Interestingly, mice in which tau overexpression was combined with overexpression of mutant APP (double transgenic) or with both mutant APP and mutant presenilin (triple transgenic) developed both plaques and tangles. Moreover, neurofibrillary tangles were much more prevalent and widespread in double- or triple-transgenic mice than in transgenic mice expressing mutant tau alone (Figure 11-8). Thus, while plaques and tangles can form by independent mechanisms, increased A $\beta$  production facilitates neurofibrillary tangle formation. Indeed, removing one or both copies of the endogenous mouse gene encoding tau can alleviate behavioral and synaptic defects caused by APP overexpression. This suggests that some symptoms caused by the overproduction of APP or A $\beta$  may be mediated by dysregulation of tau.

Despite the utility of mouse AD models in creating plaques and tangles and in investigating their relationships, they do not recapitulate the prominent neuronal death and brain atrophy of human AD (see Figure 11-1). This may result from the limited lifespan of mice or physiological differences between rodent and primate brains. Thus, development of primate models will be valuable to further explore AD pathogenesis and test therapeutic strategies.

## 11.5 An apolipoprotein E (ApoE) variant is a major risk factor for Alzheimer's disease

Mutations in genes encoding APP and presenilins, while very helpful in establishing a causal relationship between A $\beta$  production and AD, account for less than 2% of AD cases, which are usually early onset. Almost all other AD cases are late onset and are sporadic. On the other hand, twin studies of large AD populations indicated a high degree of heritability (see Section 1.1), from 60 to 80%. This suggests that AD has a strong genetic component, although environmental factors are not negligible. What additional genes contribute to AD?

Thus far, genetic analyses have not revealed FAD genes with Mendelian inheritance patterns beyond those encoding APP and presenilins. This suggested that most late-onset AD cases are caused by combinations of multiple genetic factors or by genetic factors interacting with environmental factors. By far the most



**Figure 11–9** ApoE is found in amyloid plaques, and the  $\varepsilon 4$  allele is a major risk factor for AD. (A) Adjacent postmortem brain sections of an AD patient stained with antibodies against ApoE (left) and  $\text{A}\beta$  (right). ApoE is localized to the amyloid plaques (arrows). (B) As the copy number of *ApoE*  $\varepsilon 4$  increases, the age of AD onset becomes younger. For instance, about 50% of AD patients lacking the  $\varepsilon 4$

allele had been diagnosed with AD by age 86. The median age of AD onset drops to 73 years for patients with one  $\varepsilon 4$  allele and 66 years for those with two  $\varepsilon 4$  alleles. (A, from Strittmatter et al. [1993] Proc Natl Acad Sci USA 90:1977–1981; B, adapted from Corder EH, Saunders AM, Strittmatter WJ et al. [1993] Science 261:921–923. With permission from AAAS.)

important genetic risk factor identified to date is an individual's allele composition for a gene called *ApoE* which encodes **apolipoprotein-E (ApoE)**. A component of high-density lipoproteins in the brain, ApoE is involved in lipid transport and metabolism. The most common allele of *ApoE* in the human population is  $\varepsilon 3$ . A less common allele,  $\varepsilon 4$ , differs from  $\varepsilon 3$  by a single amino acid. In the early 1990s, ApoE was found to bind to  $\text{A}\beta$  and to be present in amyloid plaques (Figure 11–9A). Genetic studies that examined the relationship between *ApoE* allele composition and AD revealed that the  $\varepsilon 4$  allele frequency increased from approximately 15% in the general population to 40% in AD patients. Compared to the common  $\varepsilon 3/\varepsilon 3$  allele combination, individuals with a single  $\varepsilon 4$  allele have a more than threefold greater chance of developing AD, while individuals with two  $\varepsilon 4$  alleles have a 12-fold greater chance of getting AD. Furthermore, among AD patients, as the copy number of  $\varepsilon 4$  alleles increases, the age of disease onset decreases (Figure 11–9B).

Thus, *ApoE* is a **genetic susceptibility locus** for AD. Unlike the FAD mutations in APP or presenilins, which invariably cause AD if the carrier lives long enough, ApoE  $\varepsilon 4$  does not definitively cause AD but increases the likelihood of developing the disease. However, given the relatively high frequency of the  $\varepsilon 4$  allele in the human population, ApoE  $\varepsilon 4$  contributes far more to the incidence of AD than do mutations in APP and presenilins. Interestingly, a less common allele, *ApoE*  $\varepsilon 2$ , appears to be protective against AD, as the  $\varepsilon 2$  allele frequency in the general population is 8%, but drops to 4% in the AD population.

The mechanism by which ApoE  $\varepsilon 4$  increases the chance of developing AD is not well understood. One proposed function for ApoE is that by binding to  $\text{A}\beta$ , ApoE regulates its metabolism and clearance. Further work is required to clarify how the  $\varepsilon 4$  and  $\varepsilon 3$  alleles differentially affect  $\text{A}\beta$  metabolism, and whether ApoE affects  $\text{A}\beta$ -independent processes. Nevertheless, the discovery of ApoE's association with AD has provided a paradigm for studying complex genetic disorders, in which mutations in individual genes increase the susceptibility for a disease rather than causing the disease outright. We will see many examples of such susceptibility variants later in this chapter.

## 11.6 Microglia dysfunction contributes to late-onset Alzheimer's disease

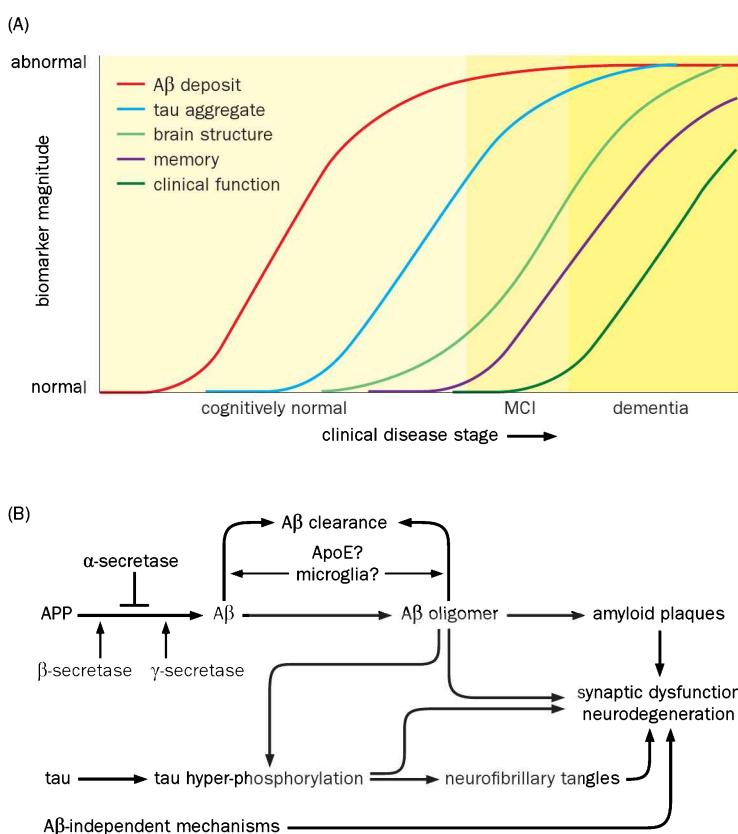
Recent genome-wide association studies and whole-genome sequence analyses (see Box 11–3) have identified additional genes that confer risks to late-onset

Alzheimer's disease. While most of these genetic variants have less effect on AD risk levels than does ApoE  $\epsilon 4$ , subjects with one copy of a particular variant in the gene encoding TREM2 (triggering receptor expressed on myeloid cells 2) have an AD risk similar to those with one copy of *Apoe*  $\epsilon 4$ , although the TREM2 variant frequency in the general population is far lower (<1%) than that of ApoE  $\epsilon 4$ . TREM2 is normally expressed at high levels in immune cells, including brain microglia that play an important role in clearing damaged cells and debris (see Figure 1–9). Specifically, TREM2 is known to stimulate phagocytosis and suppress inflammation. As another example suggesting a link between microglia and AD, several studies have associated CD33, a cell-surface antigen in immune cells as well as microglia, with late-onset AD. CD33 inhibits microglia uptake of  $A\beta_{42}$  in culture, and *Cd33* mutant mice exhibit reduced levels of  $A\beta$  plaques in a mouse model of AD. Together, these findings suggest that microglia dysfunction also contributes to AD pathogenesis, possibly via abnormal  $A\beta$  clearance.

## 11.7 How can we treat Alzheimer's disease?

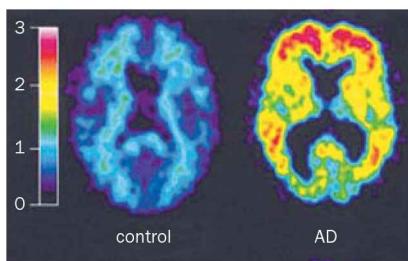
An ultimate goal of studying human diseases is to find effective ways to cure or prevent them. By the time AD is typically diagnosed, neurodegeneration may be too extensive for the disease to be cured (Figure 11–10A), although it may still be possible to halt further progression and treat AD symptoms. The most likely path to reducing the impact of AD is early diagnosis and prevention. Delaying AD onset by even a few years could have a very significant impact on the quality of life for patients and the burden on their families and society.

While we are still far from developing a cure, past decades of AD research have provided valuable clues about potential treatments. For example, multiple lines of evidence suggest that key pathogenic events may include increased  $A\beta$  levels or enhanced  $A\beta$  aggregation. Thus, this pathway has been the primary focus of intervention. Strategies to reduce  $A\beta$  toxicity include developing drugs to inhibit  $\beta$ - or  $\gamma$ -secretase activity, increase clearance of  $A\beta$ , or neutralize  $A\beta$  activity (Figure 11–10B). It will be also important to determine the nature of potential  $A\beta$ -independent pathway(s) to identify more drug targets for AD intervention.



**Figure 11-10 Temporal progression, pathogenic pathways, and potential sites of intervention for Alzheimer's disease.**

(A) A proposed scheme of temporal progression of AD, with x axis representing clinical stage, and y axis representing the magnitude of abnormalities of biomarkers such as  $A\beta$  deposit and tau aggregate. MCI, mild clinical impairment. (B) The top pathway summarizes the  $A\beta$  hypothesis. The roles of ApoE and microglia in  $A\beta$  clearance remain to be further elucidated. The middle pathway depicts the production of neurofibrillary tangles. The arrow between the top and middle pathways represents evidence that tau can mediate at least some of the  $A\beta$  toxicity in animal models (see Section 11.4). The bottom pathway emphasizes that AD pathogenesis may involve  $A\beta$ -independent mechanisms. Highlighted in blue are some of the therapeutic targets currently being pursued to reduce the  $A\beta$  level, including antibodies against  $A\beta$  and inhibitors or modifiers of  $\beta$ - and  $\gamma$ -secretase. Green and red, facilitative and inhibitory actions. (A, adapted from Jack CR, Knopman DS, Jagus WJ et al. [2013] *Lancet Neuro* 12:207–216. With permission from Elsevier Inc.)



**Figure 11-11 Positron emission tomography (PET) imaging can visualize A $\beta$  deposits.** Radioactively labeled benzothiazole, named Pittsburgh Compound-B (PIB), is used for PET imaging of AD brains because it enters the brain rapidly, binds selectively to aggregated A $\beta$  deposits, and is cleared rapidly. Compared with a 67-year-old normal subject (left), a 79-year-old AD patient (right) shows elevated standardized values (color-coded on the left) for PIB uptake. (From Klunk WE, Engler H, Nordberg A et al. [2004] *Ann Neurol* 55:306–319. With permission from the American Neurological Association.)

Many factors need to be considered when developing drugs for brain disorders (see Box 11-1). For instance, while a drug should have its intended effects on its therapeutic target, it should have minimal side effects and toxicity at the therapeutic dose. As a specific example, several  $\gamma$ -secretase inhibitors have been developed that effectively interfere with A $\beta$  production but failed in clinical testing due to severe side effects, possibly because  $\gamma$ -secretase has many substrates other than APP. (For example, one well-known  $\gamma$ -secretase substrate is the developmental signaling molecule Notch, which plays a key role in regulating cell fates as discussed in Section 7.3; Notch also has many important functions in adults.) It is necessary to identify  $\gamma$ -secretase inhibitors that specifically interfere with its activity toward APP cleavage, or to modify  $\gamma$ -secretase activity to bias the product toward shorter, less toxic A $\beta$ .

Even if successful drugs are developed, early diagnosis will be crucial in order to halt AD pathogenesis at the earliest stage possible. An important step is to identify **biomarkers**, which are characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Important biomarkers for AD include **positron emission tomography (PET)** using radioactive compounds that bind fibrillary A $\beta$  (Figure 11-11). (PET is a non-invasive three-dimensional imaging technique that traces the distribution of positron-emitting probes introduced into the body.) This would allow detection of amyloid deposits prior to the onset of cognitive and behavioral symptoms. Identifying biomarkers that signal AD progression, such as A $\beta_{42}$  and other metabolites in cerebrospinal fluid and plasma, can also contribute to early diagnosis. The goal is to have increasingly reliable diagnostic methods for treating preclinical AD before irreversible damage occurs (Figure 11-10A).

### Box 11-1: Rational drug development to treat brain disorders

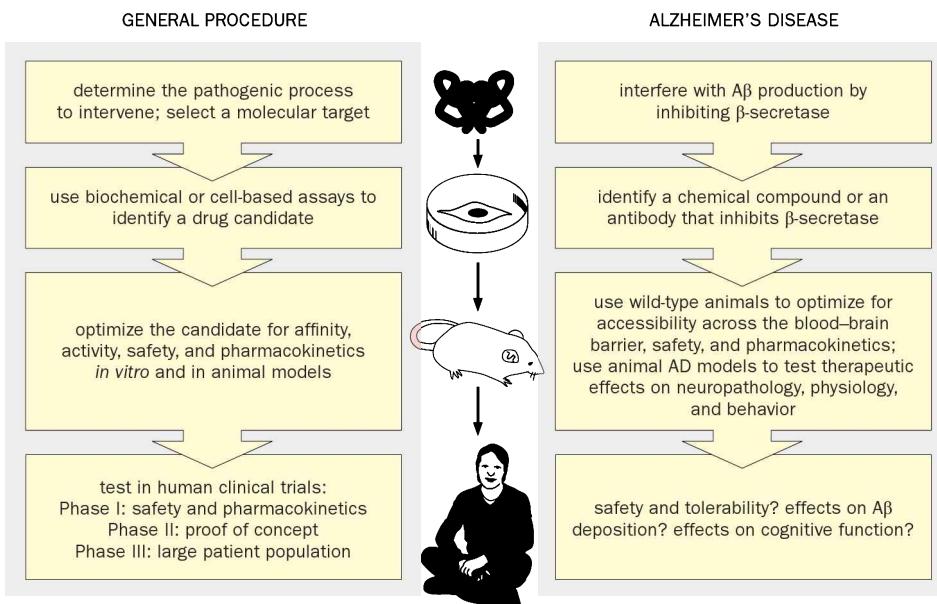
Many drugs currently used for treating brain disorders, including most drugs for psychiatric disorders, were discovered serendipitously, typically when clinicians noticed unintended but desirable effects during the treatment of another condition (see Sections 11.15–11.17). However, basic research in neuroscience and advances in the pharmaceutical and biotechnology industries during the past decades are changing this picture. Therapeutics can be developed through a rational process to make them more effective in treating the causes of a specific disease while limiting unwanted side effects. Identifying the mechanisms of disease pathogenesis is a prerequisite for rational therapeutic intervention. In this box, we focus on common steps of drug development (Figure 11-12), assuming that a key pathogenic process has already been identified.

The first step is to choose a specific molecular target for intervention. Some biological processes are more amenable than others to pharmacological intervention. For instance, cell-surface proteins are some of the preferred molecular targets because water-soluble chemicals and therapeutic antibodies can modulate their functions extracellularly. Once a target has been chosen, robust biological assays relevant to the disease process must be established to screen for candidate drugs, which usually belong to two large categories: small-molecule chemicals and large-molecule biologics such as peptides and antibodies. For small-molecule drugs, the first step is usually to establish *in vitro* assays that enable high-throughput screens of chemical libraries (which usually contain 10<sup>5</sup>–10<sup>6</sup> synthetic, semi-synthetic, or

naturally occurring compounds). Once a promising compound is identified, many variants can be synthesized to increase biological activity and target accessibility *in vivo* and to reduce potential side effects. Large-molecule biologics, while not selected using high-throughput screening, nevertheless undergo a similar optimization process for affinity, activity, safety, and physiological processing.

While some of the optimization steps can be conducted *in vitro*, many steps must be performed in animal models *in vivo*. Two commonly used terms for body-drug interactions are **pharmacodynamics**, which characterizes what the drug does to the body, including the intended effects on target molecules and processes as well as unintended side effects, and **pharmacokinetics**, which characterizes what the body does with the drug, including its absorption, distribution, metabolism, and excretion. Disease proxies, such as the animal models discussed in Section 11.4, are very helpful to assay a drug's therapeutic effects and to establish proof-of-concept. Animal models are also essential to evaluate potential drug toxicity. Initial animal models are likely to be rodents, but toxicity is usually also evaluated in additional models with more human-like physiology, such as dogs or nonhuman primates.

If the target resides within the nervous system, an important step is to ensure that drugs, which usually access the body through circulation in the blood, can pass through the **blood-brain barrier (BBB)**. Derived from endothelial cell tight junctions in the blood vessels of the brain, the BBB

**Box 11–1: Rational drug development to treat brain disorders**


**Figure 11–12** Process for rational drug discovery. Left, a flow chart depicting a typical drug development process. Right, a specific example illustrating the hypothetical development of a drug to treat Alzheimer's disease.

prevents the exchange of many substances between the blood and brain tissues. Small molecules can be chemically engineered to diffuse through the BBB or to enter cells directly, thereby reaching target molecules in any cellular compartments. Antibodies, on the other hand, usually access only cell-surface proteins and do not freely cross the BBB, although progress has been made recently to facilitate this process.

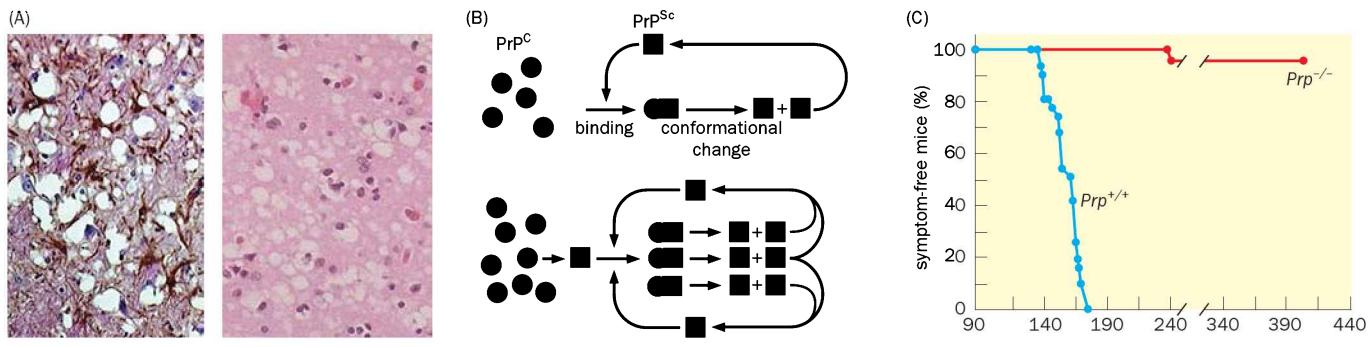
If a drug succeeds in preclinical tests in animals, including extensive safety studies, the next step is human clinical trials. Clinical trials are usually conducted in distinct phases, although some phases can be combined. Phase I studies are usually conducted with a small number of healthy volunteers and emphasize safety and drug metabolism. While Phase II studies continue to test the safety of a drug, they also gather preliminary data on the drug's effectiveness

in a relatively small number of patients, comparing the drug with a placebo control. Phase III studies collect more information about safety and effectiveness from a large patient population. In the United States, the Food and Drug Administration (FDA) oversees clinical trials and approves drugs for marketing. Drug development is a long process, averaging 10 years from initial target selection to approval for use in intended patients. This time window can be considerably longer for diseases that progress slowly, such as neurodegenerative diseases.

The process outlined above has been highly successful in identifying drugs that treat diseases such as cancer, immune disorders, and infectious diseases. Rationally designed drugs are also in the pipeline for treatment of neurodegeneration, including AD.

## 11.8 Prion diseases are caused by propagation of protein-induced protein conformational change

Just as abnormal A<sub>Beta</sub> aggregation is characteristic of Alzheimer's disease, research in the past decades has shown that **proteinopathy**—altered protein conformations, interactions, and homeostasis—appears to be a common feature of many neurodegenerative diseases. We discuss several examples in the next two sections. Among the most enigmatic causes of neurodegenerative disease are **prions** (pronounced *PREE-ons*, which stands for *proteinaceous infectious particles*). Three seemingly separate diseases share prions as the causative agent. The first is **scrapie**, which is known to infect sheep and goats after a prolonged incubation period following exposure to tissues from diseased animals; a variant of scrapie that affects cattle is colloquially known as 'mad cow disease.' The second is an infectious human disease called **kuru**, which occurred in Papua New Guinea tribes that observed ritual cannibalism. The third is a rare inherited human disease called **Creutzfeldt-Jakob disease (CJD)**. All three diseases are associated



**Figure 11-13 Prion diseases are caused by protein-induced protein conformational change.** (A) Pathology seen in postmortem brain sections of a scrapie-affected sheep (left) and a human Creutzfeldt-Jakob disease (CJD) patient (right); note the numerous sponge-like holes. (B) The prion hypothesis. Top: infectious PrP<sup>Sc</sup> (squares) can induce PrP<sup>C</sup> (circles) to adopt the PrP<sup>Sc</sup> conformation, thereby propagating PrP<sup>Sc</sup>. Bottom, in genetic prion diseases such as CJD, mutant PrP<sup>C</sup> proteins (orange circles) spontaneously adopt the PrP<sup>Sc</sup> conformation (orange square) on occasion, thereby becoming able to convert both mutant and wild-type PrP<sup>C</sup>

into PrP<sup>Sc</sup>. (C) Control mice (*Prp*<sup>+/+</sup>) that receive intracerebral inoculation of mouse-adapted prions invariably die within 6 months. However, their *Prp*<sup>-/-</sup> littermates are resistant to prion infection; following intracerebral inoculation, most live beyond a year with no prion pathology. Thus, endogenous PrP is essential for the effect of prion infection. (A, courtesy of Robert Higgins (left) and the CDC (right); B, adapted from Prusiner SB [1991] *Science* 252:1515–1522; C, adapted from Büeler H, Aguzzi A, Sailer A et al. [1993] *Cell* 73:1339–1347. With permission from Elsevier Inc.)

with massive neurodegeneration and neuronal death that creates sponge-like holes in the brain. For this reason, they are called spongiform encephalopathies (Figure 11-13A).

The nature of the infectious scrapie agent was heavily debated for several decades. A breakthrough came in the 1980s when reliable animal models were developed as bioassays for biochemical purification of the scrapie agent. Animals that had been inoculated with infected brain tissues, or with fractions of infected tissues resulting from biochemical purification, exhibited spongiform encephalopathy. Moreover, brain extracts from these newly infected animals were highly infectious when inoculated into new animals. The infectivity of these extracts was not disrupted by treatments that destroyed nucleic acids, suggesting that, unlike known infectious agents such as viruses and bacteria, the scrapie agent does not have a nucleic acid genome. This led to the **prion hypothesis**: the infectious scrapie agent is proteinaceous in nature. This was considered heretical: how could a protein be infectious without an associated genome for replication?

The infectious agent was subsequently identified as PrP<sup>Sc</sup>, a conformational variant of the cell-surface protein PrP. (The <sup>Sc</sup> superscript stands for scrapie.) It was later shown that a non-infectious conformation of PrP (termed PrP<sup>C</sup> for cellular PrP) is normally produced by most cell types. The presence of PrP<sup>Sc</sup> can cause PrP<sup>C</sup> to adopt the PrP<sup>Sc</sup> conformation (Figure 11-13B, top), which is a highly stable β-pleated sheet (recall that A<sup>β</sup> also adopts a β-pleated sheet conformation in AD). PrP<sup>Sc</sup> was proposed to propagate from cell to cell and even through the digestive system when diseased tissues are ingested by healthy animals or humans, catalyzing the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> along the way. The inherited Creutzfeldt-Jakob disease does not require infectious protein agents; instead, CJD results from mutations in the *Prp* gene that make PrP<sup>C</sup> more prone to adopt the PrP<sup>Sc</sup> conformation spontaneously (Figure 11-13B, bottom). Thus, the prion hypothesis unified the causes of scrapie, kuru, and CJD, which are collectively called **prion diseases** (Movie 11-1).

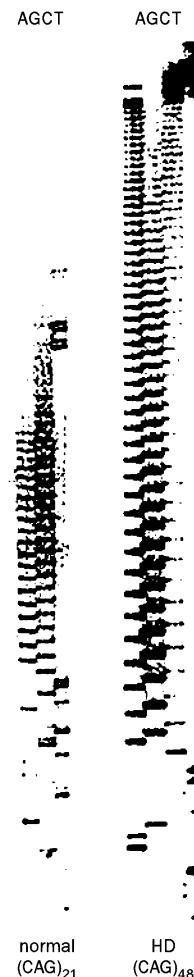
Strong support for the prion hypothesis came from *Prp* knockout mice, which were found to be resistant to PrP<sup>Sc</sup> infection, indicating that PrP<sup>Sc</sup> requires endogenous PrP<sup>C</sup> to cause disease (Figure 11-13C). Indeed, the concept of protein-induced protein conformational change and its propagation has subsequently been applied to other neurodegenerative diseases and to underlie widespread phenomena in the normal physiology of organisms ranging from yeast to humans.

### 11.9 Aggregation of misfolded proteins is associated with many neurodegenerative diseases

**Huntington's disease (HD)** is a dominantly inherited disease that usually strikes patients during midlife; it is named after George Huntington who first described the inheritance pattern in 1872. The earliest symptoms are often depression or mood swings, followed by abnormal movements, since the striatum is the most vulnerable brain area in HD. Patients later develop cognitive deficits. Death occurs 10–20 years after symptom onset. Genetically speaking, HD is one of the simplest neurological diseases, as it is caused by alterations in a single gene encoding a widely expressed protein named **huntingtin**. The cause of the disease is an expansion of a CAG trinucleotide repeat in the middle of the gene's coding sequence, resulting in an extended poly-glutamine (polyQ) repeat near the N-terminus of the huntingtin protein. (The nucleotide triplet cytidine–adenosine–guanosine codes for amino acid glutamine, abbreviated 'Q'.) Healthy individuals have 6 to 34 glutamine repeats, whereas HD patients have 36 to 121 repeats (Figure 11–14). Greater numbers of polyQ repeats correlate with earlier onset of HD symptoms.

Since the discovery of polyQ repeats in HD, expanded polyQ repeats in eight other proteins have been shown to cause neurodegenerative diseases, all of which are dominantly inherited, including six forms of **spinocerebellar ataxia** that affect motor functions (Table 11–1). In most cases, the polyQ repeat number that distinguishes healthy and diseased conditions is around 35. Because of this common feature, it had been hypothesized that the expanded polyQ repeats themselves could cause disease. Indeed, *in vivo* transgenic overexpression of expanded polyQ repeats alone is sufficient to cause degeneration of mouse and even *Drosophila* neurons. Proteins with long polyQ repeats form aggregates called inclusion bodies, which may be present in the nucleus, cytoplasm, or axons, depending on the specific protein affected. The host proteins in which these polyQ repeats reside also play essential roles for pathogenesis *in vivo*. Inclusion bodies of abnormally aggregated host protein isoforms that contain expanded polyQ repeats can recruit additional proteins that normally interact with the host proteins. As a result, the normal function of these interacting proteins is disrupted, accounting for the dominant nature of the expanded-polyQ disorders. The participation of specific host proteins in the pathogenesis of polyQ disorders also explains why different polyQ diseases have different symptoms, as each disrupts a different set of interacting proteins. In the case of HD, disruption of transcription, axonal transport, and mitochondrial function may all contribute to the eventual dysfunction and degeneration of neurons in the striatum, giving rise to characteristic uncontrolled movements (Huntington's chorea).

**Amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease)** is a rapidly progressing motor neuron disease that usually kills patients within

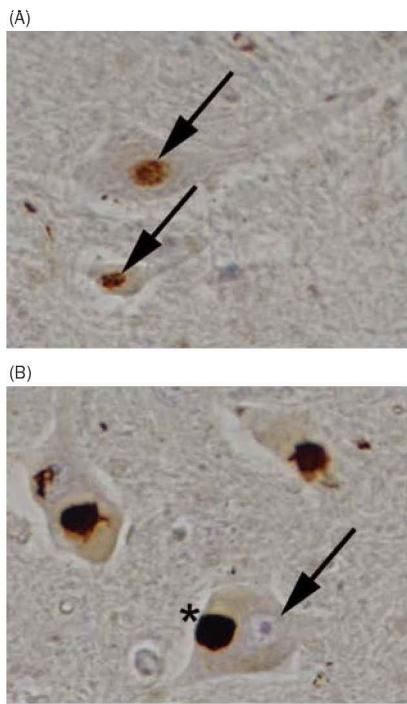


**Figure 11–14 Huntington's disease is caused by expanded poly-glutamine (polyQ) repeats.** DNA sequences of a healthy subject and a Huntington's disease (HD) patient, showing the expanded CAG repeats that result in mutant huntingtin protein having longer polyQ repeats. (From MacDonald ME, Ambrose CM, Duyao MP et al. [1993] *Cell* 72:971–983. With permission from Elsevier Inc.)

**Table 11–1: Diseases caused by poly-glutamine repeat**

Disease <sup>1</sup>	Gene product	Normal repeat length	Expanded repeat length
HD	huntingtin	6–34	36–121
SCA1	ataxin1	6–44	39–82
SCA2	ataxin2	15–24	32–200
SCA3	ataxin3	13–36	61–84
SCA6	voltage-gated Ca <sup>2+</sup> channel subunit	4–19	10–33
SCA7	ataxin7	4–35	37–306
SCA17	TATA-binding protein	25–42	47–63
SBMA	androgen receptor	9–36	38–62
DRPLA	atrophin	7–34	49–88

<sup>1</sup> Abbreviations: HD, Huntington's disease; SCA, spinocerebellar ataxia; SBMA, spinobulbar muscular atrophy; DRPLA, dentatorubral-pallidolysian atrophy. (After Orr & Zoghbi [2007] *Annu Rev Neurosci* 30:575.)



**Figure 11–15 Abnormal TDP-43 cytoplasmic inclusions in amyotrophic lateral sclerosis (ALS).** Spinal cord sections from an unaffected individual (A) and an ALS patient (B) immunostained for TDP-43 (brown). TDP-43 is normally localized to the nuclei of motor neurons (arrows in A), but in some motor neurons of ALS patients, TDP-43 is absent from the nucleus (arrow in B) and accumulates in cytoplasmic inclusion bodies (\*) in B. (Adapted from Figley MD & Gitler AD [2013] *Rare Diseases* 1: e24420. With permission from Landes Bioscience.)

a few years after symptoms emerge. Like AD, only a small fraction (~10%) of ALS cases are caused by dominantly inherited mutations in a handful of genes, whereas 90% are sporadic. Mutations that cause dominant familial ALS affect a range of different proteins, including SOD1 (*superoxide dismutase 1*, an enzyme), TDP-43 (*TAR DNA-binding protein of 43 kilodalton*, a DNA/RNA-binding protein), FUS (*fused in sarcoma*, another nucleic-acid-binding protein). The most common familial ALS is caused by an expansion of hexanucleotide repeats in the intron of a previously unstudied open reading frame, *C9orf72*. Each of the mutant proteins associated with ALS exhibits abnormal aggregation within affected motor neurons. In the case of TDP-43, for instance, normal TDP-43 is enriched in the nucleus, where it binds to DNA and RNA and regulates RNA processing; in ALS conditions, both mutant and wild-type TDP-43 accumulate in cytoplasmic inclusion bodies (Figure 11–15). This disrupts the normal function of TDP-43 in the nucleus, and at the same time recruits RNAs and proteins that interact with TDP-43 into the inclusion bodies. This may account for why TDP-43 mutations are dominant.

TDP-43-containing aggregates in cytoplasmic inclusions have been found in motor neurons not only from familial ALS with TDP-43 mutations, but also from the other familial ALS types such as those caused by the expansion of hexanucleotide repeats in *C9orf72*, as well as from the majority of sporadic ALS cases. *De novo* mutations in TDP-43 have also been found in some sporadic ALS cases. Thus, TDP-43 aggregation may represent a common pathological event in ALS that can have diverse causes.

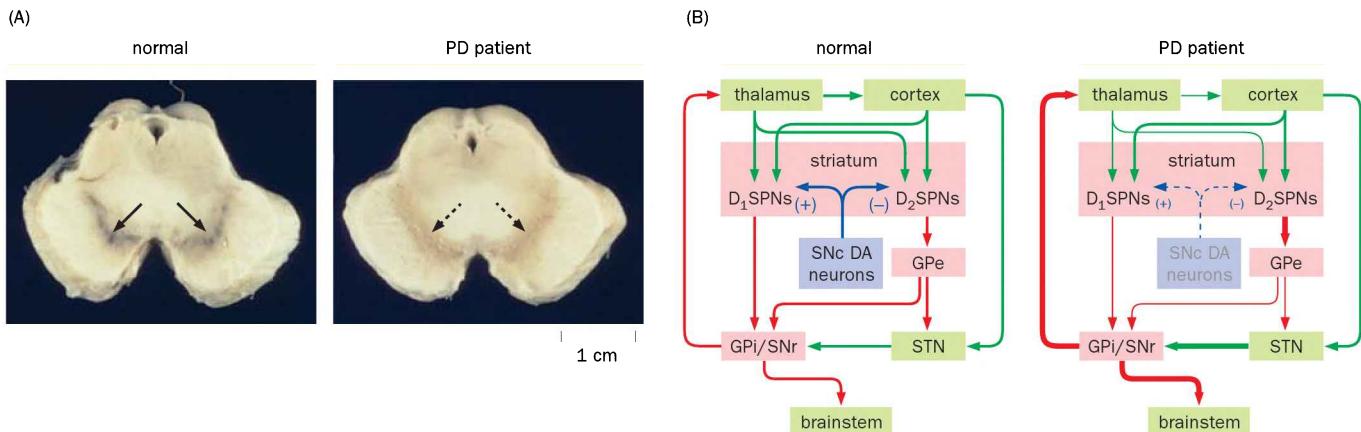
## 11.10 Parkinson's disease results from death of substantia nigra dopamine neurons

**Parkinson's disease (PD)**, first described by James Parkinson in 1817, is the second most common neurodegenerative disease after AD. PD primarily affects movement control. Characteristic PD symptoms include shaking, rigidity, slowness, and difficulty with walking. The primary cause for these symptoms is the death of dopamine neurons in a midbrain structure called the **substantia nigra** (Latin: black substance), which is named after the high levels of melanin pigment present in these dopamine neurons in healthy human subjects (Figure 11–16A). Indeed, PD studies have benefited from and contributed to our understanding of dopamine regulation of the **striatal** circuits that control movement.

As we learned in Section 8.9, striatal GABAergic spiny projection neurons (SPNs) control movement through two parallel pathways: a direct pathway that sends inhibitory signals to the globus pallidus internal segment (GPi) and the substantia nigra pars reticulata (SNr), and an indirect pathway that inhibits the globus pallidus external segment (GPe) and thereby relieves inhibition of the GPi/SNr (see Figure 8–22). Dopamine neurons in the substantia nigra pars compacta (SNc) project to the striatum and regulate the direct and indirect pathways in opposite directions: they facilitate the direct pathway through the dopamine D<sub>1</sub> receptor and inhibit the indirect pathway through the dopamine D<sub>2</sub> receptor. Thus, loss of SNc dopamine neurons in PD is predicted to cause hypo-activation of the direct pathway and hyper-activation of the indirect pathway. Both lead to excessive activation of basal ganglia output neurons in the GPi/SNr, which in turn causes excessive inhibition of their target neurons in the thalamus and brainstem and thereby inhibits movement (Figure 11–16B). This useful framework has prompted effective treatment such as deep brain stimulation that will be discussed in Section 11.13.

## 11.11 $\alpha$ -Synuclein aggregation and spread are prominent features of Parkinson's pathology

What causes dopamine neuron death in PD? In many ways PD parallels what we have learned about AD and ALS. Most PD cases are late-adult onset without a clear inheritance pattern (that is, they are sporadic), but a small fraction of PD cases are familial. In 1997, the first identified familial PD gene, inherited in an autosomal



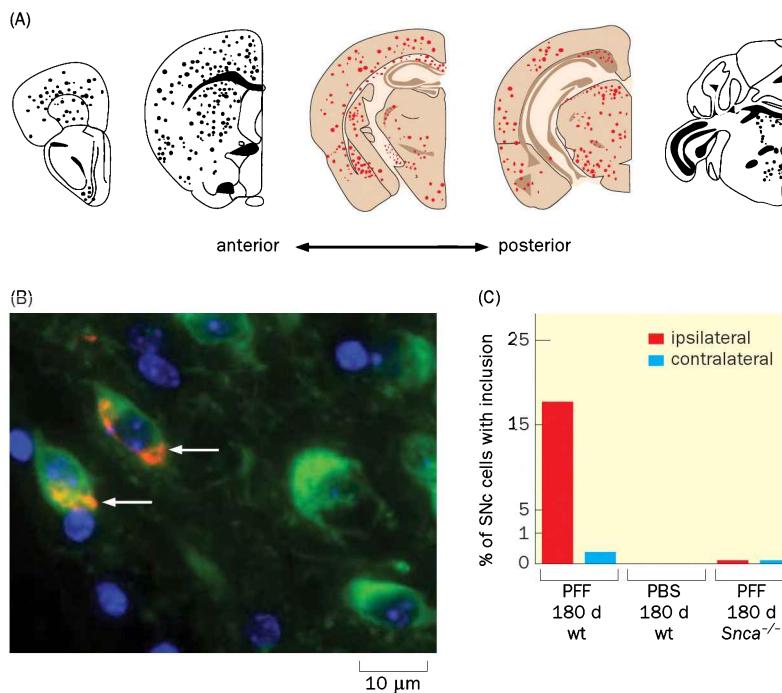
**Figure 11–16** Parkinson's disease is caused by loss of midbrain dopamine neurons, leading to dysregulation of basal ganglia circuits. **(A)** Coronal sections of postmortem midbrains from a normal subject (left) and a PD patient (right). Arrows point to the substantia nigra, enriched in pigmented dopamine neurons that appear dark in normal brains and are selectively lost in the PD brains (dotted arrows). **(B)** A comparison of simplified basal ganglia circuit models for normal and PD brains. Left, dopamine neurons in the substantia nigra pars compacta (SNC DA neurons) positively regulate synaptic transmission between cortical/thalamic input and spiny projection neurons expressing the D<sub>1</sub> dopamine receptor (D<sub>1</sub>SPNs), which project directly to the globus pallidus internal segment and the substantia nigra pars reticulata (GPI/SNr). At the same time, SNC DA neurons negatively regulate synaptic transmission between cortical/thalamic input and SPNs expressing the D<sub>2</sub> receptor (D<sub>2</sub>SPNs), which

project indirectly to GPI/SNr via the globus pallidus external segment (GPe) and the subthalamic nucleus (STN). Right, loss of SNC DA neurons in PD patients reduces the activity of D<sub>1</sub>SPNs and enhances the activity of D<sub>2</sub>SPNs, both of which contribute to hyperactivation of GABAergic output neurons in the GPI/SNr and excessive inhibition of the target neurons of GPI/SNr. Red, inhibitory projection; green, excitatory projection; blue, DA projection. Thicker or thinner arrows in the PD diagram, respectively, indicate an increase or a decrease of pathway strengths in PD compared with normal. As will be discussed in Section 11.13, lesion or deep brain stimulation of STN can alleviate PD symptoms by reducing the hyperactivation of GPI/SNr. (A, courtesy of the Duke University School of Medicine; B, see Bergman H, Wichmann T & DeLong MR [1990] *Science* 249:1436–1438 and Limousin P, Krack P, Pollak P et al. [1998] *N Engl J Med* 339:1105–1111.)

dominant fashion, was found to encode a presynaptic protein called  $\alpha$ -synuclein. Soon afterwards,  $\alpha$ -synuclein was found to be the major component of **Lewy bodies**, intracellular inclusions that have been a defining feature of PD pathology since F. H. Lewy first described them in 1912 (although not all forms of PD exhibit Lewy body pathology). Familial PD mutations in  $\alpha$ -synuclein (single amino acid changes) promote aggregation of  $\alpha$ -synuclein *in vitro*. Indeed, increasing the copy number of the wild-type  $\alpha$ -synuclein gene in humans is sufficient to cause PD with Lewy body pathology. Thus, PD can be caused by overproduction of wild-type  $\alpha$ -synuclein or by production of mutant  $\alpha$ -synuclein prone to aggregation.

Postmortem analyses of brains from patients who died at different stages of PD suggest that the distribution of Lewy body pathology follows a stereotyped spatiotemporal sequence: neurons with  $\alpha$ -synuclein pathology are found mostly in the brainstem of early-stage PD patients, but are more widespread in the forebrains of patients with more advanced PD. In fact, substantia nigra dopamine neurons are not the first to exhibit  $\alpha$ -synuclein pathology in this sequence, although they might be the most vulnerable. This suggests that  $\alpha$ -synuclein aggregates may spread from neuron to neuron. This hypothesis was spurred by findings from a PD patient whose brain received a transplant of fetal dopamine neurons, a treatment strategy that we will discuss in Section 11.13 below. When the patient died 14 years after surgery, the transplanted fetal neurons contained  $\alpha$ -synuclein-enriched Lewy body-like structures. Additional support for this hypothesis came from more recent studies in mice: focal injection into wild-type mice of  $\alpha$ -synuclein fibrils that were preformed *in vitro* caused the spread of  $\alpha$ -synuclein pathology across the brain, including to SNC dopamine neurons (Figure 11–17). Remarkably, injecting  $\alpha$ -synuclein fibrils into  $\alpha$ -synuclein knockout mice did not cause spread of  $\alpha$ -synuclein pathology, suggesting that recruitment of the endogenous  $\alpha$ -synuclein is essential for the pathology, as in the case of prion diseases (see Figure 11–13C). These studies, which together suggest that PD may involve cell-to-cell spread of pathogenic proteins, raise important and as-yet-unresolved questions regarding the mechanisms by which such spread might occur for cytoplasmic proteins such as  $\alpha$ -synuclein.

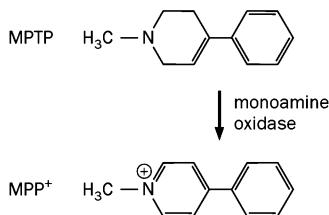
**Figure 11–17 Cell-to-cell spread of  $\alpha$ -synuclein pathology.** (A) Serial coronal brain maps of a wild-type mouse 180 days after injection of preformed  $\alpha$ -synuclein fibrils (PFF) into the dorsal striatum revealed the development of  $\alpha$ -synuclein pathology across the brain. Cells with  $\alpha$ -synuclein aggregates are shown in red; the injection site is marked by a light red circle in the 2nd map from left. (B) A high-magnification image of the substantia nigra, showing two dopamine neurons with aggregated  $\alpha$ -synuclein in Lewy body-like inclusions (arrows). Dopamine neurons are visualized in green by immunostaining against tyrosine hydroxylase, a marker for dopamine neurons.  $\alpha$ -synuclein is visualized in red using an antibody that preferentially stains aggregated  $\alpha$ -synuclein. (C) Substantia nigra pars compacta (SNC) cells that exhibited  $\alpha$ -synuclein pathology were located primarily on the ipsilateral side of the injection. Injecting phosphate-buffered saline (PBS) into wild-type (wt) or injecting PFF into  $\alpha$ -synuclein knockout ( $Snc$ a<sup>-/-</sup>) mice did not cause  $\alpha$ -synuclein pathology. (Adapted from Luk KC, Kehm V, Carroll J et al. [2012] Science 338:949–953. With permission from AAAS.)



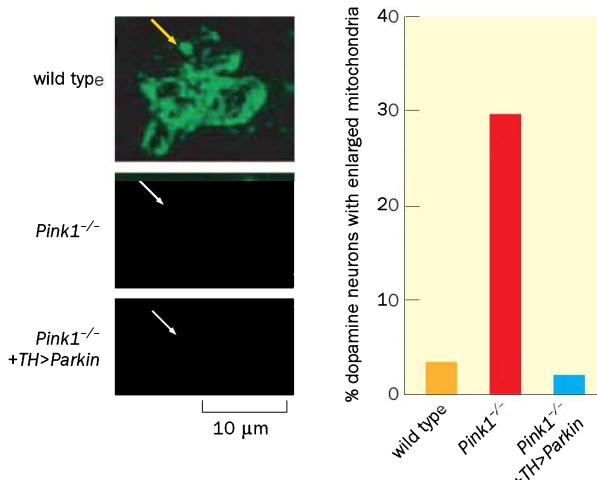
## 11.12 Mitochondrial dysfunction is central to the pathogenesis of Parkinson's disease

Prior to molecular-genetic studies of familial PD in the 1990s, Parkinson's disease was thought to be environmentally induced. A striking example came in the early 1980s, when MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a contaminant in the chemical synthesis of the opioid-like drug MPPP (1-methyl-4-phenyl-propionoxyphiperidine), caused PD-like symptoms in a number of drug users. Subsequent animal and biochemical studies confirmed MPTP toxicity and established the mechanisms: MPTP freely crosses the BBB and is converted to MPP<sup>+</sup> (1-methyl-4-phenylpyridinium) by **monoamine oxidase**, which normally oxidizes monoamine neurotransmitters leading to their degradation. MPP<sup>+</sup> is selectively accumulated via the plasma membrane dopamine transporter in dopamine neurons, where it potently inhibits mitochondrial complex I function, thus selectively killing the dopamine neurons (Figure 11-18). Biochemical assays of postmortem tissue from sporadic PD patients have also indicated complex I deficiency, thereby implicating mitochondrial defects in dopamine neurons as a cause for PD.

While the majority of PD cases are sporadic, human genetic studies since the 1990s have revealed mutations in multiple genes that are associated with familial PD. In addition to the dominant  $\alpha$ -synuclein mutations discussed above, familial PD can also be caused by autosomal recessive mutations, that is, disease occurs only if mutations in both alleles lead to loss of gene function (see Box 11-3 for more details). PD-linked recessive mutations have been identified in two evolutionarily conserved genes: *Pink1*, which encodes a mitochondrion-associated kinase, and *Parkin*, which encodes an enzyme in the ubiquitin-proteasome system for protein degradation. The relationship between *Pink1* and *Parkin* was first revealed by studying their homologs in the fruit fly *Drosophila*. *Pink1* mutant flies could not fly, died young, and exhibited degeneration of muscles and dopamine neurons with abnormal mitochondrial morphology and function (Figure 11-19). *Parkin* mutant flies exhibited similar defects. Notably, defects in *Pink1* mutants were rescued by overexpression of *Parkin*, but defects in *Parkin* mutants were unaffected by overexpression of *Pink1*. These data suggest that *Pink1* and *Parkin* act in a common pathway to regulate mitochondrial function, with *Parkin* acting downstream of *Pink1*. This relationship has subsequently been confirmed in mammalian systems. Further studies in *Drosophila* and mammals suggest that



**Figure 11–18 PD-like symptoms caused by a chemical toxin.** After passing through the blood-brain barrier, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is converted to MPP<sup>+</sup> (1-methyl-4-phenylpyridinium) by monoamine oxidase. MPP<sup>+</sup> selectively accumulates in dopamine neurons through the action of the plasma membrane dopamine transporter, inhibits mitochondrial function, and kills the dopamine neurons.



**Figure 11–19** **Pink1 and Parkin act in a common pathway in mitochondrial function.** Clusters of *Drosophila* dopamine neurons visualized by expressing a mitochondrion-targeted GFP transgene under the control of the tyrosine hydroxylase (TH) promoter. In each panel, an arrow points to an individual mitochondrion. In the *Pink1* mutant (*Pink1*<sup>-/-</sup>), mitochondria were abnormally enlarged compared with wild type. This mitochondrial defect in *Pink1* mutant dopamine neurons was rescued by overexpression of Parkin, driven by the TH promoter (*TH>Parkin*). Results are quantified at right. These data suggest that *Pink1* acts upstream of Parkin in the same pathway. (Adapted from Park J, Lee SB, Lee S et al. [2006] *Nature* 441:1157–1161. With permission from Macmillan Publishers Ltd. See also Clark IE, Dodson MW, Jiang C et al. [2006] *Nature* 441:1162–1166 and Yang Y, Gehrke S, Imai Y et al. [2006] *Proc Natl Acad Sci USA* 103:10793–10798.)

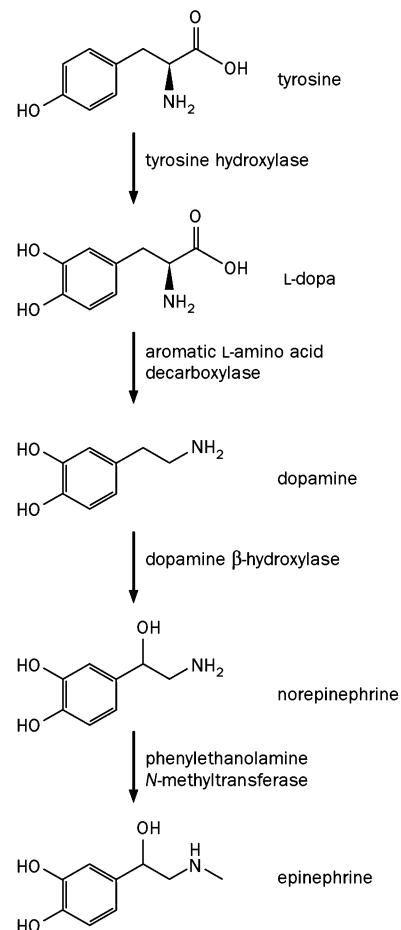
the *Pink1*/Parkin pathway regulates multiple aspects of mitochondrial dynamics, including mitochondrial fission, fusion, and movement along microtubules, as well as removal of damaged mitochondria. Studies of *Pink1* and Parkin reinforced a central role for mitochondrial dysfunction in Parkinson's disease pathogenesis.

In addition to  $\alpha$ -synuclein, Parkin, and *Pink1*, mutations in several other genes have been identified in familial PD, together accounting for 10–15% of PD cases. The relationships among these different familial PD genes are the focus of intensive investigation. Given our discussions above implicating  $\alpha$ -synuclein and mitochondrial dysfunction in both familial and sporadic PD, some of the outstanding questions include: Does  $\alpha$ -synuclein aggregation cause mitochondrial dysfunction? Does mitochondrial dysfunction cause  $\alpha$ -synuclein aggregation? Do these two processes act synergistically? How might they eventually lead to the death of dopamine neurons?

### 11.13 Treating Parkinson's disease: L-dopa, deep brain stimulation, and cell-replacement therapy

Despite the molecular complexity of Parkinson's disease, the selective loss of substantia nigra dopamine neurons as a common outcome suggested a possible treatment strategy: boosting dopamine levels. Starting in the 1960s, the successful use of **L-dopa** to treat PD symptoms has inspired generations of researchers and clinicians to find better treatments for PD and other devastating brain disorders.

Dopamine is synthesized from the amino acid **L-tyrosine** in two enzymatic steps (Figure 11–20). The first step, catalyzed by **tyrosine hydroxylase**, converts L-tyrosine to L-dopa, which is then acted upon by aromatic L-amino acid decarboxylase to yield dopamine. Tyrosine hydroxylase is the rate-limiting enzyme in the biosynthesis of **catecholamines**, a class of molecules that includes the neurotransmitters dopamine, norepinephrine, and epinephrine (Figure 11–20; see also Section 3.11; epinephrine also acts as a hormone, see Section 3.19). First known as an intermediate precursor for norepinephrine and epinephrine, dopamine was not recognized as a neurotransmitter until the late 1950s. Shortly thereafter, it was discovered that dopamine levels were markedly reduced in the striatum of post-mortem PD brains, suggesting a selective defect of the dopamine system in PD.



**Figure 11–20** **Biosynthetic pathway of catecholamines.** Tyrosine hydroxylase is the rate-limiting enzyme in the pathway. Whereas dopamine does not cross the blood-brain barrier, L-dopa does. Injection of L-dopa has been used to boost the synthesis of dopamine by the remaining dopamine neurons as a therapy for dopamine deficiency in Parkinson's disease.

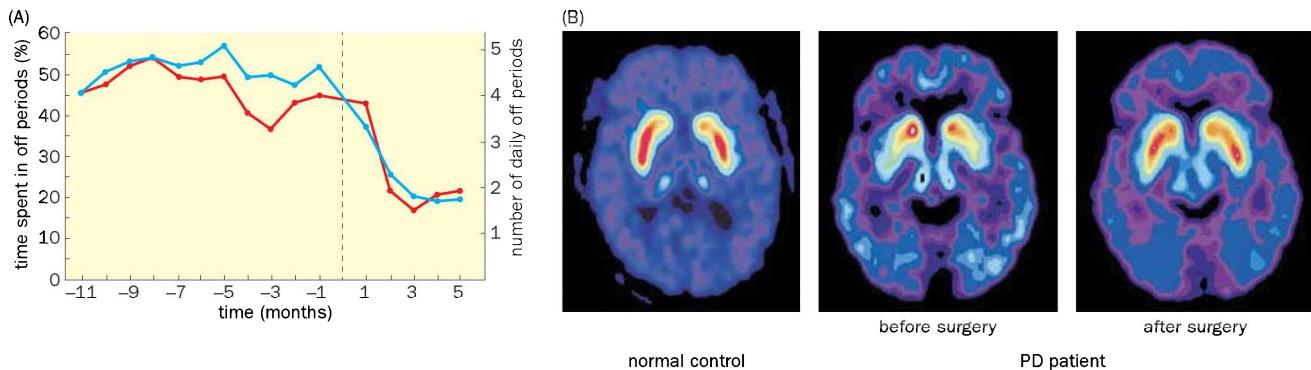
In Parkinson's disease, loss of dopamine neurons causes a decline in dopamine release. Thus, one strategy to increase dopamine release by the remaining dopamine neurons is to bypass the rate-limiting step of dopamine synthesis. It was found that dopamine cannot effectively cross the blood-brain barrier, but its immediate precursor L-dopa can. Through trial and error to optimize the therapeutic dose and reduce side effects, a treatment protocol was developed in the 1960s that is still widely used today: L-dopa administration drastically improves movement control in most early-stage PD patients.

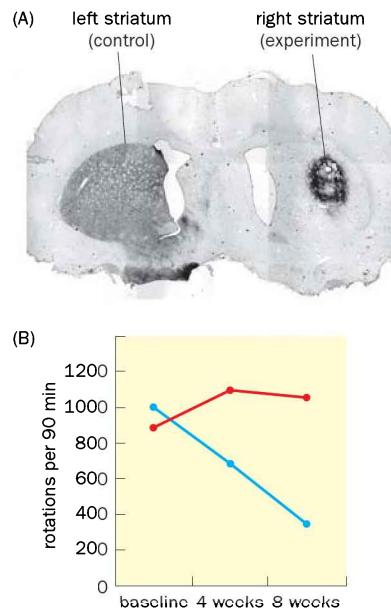
Unfortunately, L-dopa is only effective at ameliorating PD symptoms for a few years. The eventual decline in L-dopa efficacy is likely because it must be converted to dopamine by the remaining dopamine neurons, whose progressive death is not halted by the treatment. An alternative treatment strategy is **deep brain stimulation (DBS)**, which is designed to compensate for the alteration of circuit dynamics in PD due to the loss of dopamine modulation and excessive output of GPi/SNr (see Figure 11-16B). For this treatment, electrodes are surgically implanted to stimulate neurons and axons in specific nuclei. The exact mechanisms by which deep brain stimulation affects the basal ganglia circuitry in the PD brain are likely to be complex. Excitatory and inhibitory neurons as well as axons-in-passage are all stimulated simultaneously, and firing of target neurons can be affected in opposite directions depending on stimulation frequency and distance to the electrode. Clinically, deep brain stimulation of neurons and axon fibers in the STN or GPi can alleviate movement-related symptoms in late-stage PD patients when L-dopa treatment becomes less effective. In the case of STN stimulation, DBS likely causes inhibition of STN output, thus counteracting the increased excitatory input and decreased inhibitory input to GPi/SNr in PD patients (see Figure 11-16B); indeed, earlier studies had shown that STN lesion or high-frequency stimulation could alleviate PD-like symptoms in monkeys treated with MPTP, providing an important basis for human clinical trials. The success of DBS in treating PD has inspired its clinical trials for treating a number of psychiatric disorders such as depression and obsessive-compulsive disorders, even though there is far less knowledge about the underlying circuitry in these psychiatric disorders compared with PD.

A radically different strategy for PD treatment is to replace dying dopamine neurons with new dopamine neurons. This **cell-replacement therapy** has several requirements. First, a reliable source of dopamine neurons must be identified. Additionally, transplanted dopamine neurons must survive in the host, have access to their targets, and release appropriate levels of dopamine. Studies in animal PD models have shown that fetal tissues derived from midbrain areas that contain dopamine neurons can survive after being grafted into the host striatum. (To bypass the requirement for correct axonal projections to targets, dopamine neurons are usually transplanted directly to the striatum.) Remarkably, these grafted cells can release dopamine and improve motor control. Subsequently, small clinical trials in humans have reported improvement of clinical symptoms and long-term increase in dopamine release after transplantation (Figure 11-21).

There are several limitations to using human fetal tissue in cell-replacement therapy. Large numbers of dopamine neurons are required for effective therapy,

**Figure 11-21 Embryonic dopamine neuron transplantation as a treatment of Parkinson's disease. (A)** PD patients fluctuate between 'off' periods when motor function is severely impaired, and 'on' periods when motor function is relatively normal. This figure shows the self-report of a PD patient describing the fraction of awake time spent in off periods (red trace; scale on left axis) and the number of off periods per day (blue trace; scale on right axis) in the months before and after receiving transplanted dopamine neurons derived from fetal tissue. (Time of transplantation is indicated by the dashed line.) The duration and frequency of off periods are markedly reduced after transplantation. **(B)** Positron emission tomography scan of radioactive fluorodopa uptake into dopamine neuron terminals in the striatum of a normal subject (left) and a PD patient before (middle) and 12 months after (right) transplantation of fetal dopamine neurons. Before surgery, radioactivity (shown in red) in the brain of the PD patient was restricted to the caudate (medial striatum); after the bilateral transplantation, radioactivity was extended to the putamen (lateral striatum), more similar to the distribution in the normal control. (A, adapted from Lindvall O, Brundin P, Widner H et al. [1990] *Science* 247:574–577; B, from Freed CR, Greene PE, Breeze RE et al. [2001] *N Engl J Med* 344:710–719. With permission from the Massachusetts Medical Society.)





**Figure 11-22 Transplantation of dopamine neurons derived from induced pluripotent stem (iPS) cells can improve motor function in animal models.**

(A) Coronal section of a rat brain at the level of the striatum. The right hemisphere lacks endogenous dopamine neurons as a result of chemical ablation, and serves as a host for transplantation of iPS-derived dopamine neurons. The left hemisphere serves as a control. Tyrosine hydroxylase staining (dark signal) shows that transplanted iPS-derived dopamine neurons survive and produce tyrosine hydroxylase 4 weeks after transplantation.

(B) Application of a drug that enhances dopamine action induces rotation in animals in which dopamine neurons were ablated in one hemisphere (red trace); this is caused by asymmetric activation of striatal circuits in two hemispheres. This defect was ameliorated after transplanting iPS-derived dopamine neurons (blue trace). (Adapted from Wernig M, Zhao JP, Pruszak J et al. [2008] Proc Natl Acad Sci USA 105:5856–5861. Copyright National Academy of Sciences, USA.)

and these neurons need to be free of other cell types. Contamination by additional cell types is a major side effect of fetal transplantation that has prevented further clinical development. Moreover, optimal survival of transplanted tissues from different individuals requires patients to take immunosuppressant drugs, which can compromise their immune system and increase susceptibility to infection.

Recent progress in stem cell research has provided an exciting potential means by which large numbers of dopamine neurons might be produced *in vitro*, in some cases by using somatic cells derived from the patients themselves and thereby avoiding the problem of tissue rejection. For example, embryonic stem cells and induced pluripotent stem cells derived from fibroblasts (see Box 11-2) can differentiate into tyrosine hydroxylase-positive dopamine neurons *in vitro*. These induced dopamine neurons can survive after being grafted into host striatum that lacks endogenous dopamine neuron projections and can improve motor function of the recipient animal (Figure 11-22). Although much work is needed to assess the safety, reliability, and robustness of this approach, cell-replacement therapy may offer a promising avenue for treating Parkinson's disease in the future.

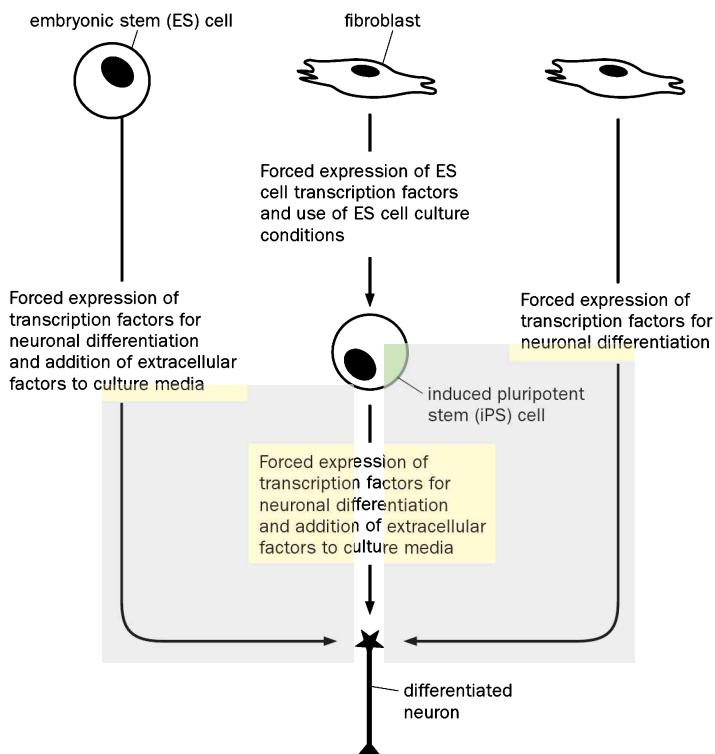
#### Box 11-2: Producing neurons from embryonic stem cells, induced pluripotent cells, and fibroblasts

As we learned in Section 7.1, different neuronal types are produced from progenitors that are located in defined parts of the neuroepithelia and are specified by signals that pattern the nervous system. The ectodermal progenitors from which neural progenitors originate derive from **pluripotent cells**, which can give rise to all cell types in the embryo. These pluripotent cells, called **embryonic stem (ES) cells**, can be cultured *in vitro*. By overexpressing specific genes and culturing ES cells with specific growth factors under conditions mimicking development *in vivo*, researchers can coax ES cells to differentiate in culture into specific types of neurons, including dopamine neurons that express tyrosine hydroxylase and release dopamine (Figure 11-23, left). These *in vitro* differentiated neurons can in principle be used for cell-replacement therapies. The drawbacks associated with ES cell-based therapy include ethical difficulties

related to the use of human embryos and potential tissue rejection after transplantation into patients.

A major breakthrough came in 2006, when researchers reported the ability to convert embryonic and adult fibroblasts into **induced pluripotent stem (iPS) cells** by forced expression of four transcription factors involved in maintaining the pluripotency of ES cells (Figure 11-23, middle top). These iPS cells resemble ES cells in many ways—they can be induced to differentiate into many different cell types *in vitro*, and they can support germ line transmission after transplantation into blastocysts just like ES cells (see Section 13.7). Fibroblast-derived iPS cells can also be induced to re-differentiate into dopamine neurons (Figure 11-23, middle bottom) and, when transplanted into the striatum, can ameliorate Parkinson-like symptoms in rodent models

(Continued)

**Box 11–2: Producing neurons from embryonic stem cells, induced pluripotent cells, and fibroblasts**


**Figure 11–23 Multiple ways of producing differentiated neurons *In vitro*.** Left, embryonic stem (ES) cells that are forced to express specific transgenes can be induced to become specific neuronal types by providing required extracellular factors and defined cell-culture conditions. For example, dopamine neurons can be produced from ES cells that first overexpress a transcription factor essential for dopamine neuron differentiation and subsequently undergo a multi-stage culture protocol with specific growth factors (see Kim JH, Auerbach JM, Rodriguez-Gomez JA et al. [2002] *Nature* 418:50–56). Middle, fibroblasts can be de-differentiated into iPS cells by forced expression of a cocktail of transcription factors normally expressed in ES cells (see Takahashi K & Yamanaka S [2006]

*Cell* 126:663–676). The resulting iPS cells can then be induced to re-differentiate into dopamine neurons following a protocol similar to the ES cell → dopamine neuron path shown on the left (see Wernig M, Zhao JP, Pruszak J et al. [2008] *Proc Natl Acad Sci USA* 105:5856–5861). Right, fibroblasts can be converted directly to neurons by transfecting a cocktail of neuronal differentiation factors (see Vierbuchen T, Ostermeier A, Pang ZP et al. [2010] *Nature* 463:1035–1041), including dopamine neurons (see Caiazzo M, Dell’Anno MT, Dvoretzka E et al. [2011] *Nature* 476:224–227 and Pfisterer U, Kirkeby A, Torper O et al. [2011] *Proc Natl Acad Sci USA* 108:10343–10348).

(see Figure 11–22). In principle, if patients carry familial PD mutations, such mutations can also be corrected at the iPS-cell stage by replacing the mutant gene with a wild-type copy through homologous recombination (see Section 13.7) before expansion and re-differentiation.

More recently, researchers described methods to trans-differentiate fibroblasts directly into neurons by over-expressing a set of transcription factors known to be important for neuronal differentiation, bypassing the de-differentiation and re-differentiation procedures with iPS cells as an intermediate (Figure 11–23, right). Transcription factor cocktails have been identified that can induce fibroblasts from healthy subjects and PD patients to differentiate into specific types of neurons, including dopamine neurons. These methods pave the way toward the eventual goal of using patient-derived cells to produce neurons for cell-replacement therapy, which would avoid the problem of transplant rejection. Compared to these direct induction procedures, iPS-cell-based strategies benefit from easy proliferation of iPS cells *in vitro* and can therefore generate

large cell populations for cell-replacement therapy. On the other hand, some iPS cells may have the potential to produce tumors after transplantation due to their pluripotency and proliferation capacity. Researchers are optimizing these procedures for production efficiency, effectiveness, and safety.

Neurons produced from fibroblasts *in vitro*, whether through direct induction or via iPS cells, have wide applications in disease research far beyond cell-replacement therapies. For example, neurons derived from patients with specific brain disorders can be examined, in culture or *in vivo* after transplantation into animal models, for potential defects in morphological development, electrophysiological characteristics, synaptic transmission, and synaptic plasticity. Once specific phenotypes have been identified, researchers can use *in vitro* assays, including high-throughput drug screens, to identify strategies for correcting the phenotypes. Drugs that improve the phenotypes in culture can serve as prime candidates for testing in animal models and for clinical trials (see Box 11–1).

### 11.14 The various neurodegenerative diseases have common themes and exhibit unique properties

Despite being associated with distinct proteins and disease symptoms, a broad suite of neurodegenerative diseases—including AD, prion diseases, polyQ diseases, ALS, and most forms of PD—share in common the abnormal aggregation of misfolded proteins or cleaved fragments. Familial mutations tend to facilitate such aggregation. These protein aggregates, or their intermediates, are either toxic by themselves or alter the localization or function of their normal interacting partners, thus disrupting protein homeostasis and causing toxic gain-of-function phenotypes. In some cases, loss-of-function of the misfolded protein may further exacerbate the gain-of-function effects. Much is to be learned about how misfolding occurs, what structure(s) define the toxic species, and which downstream effects are specific to each disease.

In the case of the prion, PrP in the pathogenic conformation ( $\text{PrP}^{\text{Sc}}$ ) serves as a seed to convert normal  $\text{PrP}^{\text{C}}$  into additional pathogenic  $\text{PrP}^{\text{Sc}}$ , causing the disease to spread and become infectious. Although no other neurodegenerative disorders are known to be infectious, the concept of seeding-induced conformational changes that result in misfolded protein aggregates and cell-to-cell spread of misfolded proteins may apply to other diseases such as PD (Section 11.11) and contribute to their progression. Much remains to be investigated about the mechanisms of cell-to-cell spread and the factors that promote or inhibit such spread.

One property that distinguishes different diseases is the neuronal types that degenerate in each disease. AD causes widespread degeneration encompassing many types of neurons in the cerebral cortex, hippocampus, and amygdala. HD primarily causes striatal neuron degeneration. ALS preferentially affects motor neurons. PD results from degeneration of dopamine neurons in the substantia nigra, at least initially. Many of the causal genes that are mutated in familial forms of the diseases, such as APP and presenilins in AD, PrP in prion diseases, huntingtin in HD, SOD1 and TDP-43 in ALS, and  $\alpha$ -synuclein in PD, are ubiquitously expressed. It remains largely a mystery how mutations in these widely expressed genes primarily damage specific neuronal cell types, thus causing specific diseases. One contributing factor could be that each disease is caused by aggregated proteins interacting with and interrupting the function of a unique set of partners that are preferentially required in specific neuronal types.

Sadly, the development of effective therapies for neurodegenerative diseases has thus far been limited to early-stage PD. However, approaches being pioneered in PD research and intense ongoing research discussed in previous sections may ultimately yield successful treatments for a broader range of neurodegenerative diseases.

---

## PSYCHIATRIC DISORDERS

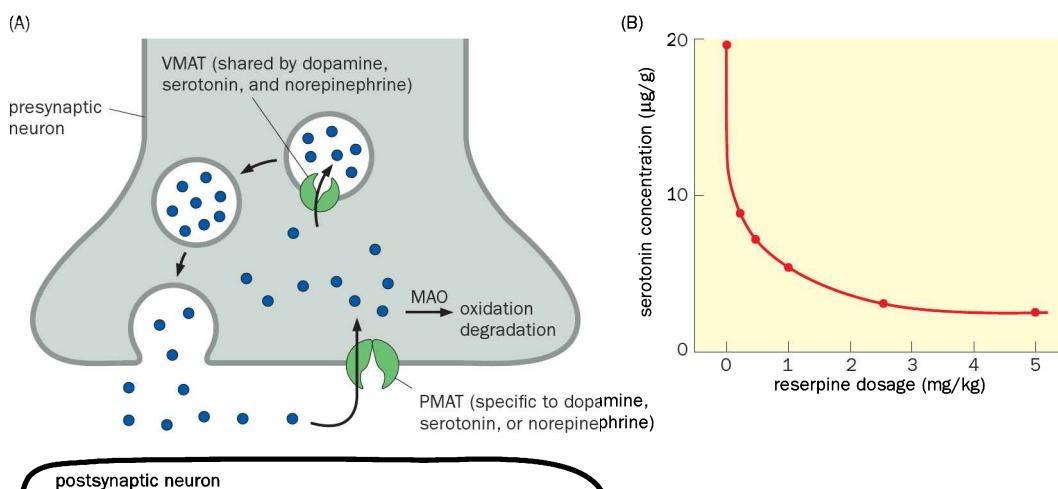
Disorders of the nervous system have traditionally been divided into neurological and psychiatric. Neurological disorders are usually associated with structural, biochemical, or physiological symptoms, as in the neurodegenerative diseases we studied. By contrast, psychiatric disorders have historically included those that affect the mind—how we perceive, feel, think, and act—without established physical basis. As the brain and the mind are inseparable and as we gain more understanding about both, the distinctions between neurology and psychiatry become increasingly blurred and somewhat arbitrary. However, traditionally defined psychiatric and neurological disorders have historically been studied using different approaches. For example, studies of neurodegenerative diseases, traditionally considered neurological disorders, start with pathology. Scientists try to understand the mechanisms underlying pathological changes, in the hope of designing treatments to interfere with the pathogenic process. By contrast, most therapeutic drugs for psychiatric disorders have been discovered through fortunate chance. By studying how such drugs act, researchers attempt to uncover the mechanisms that may underlie these disorders. Below, we illustrate this path of discovery for four classes of psychiatric disorders: schizophrenia, mood disorders, anxiety disorders, and addiction.

### 11.15 Schizophrenia can be partially alleviated by drugs that interfere with dopamine function

**Schizophrenia** is among the most costly psychiatric disorders, with a lifetime prevalence of 1% in the general population. The onset is usually during adolescence or early adulthood, and patients are typically affected for the rest of their lives. The most typical symptoms include hallucinations and delusions, often leading to paranoia. These psychotic disturbances (**psychosis**) are the symptoms most commonly associated with the disorder and are characterized as positive symptoms, referring to their presence in patients but not in healthy people. Schizophrenia is also associated with a set of negative symptoms including social withdrawal and lack of motivation, as well as cognitive impairment in memory, attention, and executive functions. The negative symptoms and cognitive impairments are usually more disabling to patients' quality of life because there is currently no treatment.

Before the 1950s, there was no treatment for schizophrenia other than confining patients to mental asylums, often for decades. Then came the fortuitous discovery of the first antipsychotic drugs: chlorpromazine and reserpine. **Chlorpromazine** was chemically synthesized as a potential anesthetic, and **reserpine** is the active ingredient purified from the snakeroot plant for treating hypertension. Both drugs were found to alleviate positive symptoms of schizophrenia patients, albeit with similar side effects, namely motor control deficits similar to those of Parkinson's disease.

Subsequent studies showed that reserpine acts by interfering with the metabolism of all three monoamine neurotransmitters: dopamine, norepinephrine, and serotonin (Figure 11–24A; see also Section 3.11 and Figure 3–16). After release into the synaptic cleft, these monoamine neurotransmitters are taken back into the presynaptic terminal by specific **plasma membrane monoamine transporters** (PMATs, see Section 3.8 and Section 11.16) of neurotransmitters. A **vesicular monoamine transporter** (VMAT) shared by all three monoamines then transports these neurotransmitters from the cytosol into synaptic vesicles for the future rounds of synaptic transmission. Reserpine acts as an inhibitor of VMAT, thus blocking monoamine neurotransmitter recycling. Monoamine transmitters retained in the cytosol after VMAT blockade are inactivated by the



**Figure 11–24 Metabolism of monoamine neurotransmitters in the presynaptic terminal and the effect of reserpine. (A)** Schematic drawing of monoamine neurotransmitter metabolism in the presynaptic terminal. After release to a synaptic cleft, each monoamine neurotransmitter is taken up by its specific plasma membrane monoamine transporter (PMAT) to the presynaptic cytosol. There, neurotransmitter molecules are either taken up by the vesicular monoamine transporter (VMAT) into synaptic vesicles for

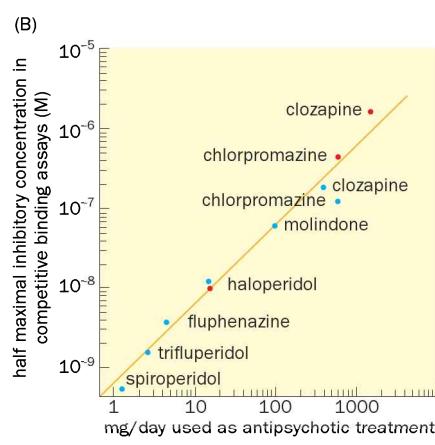
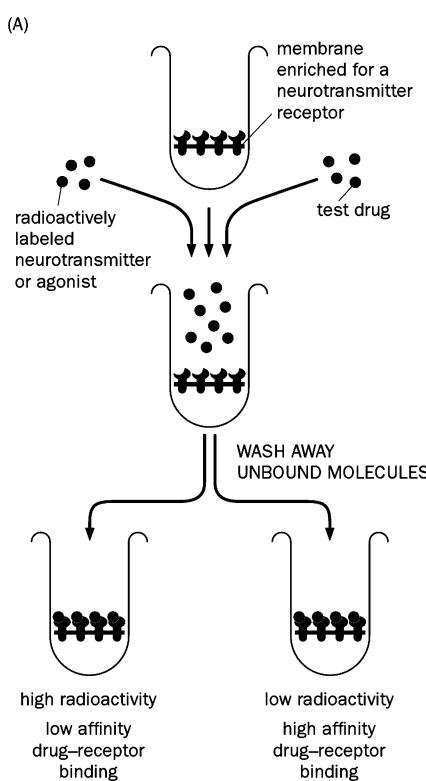
reuse, or oxidized by the monoamine oxidase (MAO) for degradation.

**(B)** Reserpine's effect was first discovered based on its ability to deplete serotonin levels. In this experiment, the level of serotonin released from the intestine (where serotonin is used as a major neurotransmitter in the enteric nervous system) was found to decrease progressively after rabbits received increasing amount of reserpine. Reserpine exerts this effect by inhibiting VMAT. (Adapted from Pletscher A, Shore PA & Brodie BB [1955] *Science* 122:374–375.)

**monoamine oxidase** enzyme, which removes the amine group and thereby allows its products to be further metabolized (Figure 11–24A). By inhibiting VMAT, reserpine effectively depletes the levels of dopamine, norepinephrine, and serotonin (Figure 11–24B). Indeed, depletion of norepinephrine in the sympathetic neurons that promote cardiac muscle contraction (see Section 8.12) underlies reserpine's effect on hypertension. Its ability to deplete dopamine also explains why reserpine treatment causes Parkinson-like symptoms.

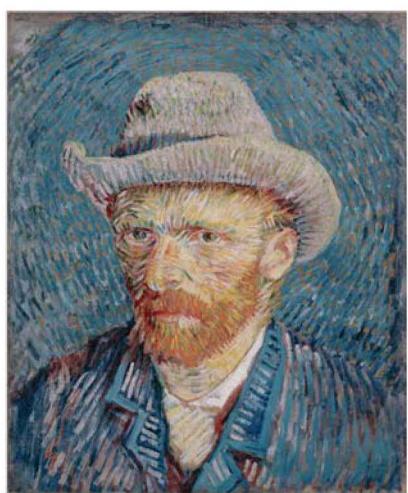
Chlorpromazine acts differently. In the 1970s, competitive binding assays were established to test drug effectiveness: a given drug would compete against a particular neurotransmitter for binding to brain membrane extracts presumed to contain high concentrations of neurotransmitter receptors (Figure 11–25A). When chlorpromazine and other antipsychotic drugs were tested in such competitive binding assays against different neurotransmitter receptors, it was found that a drug's affinity for dopamine receptors (but not for serotonin or adrenergic receptors) correlated well with its effectiveness as an antipsychotic (Figure 11–25B). Subsequent work suggested that the blockade of a specific dopamine receptor subtype, the D<sub>2</sub> dopamine receptor, correlated best with antipsychotic effects. Unfortunately, blocking the D<sub>2</sub> dopamine receptor also leads to Parkinson-like symptoms. New generations of antipsychotic drugs with milder side effects on movement control have been developed, although all of these have significant metabolic side effects.

The fact that reserpine and chlorpromazine both reduce dopamine function through distinct mechanisms suggested that dopamine system abnormalities contribute to the positive symptoms of schizophrenia. This hypothesis was further supported by cases of drug-induced psychosis. Two commonly abused drugs, cocaine and amphetamine, are known to induce delusional paranoia similar to the positive symptoms of schizophrenia. These **psychostimulants** produce transient euphoria and suppress fatigue, but are potently addictive because they modulate the brain's reward system. As we will learn in Section 11.18, both amphetamine and cocaine increase dopamine levels at their target sites. As in schizophrenia, the positive symptoms induced by these psychostimulants can be effectively treated with antipsychotic drugs that reduce dopamine receptor function. Additional support for the involvement of dopamine in schizophrenia came



**Figure 11–25 Competitive binding assay used to test drug action.**

(A) Illustration of the competitive binding assay to determine whether a drug binds specifically to receptors for a given neurotransmitter or agonist. A fixed amount of radioactively labeled neurotransmitter (or known agonist for the receptor) and variable amounts of drugs are used for competitive binding (a 1:1 ratio of the drug and agonist is illustrated for simplicity). Retention of a large fraction of the radioactivity by the receptors indicates low affinity of the drug to the receptor (left), whereas retention of a small fraction of radioactivity indicates that the drug outcompetes the radiolabeled neurotransmitter or agonist and binds the receptor with greater affinity (right). (B) The efficacy of various antipsychotic drugs, as measured by the amount required for effective treatment, correlates well with their ability to compete with radioactively labeled dopamine (blue data points) or haloperidol (itself an antipsychotic drug; red data points) for binding to dopamine receptors from brain extract. The diagonal line represents a 1:1 relation between the blocking molarity and the clinical dose. (Adapted from Seeman P, Chau-Wong M, Tedesco J et al. [1975] Proc Natl Acad Sci USA 72:4376–4380.)



**Figure 11–26 Self-portrait by Vincent van Gogh.** A brilliant artist, van Gogh suffered from illnesses that may have included bipolar disorder—he was prolific during normal and possibly manic phases, but committed suicide at the age of 37, probably during a depressive episode.

from PET imaging studies that correlated acute psychotic states with increased dopamine levels.

Current antipsychotic drugs are not effective for treating about a third of schizophrenia patients, suggesting a considerable degree of heterogeneity in the disorder. For those that respond, antipsychotic drugs are only effective in reducing positive symptoms, and have no effect on negative symptoms or cognitive impairment. This is likely because schizophrenia alters more than just the dopamine system. Indeed, drugs that act as NMDA receptor antagonists, such as phencyclidine (PCP) and ketamine, also induce psychosis that resembles schizophrenia, with associated negative symptoms, leading to the proposal that a reduction of NMDA receptor function also contributes to schizophrenia.

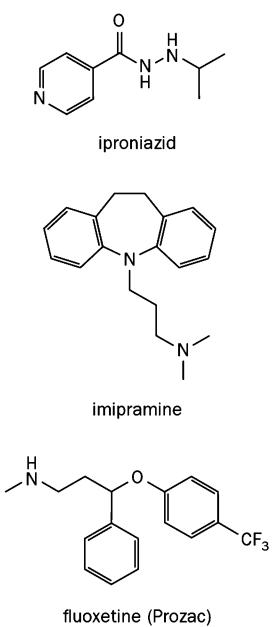
Although informative, studies investigating drug action have not revealed the root causes of schizophrenia. Recent structural magnetic resonance imaging studies indicate that schizophrenia is associated with significant thinning of the cerebral cortex. The most affected cortical areas include the **prefrontal cortex**, an executive control center that integrates multisensory information, processes working memory, and performs complex executive functions such as goal selection and decision making. Cortical thinning has been suspected to be due to excessive synaptic pruning that is normally associated with cortical development (see Section 7.14), suggesting a neurodevelopmental origin of schizophrenia. Indeed, prior to first psychosis, schizophrenia patients usually already exhibited considerable social, mood, and cognitive impairment collectively known as schizophrenia prodrome. As will be discussed in Section 11.19, schizophrenia has a strong genetic contribution. Identifying genetic factors and studying their mechanisms of action may shed more light on the causes of this calamitous mental disorder and thereby suggest more effective therapeutic strategies.

## 11.16 Mood disorders have been treated by manipulating monoamine neurotransmitter metabolism

We all experience moments of happiness and sadness. However, a sizable fraction of the general population suffers from mood disorders that at times appear to take over their lives. Mood disorders fall into two major categories: bipolar disorder and major depression. Individuals with **bipolar disorder** swing between manic and depressive phases. During the manic phase, patients feel grandiose and tireless; this is then interrupted by the depressive phase of feeling sad, empty, and worthless. The artist Vincent van Gogh (Figure 11–26) is one of many historical figures who are suspected to have suffered from bipolar disorder, which has a lifetime prevalence of 1%. **Major depression** has only the depressive phase (and is thus also termed unipolar), and is more common than bipolar disorder, with a lifetime prevalence of more than 5%. Both of these mood disorders can be life threatening, as they account for a large fraction of suicides.

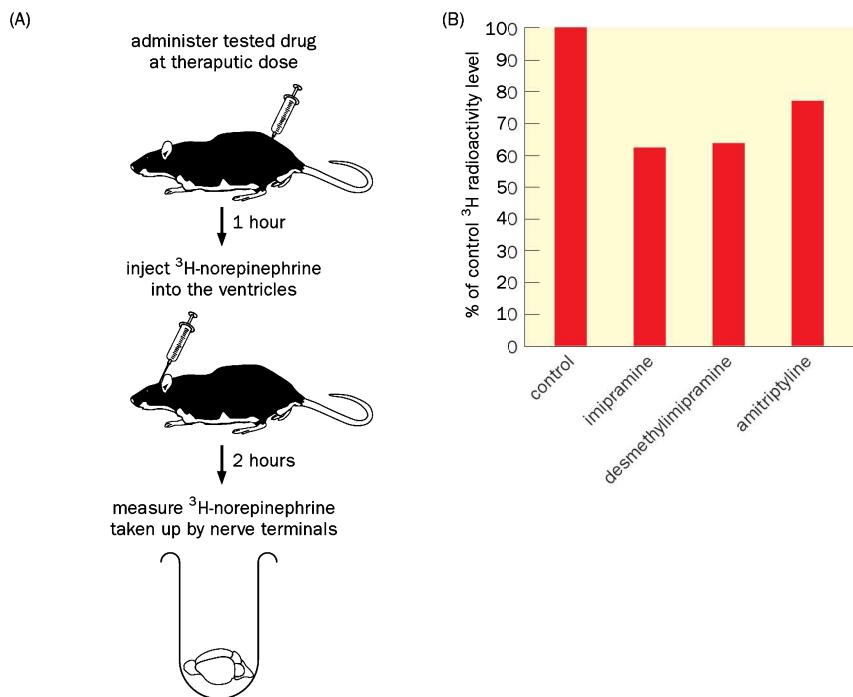
Like the first antipsychotics, the first antidepressant was discovered serendipitously in the 1950s. **Iproniazid** (Figure 11–27) was originally introduced to treat tuberculosis. Physicians reported that iproniazid-treated patients were happier despite the drug's ineffectiveness as a tuberculosis treatment. This and other clues from animal studies led to trials of iproniazid for depression, with the finding that the drug significantly improved patients' depressive states. Further studies showed that iproniazid acts by inhibiting monoamine oxidase (see Figure 11–24A), hence increasing the concentration of monoamines in presynaptic terminals and synaptic clefts. These findings suggest that elevation of one or more of the monoamine neurotransmitters—serotonin, norepinephrine, or dopamine—may have a therapeutic effect on depression. However, because monoamine oxidase inhibitors indiscriminately increase monoamine levels, they have many side effects. Over the years, they have been replaced by tricyclic antidepressants such as **imipramine**, named for their characteristic three-ring molecular structures (Figure 11–27).

Imipramine was synthesized as a variant of chlorpromazine with the hope of identifying a more effective antipsychotic. Although imipramine had no effect on treating psychosis, it had a pronounced effect on depression. Further studies led to the discovery that imipramine and other tricyclic antidepressants inhibit



**Figure 11–27 Structures of representative antidepressants.**

Resembling the structure of monoamines (see Figure 3–16), iproniazid inhibits monoamine oxidase, whereas imipramine inhibits the plasma membrane transporters for serotonin and norepinephrine, and fluoxetine selectively inhibits the plasma membrane transporter for serotonin.



**Figure 11-28 Antidepressants inhibit norepinephrine uptake by the brain.** **(A)** Assay procedure. Because norepinephrine does not cross the blood-brain barrier, it was injected into ventricles to access neurons.  $^3\text{H}$ -norepinephrine molecules retained in the brain 2 hours after injection indicated nerve terminal uptake. Those that were not taken up by nerve terminals were presumably to be metabolized. **(B)** Compared to controls, application of three clinically effective antidepressants markedly reduced brain uptake of radioactively labeled norepinephrine. In the same assay, clinically ineffective antidepressants did not inhibit norepinephrine uptake (not shown). (Adapted from Glowinski J & Axelrod J [1964] *Nature* 204:1318–1319. With permission from Macmillan Publishers Ltd.)

the plasma membrane monoamine transporters (PMATs; see Figure 11-24A) that allow neurotransmitter reuptake from presynaptic terminals. Originally discovered by studying the action of norepinephrine on the targets of sympathetic nerves, this pump-like reuptake turns out to be a general mechanism by which the action of neurotransmitters (particularly monoamines) is terminated. The effect of various drug treatments on reuptake can be determined using a quantitative assay based on how much experimentally administered radioactively labeled norepinephrine brain tissues retain (Figure 11-28A). Antidepressants such as imipramine reduced the norepinephrine level retained in brain tissues compared with controls and with other drugs that lack antidepressant effects (Figure 11-28B), indicating that imipramine inhibits norepinephrine reuptake.

Each monoamine neurotransmitter has its own PMAT encoded by a distinct gene. Inhibiting its reuptake system prolongs a neurotransmitter's actions. Imipramine affects PMATs for norepinephrine as well as for serotonin. Drugs developed subsequently, such as **fluoxetine** (brand name Prozac; Figure 11-27) block serotonin reuptake selectively. These **SSRIs (selective serotonin reuptake inhibitors)** are the most widely used antidepressants today. Thus, enhancing the actions of monoamine neurotransmitters, notably serotonin, can have significant effects in relieving depressive states. As discussed in Box 8-1, serotonin (and norepinephrine) neurons are clustered in the brainstem nuclei, but their axons project throughout the central nervous system from the forebrain to the spinal cord, enabling these neurons to modulate many excitatory and inhibitory target neurons. The primary target neurons and circuits relevant for mood regulation remain to be elucidated.

### 11.17 Modulating GABAergic inhibition can alleviate symptoms of anxiety disorders

**Anxiety disorders**, the most prevalent class of psychiatric disorders, include generalized anxiety, various kinds of phobias and panic disorders caused by irrational fear, and obsessive-compulsive disorder (OCD). Generalized anxiety disorder alone has a lifetime prevalence of more than 5%; patients with this disorder exhibit persistent worries about impending misfortunes, often with physical symptoms such as fatigue, muscle tension, and sleep disturbance.

**Barbiturates** and their derivatives were the earliest classes of drugs to treat anxiety disorders. Barbiturates are also potent sedatives, and a more serious