

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. ^3H -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

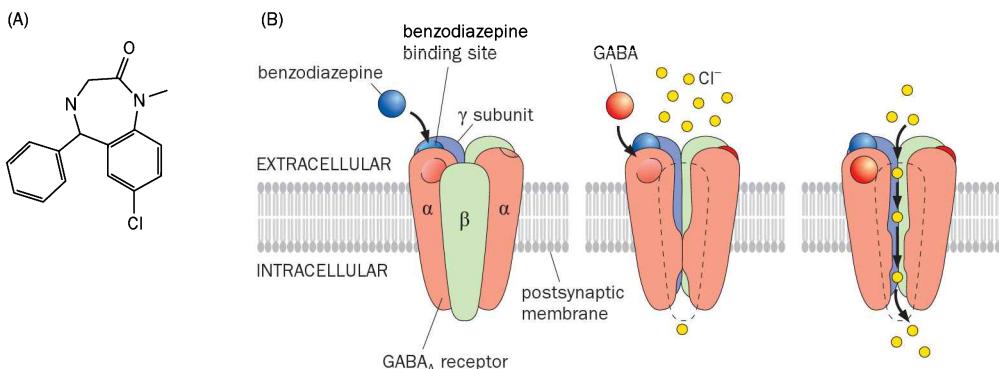


Figure 11-29 Benzodiazepines act as allosteric agonists on the GABA_A receptor. (A) Structure of diazepam (Valium), a benzodiazepine. **(B)** Schematic of benzodiazepine action as an allosteric agonist for the GABA_A receptor. Here, the GABA_A receptor pentamer consists of two α subunits, two β subunits, and one γ subunit, the most commonly occurring subunit composition. Binding

of a benzodiazepine to the GABA_A receptor at the interface of α and γ subunits does not open the channel (left), but enhances the receptor's affinity for GABA. The GABA_A receptor channel is opened by binding of two GABA molecules at the interface of the α and β subunits (middle and right). To visualize the channel opening, the β subunit at the front is removed in the middle and right panels.

concern is that overdose is lethal. Once again, fortuitous discoveries since the 1950s produced a new class of molecules called **benzodiazepines** (Figure 11-29A), which are effective in relieving anxiety symptoms but which have a reduced sedating effect. Importantly, benzodiazepine overdose induces lengthy sleep, but the lethal dose is much higher than that of barbiturates. Benzodiazepines have therefore largely replaced the use of barbiturates today.

Interestingly, barbiturates and benzodiazepines act on the same types of molecule to exert their anxiolytic (anxiety-reducing) effects: both classes of drugs bind to the ionotropic GABA_A receptors and enhance GABA transmission (see Section 3.17). Barbiturates, benzodiazepines, and GABA have distinct binding sites on the GABA_A receptors. At high concentrations, barbiturates can activate the GABA_A receptor independent of GABA, causing chloride influx through the GABA_A channels and hyperpolarization of target neurons. In contrast, benzodiazepines enhance the receptor's affinity for GABA (as well as for GABA agonists such as barbiturates or alcohol) but do not activate the GABA_A receptor by itself. Thus, benzodiazepines act as **allosteric agonists** by enhancing the action of endogenous GABA (Figure 11-29B). This accounts for the relative safety of benzodiazepines: their maximal effect is limited by the amount of endogenous GABA.

Although benzodiazepines have fewer side effects than barbiturates, they still induce sedation. Is it possible to isolate the anxiolytic and sedative effects? Molecular-genetic studies of benzodiazepine action have offered some clues. The benzodiazepine-binding site is located at the interface of the α and γ subunits of the pentameric GABA_A receptor (Figure 11-29B; see also Figure 3-21). In humans and mice, the six separate genes that encode the GABA_A receptor subunits α 1 through α 6 are differentially expressed in the brain. Subunits α 1, α 2, α 3, and α 5 possess a specific histidine residue (located at position 101 of α 1 and α 2) that renders them sensitive to benzodiazepines, whereas α 4 and α 6 have an arginine at the same position, which disrupts benzodiazepine binding. In other words, benzodiazepines only affect brain regions that express high levels of α 1-, α 2-, α 3- or α 5-containing GABA_A receptors but not those that are enriched only for α 4- or α 6-containing GABA_A receptors. Replacing the conserved histidine (H) with arginine (R) can cause a benzodiazepine-sensitive subunit to become insensitive without affecting GABA_A receptor function. This property offered a means to determine the contributions of individual α subunits to the effects of benzodiazepines *in vivo*.

Using the genetic knock-in approach (see Section 13.7), researchers produced mice with a specific H101 to R101 substitution in the α 1 subunit. These mice (designated as H101R) were no longer sedated by benzodiazepines but retained benzodiazepines' anxiolytic effects. In contrast, benzodiazepines could still sedate mice with the H101R substitution in the α 2 subunit but did not show anxiolytic effects (Figure 11-30). These experiments thus suggested that in normal mice,

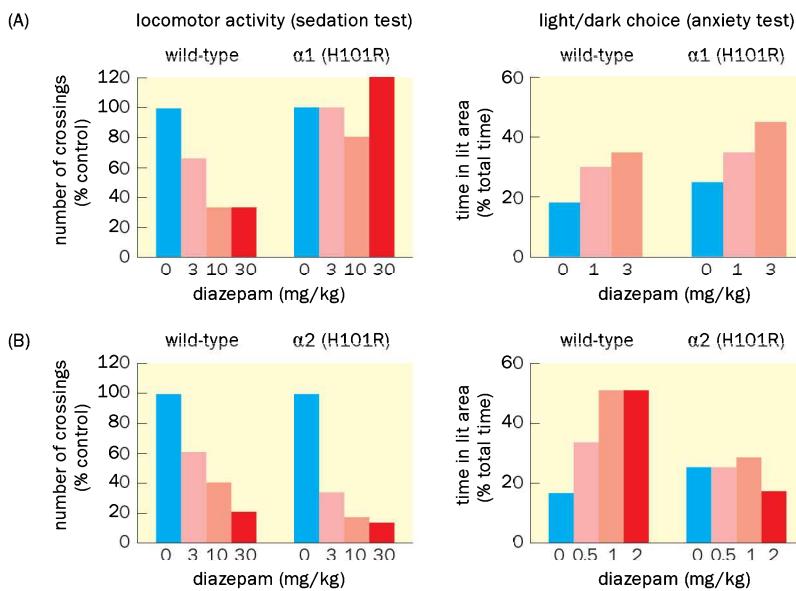


Figure 11-30 Selective functions of $GABA_A$ receptor subunits $\alpha 1$ and $\alpha 2$ in promoting sedation and relieving anxiety.

(A) Left, when the $\alpha 1$ -subunit of the $GABA_A$ receptor was rendered incapable of binding benzodiazepines by changing a key histidine residue to arginine (H101R), diazepam no longer induced sedation as assayed by locomotor activity, whereas locomotor activity of wild-type animals was reduced by diazepam in a dose-dependent manner. Right, benzodiazepine still had anxiolytic effects on mutant mice, as assayed by the time mice spent in the lit area of an open field. **(B)** When the $\alpha 2$ subunit of the $GABA_A$ receptor was rendered incapable of binding to benzodiazepine by the H101R mutation, diazepam still induced sedation in these genetically modified mice as it did in wild type (left), while the anxiolytic effects of diazepam were abolished (right). In both panels, locomotor activity was measured by the number of line crossings mice made when they moved in an open field. Anxiety was measured by the amount of time mice spent in areas that were lit versus dark (anxious mice excessively avoid lit areas). Additional anxiety test using the elevated plus-maze gave similar results (not shown). See Section 13.29 for details about these behavioral assays. (A, adapted from Rudolph U, Crestani F, Benke D et al. [1999] *Nature* 401:796–800. With permission from Macmillan Publishers Ltd; B, adapted from Löw K, Crestani F, Keist R et al. [2000] *Science* 290:131–134.)

benzodiazepines promote sedation through $\alpha 1$ -containing $GABA_A$ receptors and relieve anxiety through $\alpha 2$ -containing $GABA_A$ receptors. These differential effects are likely caused by differential expression of α subunits in brain regions that control the functions of different brain circuits. Indeed, one of the highest $\alpha 2$ -expressing areas is the amygdala, a center for regulating emotion and fear responses (see Section 10.23). Recent studies using optogenetic manipulations have begun to identify specific projections in the amygdala that mediate anxiolytic effects. In principle, drugs that specifically elevate the function of $GABA_A$ receptors containing $\alpha 2$ (but not $\alpha 1$) should be more effective and specific anxiolytics than the benzodiazepines currently in use.

GABA is the major inhibitory neurotransmitter in the brain and mediates many diverse physiological functions. In addition to treating anxiety, drugs that affect the GABAergic system have been used to treat epilepsy (see Box 11-4), pain, and sleep problems. Studies of benzodiazepine action illustrate how investigating drug action can help us tease apart GABA's diverse functions, which in turn will help inform the design of drugs that are better targeted to treat specific disorders.

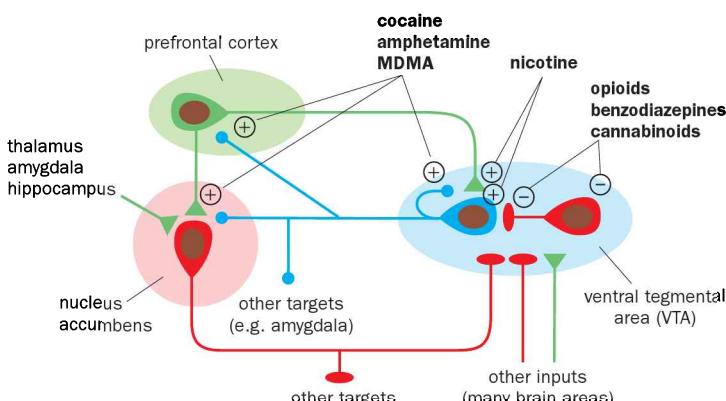
A limitation with benzodiazepines for treating anxiety disorders is that long-term use can lead to addiction (see Section 11.18). Increasing doses are required over time to achieve similar effects, and a halt of treatment results in withdrawal symptoms. SSRIs used to treat depression (see Section 11.16) also have anxiolytic effects but are not addictive, and are being used to treat certain anxiety disorders. It is not known how altering serotonin levels alleviates anxiety.

11.18 Addictive drugs hijack the brain's reward system by enhancing the action of VTA dopamine neurons

Addictive substances, such as alcohol from fermentation, opium from poppy plants, and cocaine from coca leaves, have been with human society for thousands of years. Successful chemical syntheses of active components and new methods for efficient delivery have both contributed to the recent rise in the prevalence of drug abuse. The result is a significant problem for humanity, spanning many nations and cultures. **Drug addiction** is defined as compulsive drug use despite long-term negative consequences. It is also associated with loss of self-control and propensity to relapse. What is the neurobiological basis of addiction?

Remarkably, almost all drugs of abuse have one common effect: they increase dopamine concentration at the output targets of **ventral tegmental area (VTA)** dopamine neurons, including at the VTA itself. Two major output targets of VTA dopamine neurons are the **nucleus accumbens** (ventral striatum),

Figure 11–31 Drugs of abuse increase dopamine concentration in the target areas of VTA dopamine neurons. A simplified circuit diagram illustrating connections between the ventral tegmental area (VTA), nucleus accumbens, prefrontal cortex, and other connected areas. Blue, dopamine neurons; red, GABAergic neurons; green, glutamatergic neurons. Also summarized are the action sites of most common drugs of abuse, all of which enhance dopamine concentrations in target areas. +, enhancement; –, suppression. For example, cocaine blocks dopamine reuptake from presynaptic terminals of dopamine neurons. Benzodiazepines enhance dopamine neuron firing by disinhibiting VTA GABAergic neurons. Nicotine causes increased release of glutamate at the terminals of excitatory input to dopamine neurons, and directly depolarizes dopamine neurons. (Based on Lüscher C & Malenka RC [2011] *Neuron* 69:650–663 and Sulzer D [2011] *Neuron* 69:628–649.)

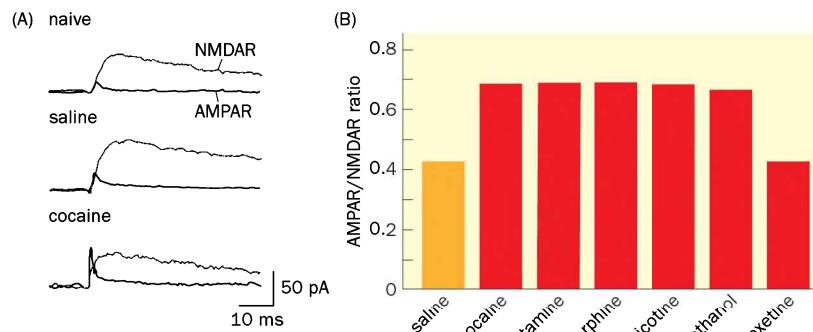


an area best known for processing reward information (see Section 10.24), and the prefrontal cortex responsible for executive functions such as goal selection and decision making (Figure 11–31). VTA dopamine neurons also receive inputs from many parts of the brain, including glutamatergic excitatory input from the prefrontal cortex and GABAergic inhibitory input from local and nucleus accumbens neurons.

Different drugs of abuse enhance dopamine action through distinct mechanisms. For example, nicotine enhances excitatory input onto VTA dopamine neurons by presynaptic excitation—it activates nicotinic ACh receptors (see Section 3.13) located on the glutamatergic presynaptic terminals, causing increased release of glutamate and hence greater excitation of dopamine neurons. Nicotine can also excite dopamine neurons directly through nicotinic ACh receptors on the dopamine neurons themselves. In contrast, opioids, benzodiazepines, and cannabinoids act by hyperpolarizing and thereby inhibiting local GABAergic neurons in the VTA, causing disinhibition of dopamine neurons. Ethanol is known to boost dopamine concentration at the VTA and nucleus accumbens, but the exact mechanisms and site(s) of action remain unclear. The psychostimulant drugs cocaine and amphetamine act by enhancing dopamine's effects at presynaptic terminals of dopamine neurons. Cocaine blocks the **plasma membrane dopamine transporter (DAT)** for dopamine reuptake, thus increasing the dopamine concentration in the synaptic cleft post-release. Amphetamines (including MDMA, or 3,4-methylenedioxy-N-methylamphetamine, commonly known as ecstasy) have more complex effects: (1) they reverse the normally unidirectional transport mediated by the DAT (that is, from synaptic cleft to presynaptic cytosol), causing presynaptic vesicle-independent release of dopamine into the synaptic cleft; (2) they enhance dopamine biosynthesis; and (3) they inhibit dopamine degradation. All these effects enhance dopamine action (Figure 11–31).

How does enhancement of the dopamine action of VTA neurons lead to addiction? As we learned in Section 10.24, projections from VTA dopamine neurons to the nucleus accumbens play a critical role in reward-based learning. Specifically, studies in primates and rodents have shown that many VTA dopamine neurons encode reward prediction errors. This error signal is hypothesized to direct synaptic plasticity in target neurons in the nucleus accumbens and prefrontal cortex for reinforcement-based learning. If VTA dopamine neurons signal a reward, the action or behavior that immediately preceded the reward is reinforced through dopamine modulation of downstream circuits (see Figure 10–44). Drugs of abuse bypass natural signals that activate these dopamine neurons, thus dissociating the reward system from its natural stimuli. Specifically, by increasing dopamine concentration at dopamine neurons' presynaptic terminals, drug consumption mimics dopamine neuron activation; this reinforces the preceding actions, include drug consumption itself. Thus, addictive drugs hijack the brain's reward system and exploit mechanisms that otherwise regulate learning and motivational behaviors.

What are the cellular and molecular mechanisms underlying the long-lasting behavioral changes in drug addiction? As discussed in Chapter 10, learning is



mediated by synaptic plasticity in relevant neural circuits. Drugs of abuse likely act to alter synaptic weights in circuits involving VTA dopamine neurons and their targets. For example, a single *in vivo* exposure to cocaine induced marked enhancement of excitatory input onto VTA dopamine neurons, as measured by an increased ratio of AMPA receptor (AMPAR)-mediated current to NMDA receptor (NMDAR)-mediated current in subsequent whole-cell patch clamp recording in VTA slices *in vitro* (Figure 11-32A). This effect is similar to long-term potentiation in hippocampal synapses (see Section 10.7). Indeed, the cocaine-induced increase of AMPAR/NMDAR ratio was NMDAR-dependent and occluded subsequent LTP induced *in vitro*. Other drugs of abuse, including morphine, nicotine, and ethanol, cause a similar increase in the AMPAR/NMDAR ratio of VTA dopamine neurons (Figure 11-32B). Addictive drugs can likewise affect excitatory synapses onto spiny projection neurons in the nucleus accumbens, which receive input from diverse brain areas (see Figure 11-31). The VTA–nucleus accumbens–prefrontal cortex circuits are complex and heterogeneous: for instance, while some VTA dopamine neurons signal reward prediction errors, other dopamine neurons signal aversion, yet other dopamine neurons signal salience of stimuli (see Section 10.24). Identifying the specific synaptic connections and subcircuits that are modulated by drugs of abuse will be crucial in establishing causal relationships between synaptic changes and addictive behaviors.

11.19 Human genetic studies suggest that many genes contribute to psychiatric disorders

As we see from the above sections, studies on the action of various therapeutic and abuse-related drugs have enriched our understanding of normal brain function. A major limitation of relying on drug action to reveal the pathophysiology of psychiatric disorders, however, is that our understanding is limited to drug targets, which may only be related to the symptoms of the disorders rather than to their root cause. Twin studies in schizophrenia, mood, and anxiety disorders all point to significant genetic contributions (for example, Figure 11-33), with a heritability (the proportion of phenotypic differences contributed by genetic differences; see Section 1.1) of up to 80% for schizophrenia and bipolar disorders. Heritability for major depression and general anxiety disorders is lower (30–40%). Family studies also suggest that different psychiatric disorders may share common genetic factors. For example, family members of a schizophrenic patient have an increased chance of developing not only schizophrenia but also bipolar disorder. Likewise, major depression and generalized anxiety disorders often run together in families. These studies implicate strong genetic contributions to these psychiatric disorders. At the same time, factors other than inheritance also contribute significantly—these could be environmental factors, epigenetic influences, or *de novo* mutations that occur in the parental germ line or during the early embryonic development of patients (Box 11-3). Because environmental factors are multifaceted and more difficult to trace, hope has been placed on identifying genes that contribute to psychiatric disorders as a means to gain new insight into their origins and find new targets for drug development.

Figure 11-32 Exposure to drugs of abuse causes long-lasting enhancement of excitatory input to VTA dopamine neurons. (A) Dopamine neurons in VTA slices were recorded by whole-cell patch clamp 24 hours after a single *in vivo* exposure to cocaine to measure the magnitude of excitatory postsynaptic current conducted by the AMPA and NMDA receptors (AMPAR and NMDAR). The total AMPAR- and NMDAR-mediated excitatory postsynaptic current (total current) in response to stimulating input axons was measured at +40 mV (therefore the Mg²⁺ block of the NMDAR was relieved, and current was outward; see Section 3.15); then the NMDAR antagonist AP5 was added so only the AMPAR current in response to the same input stimulation was measured. The NMDAR current was calculated by subtracting AMPAR current from the total current. Compared to naive and saline injection controls, cocaine exposure caused an increase in the AMPAR current and an enhanced AMPAR/NMDAR current ratio, indicative of a potentiated synapse (see Section 10.7). (B) The AMPAR/NMDAR current ratio is similarly enhanced following *in vivo* exposure to five addictive substances, but not to exposure of non-addictive drugs such as fluoxetine. (A, adapted from Ungless MA, Whistler JL, Malenka RC et al. [2001] *Nature* 411:583–587. With permission from Macmillan Publishers Ltd; B, adapted from Saal D, Dong Y, Bonci A et al. [2003] *Neuron* 37:577–582. With permission from Elsevier Inc.)



Figure 11-33 The Genain quadruplets. Born in 1930, each of these identical quadruplet sisters was diagnosed with schizophrenia of variable severity by age 24. Several other family members also suffered from mental illnesses, suggesting a strong genetic component in these disorders. (From Rosenthal, D. [1963] *The Genain Quadruplets: A Case Study and Theoretical Analysis of Heredity and Environment in Schizophrenia*. Basic Books, New York.)

So far, a simple Mendelian inheritance pattern has not been identified in any of the psychiatric disorders we discussed thus far. (This contrasts with Huntington's disease and certain familial forms of Parkinson's or Alzheimer's diseases, which are caused by dominant or recessive mutations in single genes.) This suggests that each psychiatric disorder is caused by multiple genetic factors and that each factor in itself only increases susceptibility, similar to the case of ApoE $\epsilon 4$ in Alzheimer's disease (see Section 11.5). The recent genomic revolution (see Section 13.14) has generated new tools for identifying genetic variations that contribute to complex diseases, including psychiatric disorders (see Box 11–3). These studies have suggested that schizophrenia and bipolar disorder may result from modest contributions of multiple genetic variants, with total variants estimated to be in the hundreds. These variations can take the form of point mutations or differences in gene copy numbers; some are inherited, whereas others are produced *de novo*. The identities of some of the candidate susceptibility genes (Table 11–2) also suggest that abnormal neuronal signaling and neural development are major contributors to psychiatric disorders, and that each of these genes may increase susceptibility to multiple disorders.

For example, the *Drd2* locus, which encodes the dopamine receptor D_2 widely expressed in the brain including the spiny projection neurons that constitute the indirect pathway from the striatum (see Figure 8–22), was recently identified in a large-scale genome-wide association study (GWAS; see Box 11–3 for more details) as a risk factor for schizophrenia. As discussed in Section 11.15, the D_2 -type dopamine receptor is a major target for all effective antipsychotic drugs used today; thus, the GWAS finding provides a satisfying link between recent genomic approaches and decades of drug-based investigations. GWAS of schizophrenia also identified genetic loci that encode other neuronal signaling molecules, including multiple glutamate receptors and voltage-gated Ca^{2+} channels that play important roles in synapse-to-nucleus signaling (see Section 3.23). Genes important for neural development have also been associated with schizophrenia and bipolar disorders. These include genetic loci that encode *Satb2*, a transcription factor that regulates cerebral cortex neuronal fate and axonal projections (see Section 7.4), and *Neurexin-1* and *Teneurin-4*, transmembrane proteins that are enriched in synapses and play important roles in synapse development, organization, and wiring specificity in mice and flies (see Sections 7.11 and 7.23). A strong

Table 11–2: Selected candidate genes associated with psychiatric disorders¹

Protein encoded by candidate gene	Identified on the basis of ²	Associated with	Also associated with	Physiological functions
<i>Drd2</i> ³	Genome-wide association study (GWAS)	schizophrenia		dopamine receptor D_2 that couples dopamine binding to G protein signaling
<i>Ca_v1.2</i> ³	GWAS	schizophrenia	autism spectrum disorders	voltage-gated Ca^{2+} channel with large conductance, used in neuronal and synapse-to-nucleus signaling, and cardiac muscle contraction
<i>Satb2</i> ³	GWAS	schizophrenia		transcription factor that regulates neuronal fate and axon targeting in cerebral cortex
<i>Neurexin-1</i> ⁴	copy number variation	schizophrenia	autism spectrum disorders	cell-surface protein for synapse development and trans-synaptic signaling
<i>Teneurin-4</i> ⁵	GWAS	bipolar disorder	schizophrenia	cell-surface protein for wiring specificity and synapse development
<i>Laminin-$\alpha 2$</i> ⁶	whole-exome sequencing	schizophrenia		extracellular matrix protein for cell adhesion, axon growth, and synapse development

¹ This list represents selected findings from a large body of human genetic studies of psychiatric disorders.

² See Box 11–3 for definitions.

³ Data from Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014) *Nature* 511:421.

⁴ Data from Rujescu et al. (2009) *Hum Mol Genet* 18:988.

⁵ Data from Psychiatric GWAS Consortium Bipolar Disorder Working Group (2011) *Nat Genet* 43:977.

⁶ Data from Xu et al. (2012) *Nat Genet* 44:1365.

link between neural development and psychiatric disorders was further suggested by the fact that some psychiatric disorder susceptibility genes are also associated with neurodevelopmental disorders such as autism spectrum disorders, which we will study later in the chapter.

Rapid advances in human genetics will undoubtedly uncover many more susceptibility genes for psychiatric disorders. Progressing from these genetic variations to mechanistic understanding and rational drug design for treatment, however, poses significant challenges. As discussed earlier, animal models are instrumental for studying disease mechanisms and testing therapeutic strategies. However, many symptoms of neuropsychiatric disorders, such as hallucination, delusion, or depression, are difficult to model in animals; the small effects of each susceptibility gene further complicate efforts to create effective animal models. Researchers are developing behavioral paradigms (see Section 13.29) and physiological assays in animals that mimic specific aspects of psychiatric disorders. In addition, investigating the physiological and developmental functions of susceptibility genes may ultimately bring new insights into why their disruption contributes to psychiatric disorders and how different susceptibility genes may interact with each other and with environmental factors in affected patients.

Box 11–3: How to collect and interpret human genetics data for brain disorders

Brain disorders caused by single-gene mutations that follow Mendelian inheritance patterns are the simplest to study from a genetics perspective (Figure 11–34). We've already discussed **autosomal dominant** mutations (the phenotypes of which can result from toxic gain-of-function effects

from the mutant allele, or loss-of-function effects due to insufficient amount of normal gene products produced from the wild-type allele) and **autosomal recessive** mutations (the phenotypes of which result from loss-of-function effects due to disruption of both alleles) that give rise to familial forms of neurodegenerative diseases. Single-gene mutations can also be **sex-linked**, that is, the mutant gene is located on the X chromosome. (The human Y chromosome carries few genes.) Sex-linked mutations affect males more severely than females, because mutations on a male's single X chromosome exert their effects in every cell. In females, by contrast, one of the two X chromosomes is randomly inactivated in each cell since early development (**random X-inactivation**), so that sex-linked mutations are expressed in about half of a female's cells. Red-green colorblindness (see Section 4.13) is a good example of a sex-linked trait. Genes that cause Mendelian disorders are usually mapped by pedigree analyses and by molecular markers that are distributed across the genome (for example, see Figure 11–4A).

Most brain disorders that are defined by symptoms or pathology do not follow simple Mendelian inheritance patterns and likely have heterogeneous causes. These disorders can in principle be caused by (1) inheritance of multiple genetic variants that interact with each other, (2) ***de novo* mutations** that occur in the parental germ line and—with the exception of X-chromosome mutations inherited by a female—affect all of the patient's cells, (3) ***de novo somatic mutations*** that occur in progenitor cells and therefore affect a subset of the patient's cells derived from the progenitors, (4) environmental factors, and (5) any of the above factors acting in combination. Of these, only factor 1 (and a small fraction of factor 2; see below) contributes to heritability. Therefore, if a genetic disorder such as schizophrenia or bipolar disorder has a high heritability but no clear Mendelian inheritance pattern, then multiple inherited

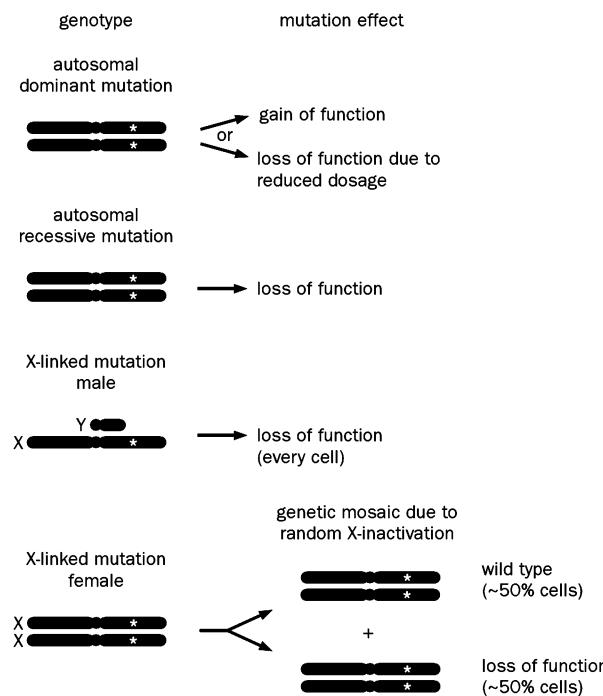


Figure 11–34 Three types of Mendelian inheritance. Left, genotypes are represented by pairs of homologous chromosomes, with one chromosome inherited from the father (blue) and one from the mother (red), in each cell (yellow oval). * designates a mutation. Right, summary of mutation effects. Black chromosomes indicate inactivated X chromosomes.

(Continued)

Box 11–3: How to collect and interpret human genetics data for brain disorders

mutations, interacting either with each other or with additional factors, must contribute to the disorder.

A conceptually simple way of identifying genes that contribute to a given disorder is to perform a **genome-wide association study (GWAS)**, taking advantage of **single nucleotide polymorphisms (SNPs)** that are present throughout the human genome. Any individual has about 3.5 million SNPs compared with the reference human genome. If a SNP is close to or within a gene whose mutations contribute to a disease, then it should be tightly linked with the disease-contributing mutation in the general population. DNA samples collected from many patients (usually thousands or more) can be compared with those from a similar number of healthy controls (ideally healthy relatives, or populations with the same ethnicity and geographic distribution) to identify the SNPs that are most strongly linked with the disease. The strength of the association can be quantified by parameters such as the **odds ratio**, which is defined as the probability of having the disease among people with the SNP divided by the probability of having the disease among people without the SNP. Given that most brain disorders have multiple genetic causes, the odds ratio is a complex function of both the heterogeneity of the patient population and the penetrance of the linked mutation that contributes to the disease. For schizophrenia and bipolar disorder, identified disease-associated SNPs have odds ratios between 1.10 and 1.25. By comparison, GWAS studies identified *Apoe ε4* as having an odds ratio of about 3.5 for Alzheimer's disease.

While SNPs have historically been detected by DNA microarray analyses (see Section 13.13), recent advances in sequencing technology have made it possible to sequence whole exomes (that is, the roughly 1% of genomic DNA sequences that corresponds to exons) or whole genomes of patients and control subjects; these methods offer powerful ways to identify disease-causing DNA variants. Whole-exome sequencing and whole-genome sequencing have revealed that *de novo* mutations contribute significantly to many brain disorders. *De novo* mutations usually occur spontaneously in the germ line of parents, with a bias toward the paternal germ line because spermatogenesis involves many more cell divisions than oogenesis, and hence presents more opportunities for DNA replication errors. By definition, *de novo* mutations do not affect the phenotypes of parents and do not contribute to heritability (except in the rare case where the mutations occur early in parental germ line development and affect the sperm or eggs inherited by more than one progeny, contributing to sibling similarities). Thus, whole-exome or whole-genome sequencing of

patients with a specific disease and their healthy parents should reveal *de novo* mutations that contribute to the disease. A complication is that *de novo* mutations occur even in healthy individuals, with an incidence of about one gene-disrupting *de novo* mutation per individual; in fact, each of us has about 100 inherited gene-disruption mutations in our genome. As a result, identifying which *de novo* mutations contribute to a given disease involves complex statistical analysis, taking into consideration the sequence conservation and possible physiological functions of affected proteins. In general, if *de novo* mutations affect the same gene in more than one patient with the same disease (as was the case for laminin $\alpha 2$ in Table 11–2), then the probability that these mutations contribute to the disease increases.

Among *de novo* mutations, **copy number variations (CNVs)** make a major contribution to brain disorders. CNVs are deletions or duplications of chromosome segments that vary in length from 500 base pairs to several megabases (Mb) and may contain coding sequences that range from a small fraction of a single gene to many genes. CNVs can also be inherited if carriers bear progeny. Frequently occurring CNVs are associated with repeat elements in the genome that cause errors in DNA recombination during the meiotic cell cycles that produce sperm and eggs. Healthy humans usually carry an average of about 1000 polymorphic CNVs; having one or three copies of most genes has no significant impact on health. However, some genes are dosage-sensitive, such that losing a copy or gaining an extra copy can contribute to or cause specific disorders. For instance, a spontaneous deletion of a 3-Mb segment on Chromosome 17 that is flanked by genomic repeats affects 1 in 15,000–25,000 people and causes a neurodevelopmental disorder called **Smith-Magenis syndrome**, characterized by mild-to-moderate intellectual disability, delayed speech, sleep disturbances, and impulse control and other behavioral problems. Despite the fact that the common deletion contains more than 30 genes, losing one copy of a single gene called *Rai1* (retinoic acid induced 1) within the common deletion interval is sufficient to cause most of the symptoms. Remarkably, duplication of this genomic region (which occurs with the same frequency as the common deletion) results in **Potocki-Lupski syndrome**, which is likely caused by an increased dose of *Rai1* and is associated with mild intellectual disability and autistic symptoms. Thus, the gene dosage of *Rai1*, which encodes a nuclear protein that regulates gene expression, is critical for proper brain development and function. As another example, deletions of one copy of part of the gene encoding Neurexin-1 markedly increase the odds of developing schizophrenia and autism (see Table 11–2).

NEURODEVELOPMENTAL DISORDERS

Whereas neurodegenerative and psychiatric disorders usually have an adult or adolescent onset, the symptoms of neurodevelopmental disorders first appear in infancy or early childhood. Depending on the types of symptoms, neurodevelopmental disorders are categorized as intellectual disabilities (ID, previously

referred to as mental retardation), autism spectrum disorders (ASD), communication disorders, attention deficit/hyperactivity disorders, learning disorders, or motor disorders. Despite this classification, recent work suggests that different neurodevelopmental disorders and some psychiatric disorders share similar underlying genetic causes. Below, we start with a general discussion of ID and ASD, two developmental disorders that are significant both in their frequency of occurrence and in their profound effects on patients and their caregivers. We then focus in greater detail on two specific syndromes that include symptoms of both ID and ASD: Rett syndrome and fragile-X syndrome. Approaches pioneered by research on these two syndromes will likely apply to studies of other neurodevelopmental disorders.

11.20 Intellectual disabilities and autism spectrum disorders are caused by mutations in many genes

Intellectual disability is characterized by deficits in general mental abilities such as reasoning, problem-solving, planning, abstract thinking, judgment, and learning from experience. ID patients usually have an intelligence quotient (IQ) of 70 or less, which is two standard deviations below the age-matched population mean (see Figure 1-2). ID is estimated to affect 1–3% of the general population.

Genetic factors, including chromosomal abnormalities and monogenic causes, account for a large fraction of ID cases, especially for those with IQs below 50. ID can also be one feature of **syndromic disorders** characterized by defined constellations of behavioral, cognitive, and physical symptoms. For example, Down syndrome is caused by having an extra copy of Chromosome 21 and is the most common genetic form of ID (affecting 1 in 500–1000 births). ID can also be caused by genetic mutations in the absence of recognizable syndromes or global structural abnormalities of the brain; these are called non-syndromic ID (NS-ID). Because the primary symptom of NS-ID is intellectual impairment, the corresponding genes may function more specifically in processes related to learning and intellectual capabilities, and are thus of considerable interest to scientists who seek to understand the biological bases of cognitive functions.

Genetic mapping studies in the past two decades have identified several dozen genes that when mutated cause NS-ID, 80% of which reside on the X chromosome. Because males have only one copy, mutations on the X chromosome affect all cells (see Figure 11-34) and are therefore technically easier to identify than autosomal recessive mutations. However, mutations in X-chromosome genes are estimated to account for only a small fraction of NS-ID cases; thus genetic causes for NS-ID may involve hundreds of genes distributed throughout the genome. The ID-associated genes identified to date encode proteins that include transcriptional regulators and cell-adhesion and signaling molecules important for brain wiring, as well as molecules known to regulate synapse development and function. Below we discuss one specific example.

A prominent class of proteins involved in ID are involved in Rho GTPase signaling. These proteins transduce extracellular signals to regulate cytoskeletal changes that underlie axon growth and guidance, dendrite morphogenesis, and synapse development (see Box 5-2). Rho GTPase signaling pathway members associated with NS-ID or syndromic ID include guanine nucleotide exchange factors (GEFs) that activate GTPases, GTPase activating proteins (GAPs) that deactivate GTPases, and protein kinases downstream of GTPases (Figure 11-35A). One of the first X-linked NS-ID genes to be identified encodes a protein called oligophrenin, which acts as a GAP for Rho GTPases. Oligophrenin is widely expressed in the nervous system and is distributed in axons, dendrites, and dendritic spines (Figure 11-35B). RNAi knockdown in cultured rat hippocampal neurons resulted in decreased spine length (Figure 11-35C) and impaired synaptic transmission and synaptic plasticity. Oligophrenin knockout mice exhibited a variety of cognitive defects, including impaired spatial learning in the Morris water maze assay (Figure 11-35D; see also Figure 10-32). Thus, in the case of oligophrenin, the cognitive deficits observed in human patients may be caused in part by impaired synapse structure and function.

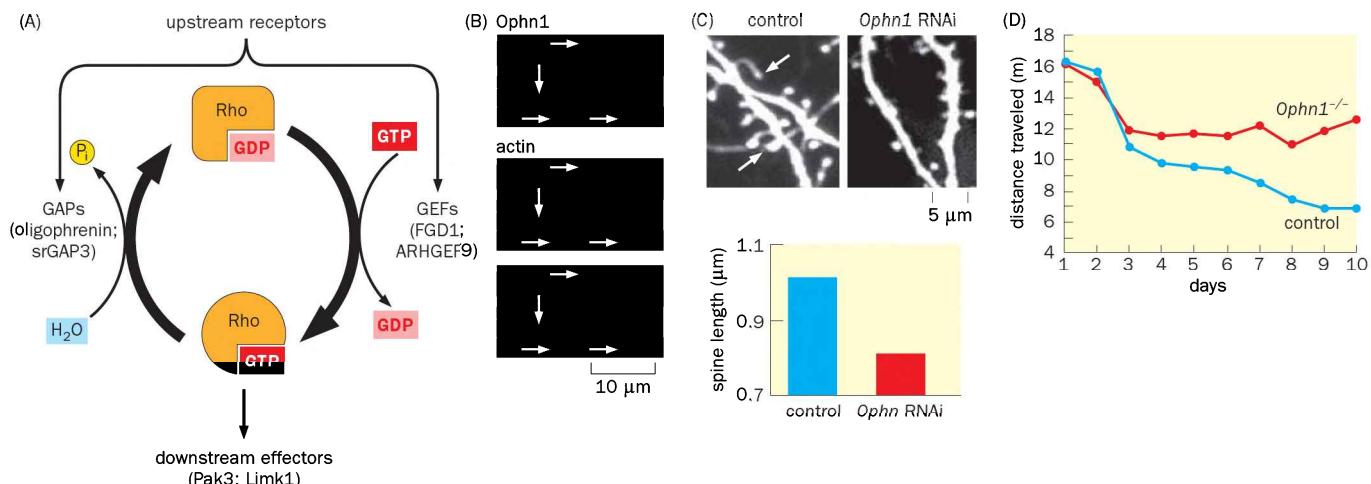


Figure 11-35 Defects in Rho GTPase signaling can cause intellectual disabilities.

Intellectual disabilities. (A) Schematic of Rho GTPase signaling pathway. Mutations in two GTPase activating proteins (GAPs), two guanine nucleotide exchange factors (GEFs), and two downstream kinases are associated with intellectual disabilities. The protein names corresponding to these mutations are in parentheses. (B) Oligophrenin1 (Ophn1), a RhoGAP, is highly concentrated in axons (cyan arrow), dendrites (white arrow), and dendritic spines (yellow arrows) of cultured rat hippocampal neurons. These cultures are doubly stained with antibodies against Ophn1 in red, and actin in green that highlights the dendritic spines enriched for F-actin.

(C) Compared with wild-type dendritic spines (arrows), dendritic spines in neurons treated with RNAi against Ophn1 show reduced length, as quantified below. **(D)** Compared with controls, Ophn1 mutant mice travel longer distances to reach the hidden platform during daily sessions in the Morris water maze. (A, based on Pavlowsky A, Chelly J & Billuart P [2012] *Mol Psychiatry* 17:682–693; B & C, adapted from Govek EE, Newey SE, Akerman CJ et al. [2004] *Nat Neurosci* 7:364–372. With permission from Macmillan Publishers Ltd; D, adapted from Khelfaoui M, Denis C, van Galen E et al. [2007] *J Neurosci* 27:9439–9450.)

Autism spectrum disorders (ASD) cover a wide range of symptoms that in total affect >1% of children. At their core, ASD is characterized by deficits in communication and reciprocal social interactions. ASD patients often show an absence or reduction in sharing of interests and emotions and have difficulty adapting their behavior to different environments. They also exhibit restricted and repetitive patterns of activities and excessive adherence to routines. About 70% of ASD patients also have ID, but others have normal intelligence, and some exhibit exceptional ability in mathematical calculation, memory, art, or music.

Genetic factors are a predominant cause for ASD. For example, compared with the general population, the relative risk of a child being diagnosed with ASD is >25-fold greater if a sibling is affected. Recent genome-wide association studies, CNV analyses, and whole-exome and whole-genome sequencing studies (see Box 11-3) have identified many independent genetic lesions associated with ASD. Similar to ID, genes associated with ASD encode proteins that regulate synapse development and synaptic function, transcription, and chromatin structures (see also Section 11.26). Most ASD-associated genes are risk factors and little is known yet about how they affect neural development. On the other hand, studies of specific syndromes whose symptoms overlap with ID and ASD can shed light on the underlying neurobiological mechanisms. These syndromes are usually caused by mutations in single genes with complete penetrance, and animal models often recapitulate significant aspects of the symptoms, making it possible to study pathogenic processes and reveal the underlying mechanisms. Below, we use studies of Rett syndrome and fragile-X syndrome to illustrate these points.

11.21 Rett syndrome is caused by defects in MeCP2, a regulator of global gene expression

First described by Andreas Rett in the 1960s in severely disabled girls who exhibited a common set of symptoms such as incessant hand wringing, **Rett syndrome** is a neurodevelopmental disorder that affects 1 in 10,000–15,000 girls during early childhood. Rett patients usually develop normally for the first 6–18 months, often achieving milestones such as walking and first words at a normal age. Their

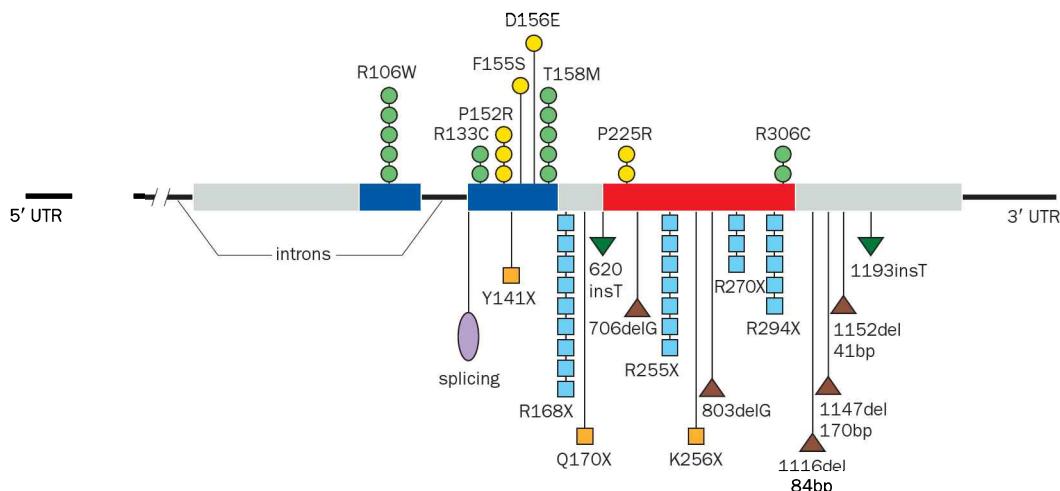


Figure 11–36 Rett syndrome is caused by mutations in the *Mecp2* gene, which encodes methyl-CpG-binding protein 2. Distribution of mutations in the coding region of the *Mecp2* gene (boxed region is the coding region, interrupted by two introns) identified in Rett patients. Above the gene structure are missense mutations, each of which changes the identity of a single amino acid. Below are a splicing mutation (oval), nonsense mutations (squares) that prematurely terminate translation, insertions (downward triangles; the inserted

nucleotide is followed by ‘ins’), and deletions (upward triangles). Mutations observed in more than one patient are represented by multiple symbols at the same position; most of these occur at mutational hot spots. The methyl-binding domain and transcriptional repression domain of MeCP2 are colored blue and red, respectively. 5' UTR and 3' UTR, 5' and 3' untranslated regions. (Adapted from Amir RE, Van Den Veyver IB, Schultz R et al. [2000] *Ann Neurol* 47:670–679. With permission from John Wiley & Sons.)

development then slows, arrests, and regresses. Patients exhibit social withdrawal, loss of language, and other autistic features. The onset of mental deficits is also accompanied by motor symptoms such as hand wringing. The condition subsequently stabilizes and patients usually live to adulthood, although with severe and persistent disability.

In 1999, genetic mapping and candidate gene sequencing revealed that Rett syndrome is caused by mutations in an X-linked gene encoding a protein called **methyl-CpG-binding protein 2 (MeCP2)** (**Figure 11–36**). Loss-of-function *Mecp2* mutations usually lead to prenatal or infant lethality in boys, who have only one X chromosome. Girls with a loss-of-function *Mecp2* mutation are genetic mosaics for MeCP2 function and develop Rett syndrome: of the two X chromosomes per cell, one is randomly inactivated (see **Figure 11–34**), so about half of their cells have defective MeCP2, and the severity of the disorder is influenced by the pattern of random X-chromosome inactivation. Rett syndrome is almost always caused by *de novo* mutations in *Mecp2*, as patients are so severely disabled that they rarely have children. Since the discovery that *Mecp2* mutations underlie Rett syndrome, specific missense mutations in *Mecp2* (that presumably have weaker effects than a complete loss-of-function) have been associated with sporadic ASD and schizophrenia. The proper level of MeCP2 expression is important, as duplication of *Mecp2* also causes severe neurodevelopmental defects.

MeCP2 is a nuclear protein that binds to methylated DNA at CpG sites (CpG refers to a cytidine followed by a guanosine in the DNA sequence). DNA methylation, a major form of epigenetic regulation of gene expression, is usually associated with target gene repression; for instance, methylation is the primary contributor to random X-chromosome inactivation. In the mammalian genome, CpGs are usually methylated except where they are present in large clusters (CpG islands), which are often associated with active transcription. Because methylation states affect gene expression, MeCP2 provides a link between chromatin structure and gene expression. MeCP2 is most abundantly expressed in the brain. In the mouse, MeCP2 expression increases greatly during the first 5 weeks of life as the final stages of neural development take place. Biochemical analysis indicated that MeCP2 binds CpG tracks along the entire genome, suggesting that MeCP2 acts as a general regulator of chromatin structure, which in turn affects global gene expression. Indeed, the number of MeCP2 molecules per neuronal

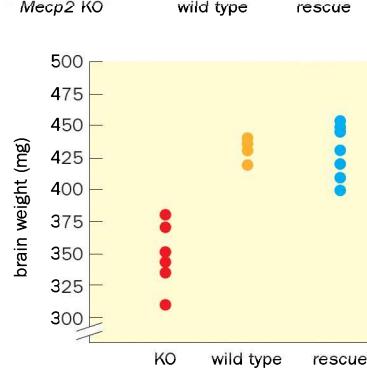
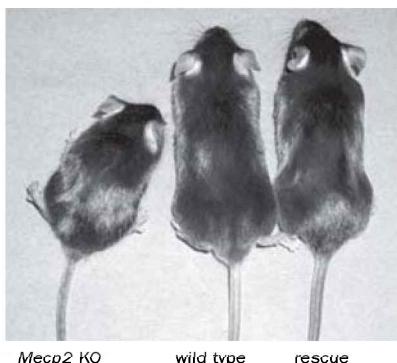


Figure 11–37 MeCP2 acts primarily in post-mitotic neurons. Compared with wild-type male mice at 8 weeks, *Mecp2* knockout (KO) males have reduced size (top) and reduced brain weight (bottom). Both phenotypes are rescued by transgenic expression of MeCP2 in post-mitotic neurons of KO mice. (Adapted from Luikenhou S, Giacometti E, Beard CF et al. [2004] Proc Natl Acad Sci USA 101:6033–6039. Copyright the National Academy of Sciences, USA.)

nucleus is similar to that of histones, the principal proteins that complex with DNA to form chromatin.

11.22 MeCP2 acts predominantly in post-mitotic neurons to regulate their maturation and function

How does loss of a global regulator of chromatin structure cause the neurological deficits characteristic of Rett patients? As discussed earlier in this chapter, animal models can provide important insights into human disease. MeCP2 is present in all vertebrates and is highly conserved in mammals. Indeed, *Mecp2* knockout mice mimic many aspects of Rett syndrome. As in humans, *Mecp2* in the mouse is located on the X chromosome. *Mecp2* mutant male mice grow normally for the first several weeks. Between 3 and 8 weeks of age, mutant male mice start to exhibit motor coordination defects, impaired growth, and reduced brain weight compared to normal mice (Figure 11–37). These symptoms become progressively worse, and most mutant males die by age 12 weeks. Female mice heterozygous for the *Mecp2* mutation, a genetic condition equivalent to that of girls with Rett syndrome, initially develop normally. They start to exhibit symptoms such as mild motor defects and inertia when several months old. Importantly, conditional knockout of *Mecp2* only in neurons and glia resulted in phenotypes essentially identical to those of *Mecp2* knockout mice. Conversely, restoring MeCP2 function only in post-mitotic neurons rescued many neurological phenotypes (Figure 11–37) and prevented the death of *Mecp2* mutant males. These experiments indicate that MeCP2 acts predominantly in post-mitotic neurons to exert its function. Furthermore, overexpression of MeCP2 in post-mitotic neurons in wild-type mice also caused severe motor dysfunction, echoing the symptoms caused by human *Mecp2* duplication.

Detailed analyses of the *Mecp2*-deficient mouse model (mostly in males) have revealed a host of defects, including reductions in the size of the brain, neurons, and dendritic spines and alterations in dendritic morphology, synaptic transmission, and synaptic plasticity. Conditional knockout (see Section 13.7) of *Mecp2* in specific neuronal populations indicated that MeCP2 plays important roles in excitatory, inhibitory, modulatory, and peptidergic neurons, as well as in glia, each of which contributes to a subset of phenotypes found in mice lacking *Mecp2* in all cells. Deletion of *Mecp2* in GABAergic neurons caused the most severe phenotypes, including many Rett syndrome features such as repetitive and compulsive behaviors (Figure 11–38A), motor dysfunction, learning deficits, and premature death. Some of the defects observed in *Mecp2* mutant GABAergic neurons may be caused by reduced expression of glutamic acid decarboxylases (*Gad1* and *Gad2*), the enzyme for GABA synthesis (Figure 11–38B). Reduction of GABAergic inhibition could also contribute to the seizures (see Box 11–4) often associated with Rett syndrome.

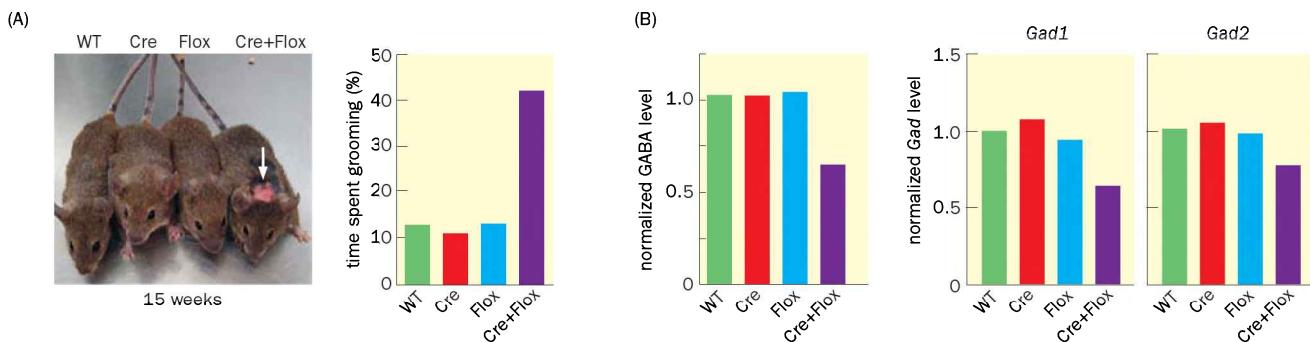


Figure 11–38 MeCP2 regulates functions of GABAergic neurons. (A) When *Mecp2* was knocked out only in GABAergic neurons using a vesicular-inhibitory-amino-acid-transporter-Cre transgene, the conditional knockout animals (Cre + Flox) frequently exhibited fur loss (arrow) due to excessive time spent self-grooming (quantified at right); controls include wild type (WT), Cre only (Cre), conditional knockout allele

without Cre (Flox). (B) Compared with controls, conditional knockout animals had reduced levels of the neurotransmitter GABA (left) and reduced mRNA levels for *Gad1* and *Gad2*, encoding two enzymes for GABA synthesis (right). (Adapted from Chao HT, Chen H, Samaco RC et al. [2010] Nature 468:263–269. With permission from Macmillan Publishers Ltd.)

11.23 Restoring MeCP2 expression in adulthood reverses symptoms in a mouse model of Rett syndrome

A key question in neurodevelopmental disorders is whether the symptoms are reversible. A given neurodevelopmental disorder may be caused by early and irreversible defects in nervous system development, with more challenging consequences for therapeutic intervention. Alternatively, it may reflect that the developing and adult nervous systems require an ongoing supply of the disrupted gene's product for maturation and function, whose symptoms might be reversible. The mouse model for Rett syndrome provided an opportunity to ask whether MeCP2 is continuously required in the adult nervous system and to determine whether defects caused by *Mecp2* deficiency can be alleviated by restoring MeCP2 expression in adults.

To answer the first question, a temporally controlled knockout scheme was employed using a drug-inducible variant of the Cre recombinase, CreER (see Section 13.7), to remove *Mecp2* gene upon drug application only in adults. Mice that lost *Mecp2* as adults developed symptoms resembling those observed in mice born with *Mecp2* knockout, including abnormal motor coordination and premature death (Figure 11–39). This experiment indicated that MeCP2 is continuously required in adults.

To determine whether late expression of MeCP2 can rescue developmental *Mecp2* deficiency, one can in principle use a *Mecp2* transgene under the control a drug-inducible promoter to test whether late expression of MeCP2 can remedy the knockout phenotypes. However, overexpression of MeCP2 also causes significant neurological defects. To restore MeCP2 expression at physiological level, a conditional transcriptional stop cassette was knocked in between the promoter and the coding sequence of *Mecp2* such that MeCP2 is normally not expressed from this modified allele. Upon drug-induced activation of CreER, the stop cassette can be excised by the recombinase (see Section 13.10), and transcription is reactivated from the endogenous promoter such that MeCP2 is expressed at physiological levels (Figure 11–40A). Remarkably, Rett-like symptoms as well as premature death were rescued after reactivation of MeCP2 in young adult males carrying the modified allele (Figure 11–40B). In female mice that were heterozygous for the modified allele and therefore similar to girls with Rett syndrome in genotype, defects in hippocampal long-term potentiation were also reverted after MeCP2 reactivation (Figure 11–40C). These results do not suggest a specific therapy to treat Rett patients; indeed we are still far from any therapy. However, these findings offer hope for effective intervention even after symptom onset.

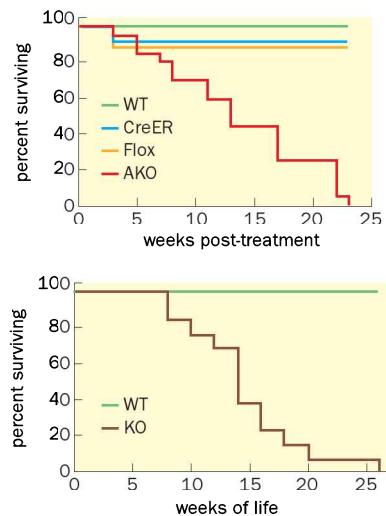


Figure 11–39 MeCP2 is required in adulthood. Top, mice in which the *Mecp2* gene is knocked out in adulthood (AKO, red) die prematurely compared with controls (WT, wild type; CreER, CreER only; Flox, conditional allele only). Bottom, survival curves for mice born with *Mecp2* knockout mutation (KO, brown) and wild-type controls (green). Note the similarity in AKO and KO survival curves from the time point at which *Mecp2* gene product is lost. (Adapted from McGraw CM, Samaco RC, & Zaghbi HY [2011] *Science* 333:186.)

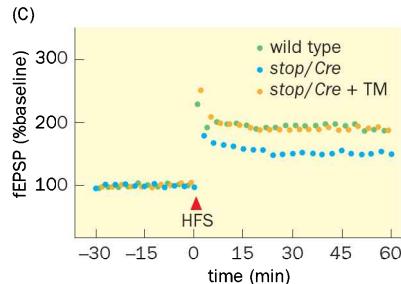
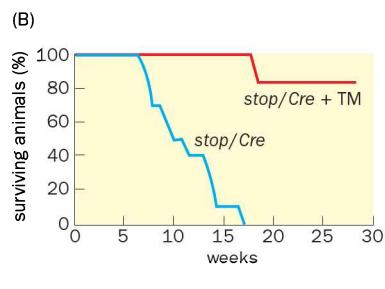
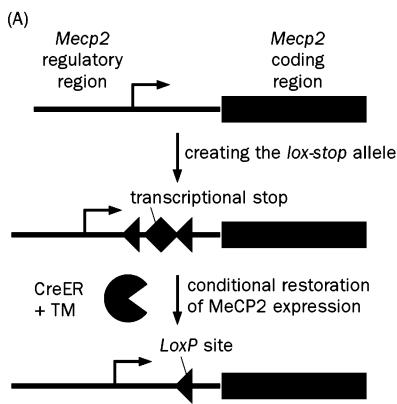
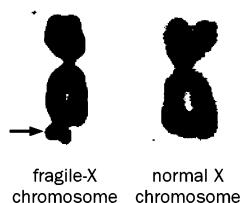


Figure 11–40 Reversing Rett symptoms by restoring MeCP2 expression in young adult mice. (A) Strategy to restore MeCP2 expression conditionally. Top, the endogenous *Mecp2* locus. Middle, a transcriptional stop flanked by two *loxP* sites is inserted between the transcriptional start and the coding region, such that no MeCP2 protein is made from this *lox*-stop allele. Bottom, upon tamoxifen (TM)-inducible CreER excision of the transcriptional stop, MeCP2 is expressed at the endogenous level (see Sections 13.7 and 13.10 for more details of the techniques). (B) Male stop/Cre mice survived after TM injection in adulthood (red curve), but died prematurely without TM injection (blue curve). (C) Restoration of MeCP2 expression in adult females (orange) rescued the long-term potentiation defects exhibited by *Mecp2* heterozygous females (blue) to wild-type level (green), as seen by the magnitude of field excitatory postsynaptic potential (fEPSP) increase in response to high-frequency stimulation (HFS). (Adapted from Guy J, Gan J, Selfridge J et al. [2007] *Science* 315:1143–1147.)

**Figure 11–41 Fragile-X chromosome.**

X chromosomes from a fraction of cells of fragile-X syndrome (FXS) patients exhibit a gap near the tip of the long arm (arrow on the left), which is not seen in normal X chromosomes (right). (Adapted from Lubs HA [1969] *Am J Hum Genet* 21:231–244. With permission from American Society of Human Genetics.)

11.24 Fragile-X syndrome is caused by loss of an RNA-binding protein that regulates translation

Fragile-X syndrome (FXS) is a leading cause of inherited intellectual disability (ID), affecting about 1 in 5000 boys. FXS patients exhibit reduced IQ and a significant developmental delay in speech and motor skills. Many patients exhibit autistic features, such as a tendency to avoid eye contact and repetitive, stereotyped behaviors; indeed, about 20–30% of FXS patients are diagnosed with autism spectrum disorders (ASD) based on behavioral criteria. The name ‘fragile-X’ came from the unusual chromosomal gap on the X chromosome of patients (**Figure 11–41**). The defective gene that causes FXS was molecularly identified in 1991 and named *Fmr1* (fragile-X mental retardation 1). A polymorphic CGG trinucleotide repeat was found in the 5' untranslated region of the *Fmr1* gene. Healthy individuals contain 6 to 54 CGG repeats, but the number expands to >200 in FXS patients. CGG repeats between 55 and 200 are called premutations; these repeats are at high risk of expansion. FXS is inherited either from mothers who carry premutations, or from mothers who carry the full mutation but are not severely affected themselves. Boys with the syndrome are most severely affected, while the severity of the syndrome in girls varies widely depending on X-inactivation patterns. Expanded CGG repeats cause extensive methylation near the *Fmr1* promoter and silencing of *Fmr1* gene expression. Therefore, FXS is caused by loss of a single protein, FMRP, encoded by the *Fmr1* gene.

FMRP is an evolutionarily conserved RNA-binding protein. A point mutation affecting a conserved residue in the RNA-binding domain of FMRP causes FXS with symptoms similar to those in patients with silenced *Fmr1* expression, demonstrating the importance of RNA binding for normal FMRP function. FMRP is highly expressed during embryonic development and is continuously expressed in all neurons throughout life. Within neurons, FMRP is localized to the cytoplasm, axons, dendrites, and postsynaptic compartments, and thus can regulate local protein translation (see Section 2.2). FMRP is enriched in polyribosomes, where it binds to mRNA molecules and represses translation *in vitro* and *in vivo* (**Figure 11–42A, B**). Normally most FMRP is phosphorylated at a conserved serine residue (S499 in the mouse). S499-phosphorylated FMRP represses translation; dephosphorylation relieves this repression. Upon specific signals such as synaptic activity, FMRP is dephosphorylated, which transiently reduces its translational

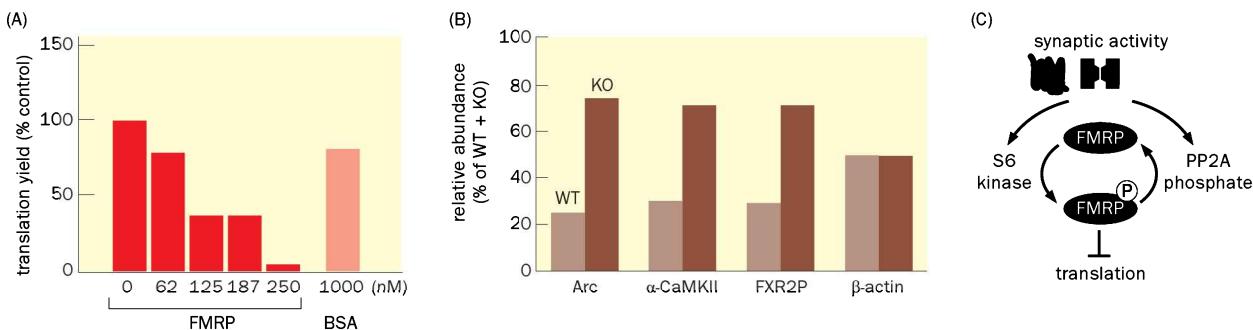


Figure 11–42 Regulation of protein synthesis in dendrites and synapses by fragile-X mental retardation protein (FMRP). (A) In an *in vitro* translation assay, purified FMRP repressed translation of total brain mRNA in a dose-dependent manner, whereas a control protein, bovine serum albumin (BSA), did not. (B) Synapse-enriched protein extracts from *Fmr1* knockout mice contained higher amounts of the proteins Arc (a postsynaptic signaling protein), α -CaMKII (α subunit of the Ca^{2+} /calmodulin-dependent kinase), and FXR2P (fragile-X mental retardation syndrome-related protein 2, encoded by a *Fmr1* paralog) than do similar extracts from wild-type mice. By contrast, the level of β -actin protein was unaffected. This suggested that FMRP normally represses translation of select mRNAs such as those that

produce Arc, α -CaMKII, and FXR2P proteins. (C) Only phosphorylated FMRP represses translation. S6 kinase and phosphatase 2A (PP2A) respectively phosphorylate and dephosphorylate FMRP. Through its effects on PP2A and S6 kinase, synaptic activity can regulate FMRP phosphorylation, and hence local translation. Type I metabotropic glutamate receptors activate PP2A more rapidly than they activate S6 kinase, thus causing transient FMRP dephosphorylation and activation. (A, adapted from Li Z, Zhang Y, Ku L et al. [2001] *Nucleic Acid Res* 29:2276–2283. With permission from Oxford University Press; B, adapted from Zalfa F, Giorgi M, Primerano B et al. [2003] *Cell* 112:317–327. With permission from Elsevier Inc.; C, based on Santoro MR, Bray SM & Warren ST [2012] *Annu Rev Pathol Mec Dis* 7:219–245.)

repression activity, thereby allowing rapid local translation (Figure 11–42C). This activity-dependent regulation of local translation contributes to synaptic plasticity and learning.

Biochemical studies that used cross-linking of bound RNA with FMRP followed by immuno-purification of FMRP have identified many specific target mRNAs associated with FMRP. These include microtubule-associated proteins involved in axonal and dendritic transport, presynaptic proteins that regulate synaptic vesicle release, postsynaptic scaffolding proteins, and components of the NMDA and metabotropic glutamate receptor (mGluR) signaling pathways for neurotransmitter reception. Interestingly, FMRP also binds to the mRNAs for many ASD-associated proteins, providing a molecular link between FXS and ASD.

11.25 Reducing mGluR signaling ameliorates fragile-X symptoms in animal models

As with Rett syndrome, *Fmr1* knockout mice recapitulated certain symptoms and pathologies of human FXS. For example, postmortem analysis revealed that dendritic spines in FXS patients tend to be longer, more tortuous, and more numerous compared to those of control subjects. Similar phenotypes have been found in *Fmr1* knockout mice, which also exhibit deficits in a variety of learning and synaptic plasticity assays.

Of particular interest in *Fmr1* research is long-term depression (LTD) in the hippocampus mediated by the type I metabotropic glutamate receptors (mGluRs). The type I mGluRs, mGluR1 and mGluR5, are known to regulate local protein translation at the postsynaptic density, leading to AMPA-type glutamate receptor endocytosis and hence long-term depression (see also Section 10.9). In *Fmr1* knockout mice, mGluR-dependent LTD was enhanced (Figure 11–43A). Given that FMRP represses translation, one interpretation of this result is that FMRP-mediated translational repression normally counterbalances mGluR-induced translation. A critical function of FMRP may be to repress translation of proteins that are normally synthesized following mGluR activation in the context of synaptic plasticity and learning (Figure 11–43B).

This ‘mGluR hypothesis’ has received support in animal model studies. For example, a prediction of this hypothesis is that a reduction of mGluR function should ameliorate FXS symptoms. Indeed, removing one copy of the gene encoding mGluR5, which is the major form of type I mGluR expressed in the hippocampus, ameliorated several phenotypes in *Fmr1* knockout mice, such as increased dendritic spine density (Figure 11–44A). Treatment of *Fmr1* knockout mice with an mGluR5 antagonist likewise improved certain behavioral phenotypes. Since *Fmr1* is a highly conserved gene, its homolog in *Drosophila* (*Dfmr1*) has also been used to explore its physiological function. *Dfmr1* mutant flies exhibit a variety of phenotypes ranging from defects in synaptic transmission to abnormalities in courtship and courtship conditioning. *Dfmr1* males do not mate as efficiently as controls, and they do not learn to reduce mating after being rejected by mated females (see Section 9.12). Remarkably, mGluR5 antagonist treatment ameliorated both phenotypes in *Dfmr1* mutants (Figure 11–44B). Thus, drugs that reduce mGluR signaling may represent a viable means of alleviating FXS symptoms.

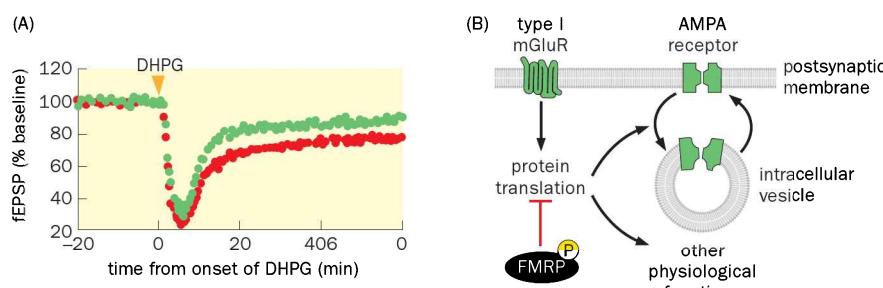


Figure 11–43 FMRP action in the context of metabotropic glutamate receptor (mGluR) signaling.

(A) Application of dihydroxyphenylglycine (DHPG), an agonist of type I mGluR, induces long-term depression (LTD) of the CA3 → CA1 synapse in hippocampal slices. The magnitude of LTD is enhanced in *Fmr1* knockout mice (red) compared to wild type (green). fEPSP, field excitatory postsynaptic potential. (B) A model of the interaction between mGluR signaling and FMRP. Type I mGluR-induced LTD involves a net removal of AMPA-type glutamate receptors from the postsynaptic surface and requires protein translation. Phosphorylated FMRP normally represses the translation of the same set of proteins. In the absence of FMRP, these normally repressed proteins are at elevated levels prior to mGluR activation and thus enhance mGluR-induced LTD. (A, adapted from Huber KM, Gallagher SM, Warren ST et al. [2002] Proc Natl Acad Sci USA 99:7746–7750; B, adapted from Bear MF, Huber KM, & Warren ST [2004] Trends Neurosci 27:370–377. With permission from Elsevier Inc.)

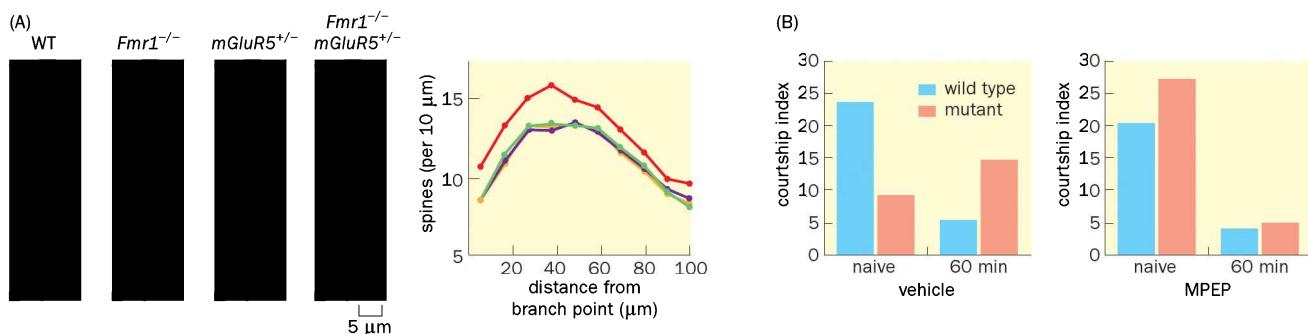


Figure 11–44 Reducing mGluR function ameliorates defects due to loss of FMRP in animal models. (A) The left panels show representative dendrites visualized by Golgi staining. The right panel shows the quantification of spine density. *Fmr1* knockout mice (*Fmr1*^{-/-}; red) had more dendritic spines compared to wild type (WT; orange). Loss of one copy of the *mGluR5* gene (*mGluR5*^{+/-}; green) in a WT background did not affect spine density, but loss of one copy of the *mGluR5* gene in an *Fmr1* knockout background (purple) rescued the supernumerary spine defect of *Fmr1* knockout. (B) Left, loss of *Drosophila Dfmr1* caused a reduction of courtship in naive males (compare the first two columns) and a defect in courtship conditioning

(see Section 9.12). Control males reduce courtship after being paired with mated females, and remember this experience 60 minutes later by displaying a reduced courtship index (compare the first and third columns). *Dfmr1* mutant flies do not reduce courtship after being paired with mated females (compare the second and fourth columns). Right, both phenotypes are rescued by treatment with 2-methyl-6-(phenylethynyl)pyridine (MPEP), an mGluR5 antagonist. (A, adapted from Dölen G, Osterweil E, Shankaranarayana Rao BS et al. [2007] *Neuron* 56:955–962. With permission from Elsevier Inc.; B, adapted from McBride SMJ, Choi CH, Wang Y et al. [2005] *Neuron* 45:753–764. With permission from Elsevier Inc.)

However, clinical trials using mGluR antagonist to treat FXS patients have not been successful so far, highlighting the difficulty of translating findings in animal models to therapies in humans (see also Section 11.4).

11.26 Synaptic dysfunction is a common cellular mechanism that underlies neurodevelopmental and psychiatric disorders

Studies of Rett and fragile-X syndromes have reinforced the theme we introduced in Section 11.20: disruption of synaptic development and function may be a common cellular mechanism for many neurodevelopmental disorders. Further support for such a common mechanism comes from the identification of other syndromic, non-syndromic, or sporadic ASD and ID genes that affect different aspects of synaptic signaling (Figure 11–45). For example, as we discussed in Chapters 3 and 7, neurexins and neuroligins form trans-synaptic complexes that regulate synapse assembly and organization. Independent genetic studies have identified mutations in human genes that encode several neurexin and neuroligin isoforms as being associated with ASD. In addition, disruption of *Shank3*, a postsynaptic scaffolding protein (see Section 3.16), has been associated with syndromic as well as sporadic ASD. Expanding on FMRP's role in regulating synaptic protein translation, a key regulator of protein translation in response to extracellular signals is **mTOR** (mammalian target of rapamycin), which in turn is negatively regulated by a complex consisting of *Tsc1* and *Tsc2* (tuberous sclerosis 1 and 2). A large fraction of patients with **tuberous sclerosis**, which is characterized by nonmalignant tumors in the brain and other organs and which results from mutations in *Tsc1* or *Tsc2*, exhibit ASD symptoms, reinforcing the notion that abnormal protein translation may contribute to ASD.

Many factors that are involved in postsynaptic events, including synapse-to-nucleus signaling (see Section 3.23), have also been implicated in neurodevelopmental disorders. For example, a gain-of-function mutation in a voltage-gated Ca^{2+} channel, $\text{Ca}_v1.2$, causes **Timothy syndrome**, with cardiac arrhythmia and autistic symptoms. Disruption of components of the Ras/MAP kinase pathway, including the small GTPase Ras or its downstream MAP kinase cascade, causes Noonan syndrome with multiple developmental defects including learning disability. Disruption of Neurofibromin 1 (NF1), a GTPase activating protein (that is, negative regulator) of Ras, causes neurofibromatosis type I, whose symptoms also include learning disability. The Ras-MAP kinase pathway regulates protein translation and is also a key signaling pathway from the synapse to the nucleus (see Figure 3–41). One nuclear effector for synapse-to-nucleus signaling is MeCP2,

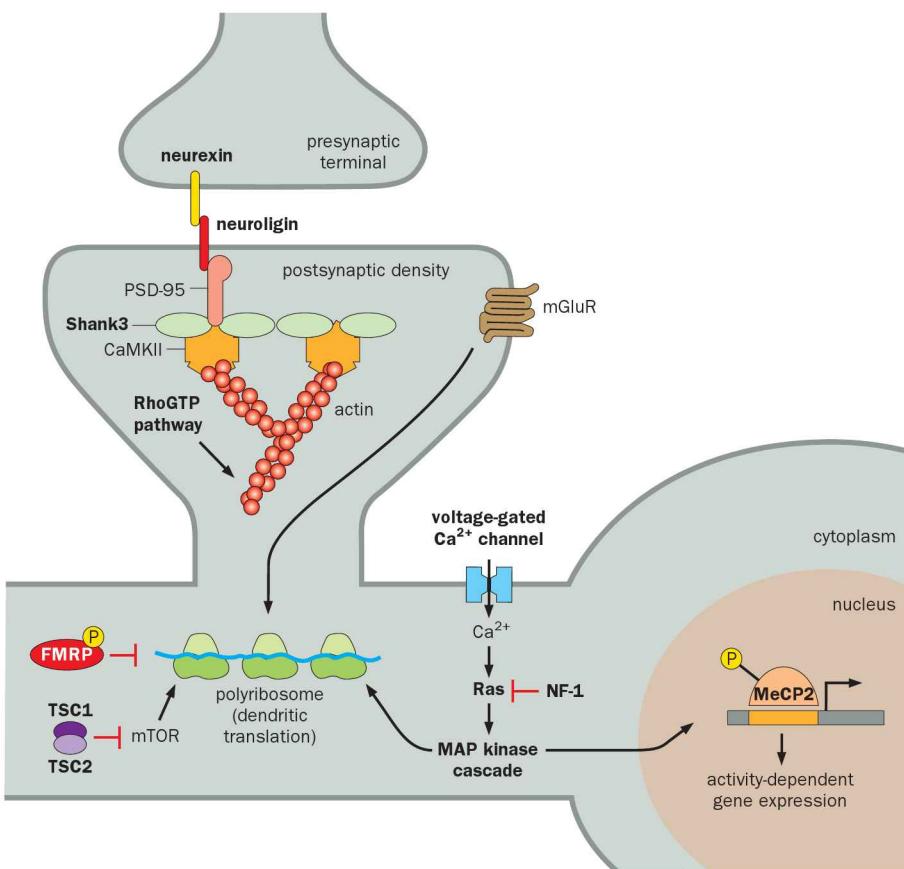


Figure 11–45 Defects in many proteins involved in synaptic signalling contribute to neurodevelopmental disorders.

Mutations in genes that encode proteins named here in bold have been implicated either in various syndromes with increased risks of intellectual disability or autism spectrum disorder (ID/ASD), or in non-syndromic or sporadic ID/ASD. For example, mutations in synaptic adhesion proteins neurexin and neuroligin, a voltage-gated Ca²⁺ channel, and a postsynaptic scaffolding protein Shank3 are associated with ASD; mutations in several components of the Rho GTPase signaling pathways cause ID; mutations in translation regulator TSC1 and TSC2 yield ASD symptoms; mutations in small GTPase Ras, its negative regulator NF-1, and effector kinases are associated with learning disabilities; and mutations in the nuclear effector of the MAP kinase cascade, MeCP2, cause Rett syndrome with features of both ASD and ID. See also Figures 3–27 and 3–41.

encoded by the causal gene for Rett syndrome. MeCP2 is phosphorylated at multiple sites in response to neuronal activity, and phosphorylation regulates its gene repressive activity.

Defects in synaptic signaling may also be a major cause for psychiatric disorders such as schizophrenia and bipolar disorders. Indeed, mutations in several genes, including those that encode Ca_v1.2, Neurexin-1, and MeCP2, have been associated with both schizophrenia and ASD (see Table 11–2). As new genes associated with brain disorders are being discovered at a rapid pace thanks to recent advances in human genetics (see Box 11–3), the overlap between genes associated with psychiatric and neurodevelopmental disorders will likely increase, as will the link between these disorders and synaptic signaling.

11.27 Studies of brain disorders and basic neurobiology research advance each other

If synaptic dysfunction is a common cellular mechanism for neurodevelopmental and psychiatric disorders, why do disruptions of different genes (and sometimes different mutations of the same gene) give rise to different disorders? Do these mutations affect all synapses equally, such that the final symptoms of these disorders are caused by overall suboptimal synaptic function? Or are synapses in specific brain areas with special circuit functions differentially affected in different disorders? We are still far from having satisfactory answers to these questions, and the answers may differ for different disorders. For example, as we learned in Section 11.22, MeCP2 disruption in inhibitory neurons appears to cause the mutation's strongest effects in a mouse model. An imbalance between excitation and inhibition has also been suggested to underlie epilepsy (see Box 11–4), ASD, and schizophrenia. As researchers use more sophisticated tools to dissect the contributions of different subpopulations of neurons, we will surely find better answers to the questions raised above. At the same time, studies that focus on specific diseases may shed light on how the normal brain functions—revealing,

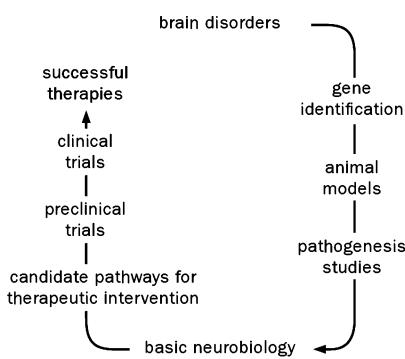


Figure 11–46 A general strategy for understanding and treating brain disorders. The right path links brain disorders to underlying basic neurobiology; the left path uses knowledge of basic neurobiological research for therapeutic intervention. (Adapted from Zoghbi HY & Bear MF [2012] *Cold Spring Harb Perspect Biol* 4:a009886.)

for instance, the specific brain regions and circuits crucial for intelligence, social interactions, and other complex cognitive functions.

Studies of genetically defined brain disorders have also introduced general strategies for treating these disorders (Figure 11–46). Identification of defective genes underlying brain disorders leads to the establishment of appropriate animal models. This enables mechanistic studies of the pathogenic process that enrich our understanding of basic neurobiology and at the same time suggest candidate pathways for therapeutic intervention. Development and clinical trials of appropriate drugs may eventually lead to successful therapies. For disorders whose underlying causes are largely unidentified, are multigenic, or are largely nongenetic, parts of this discovery-to-treatment path can still apply. Although we don't have effective therapies for most of the disorders described in this chapter, new advances in basic and disease-focused neurobiology research are being made each day, and breakthrough treatments for disabling brain disorders are anticipated in the coming decades.

Box 11–4: Epilepsy is a disorder of neuronal network excitability

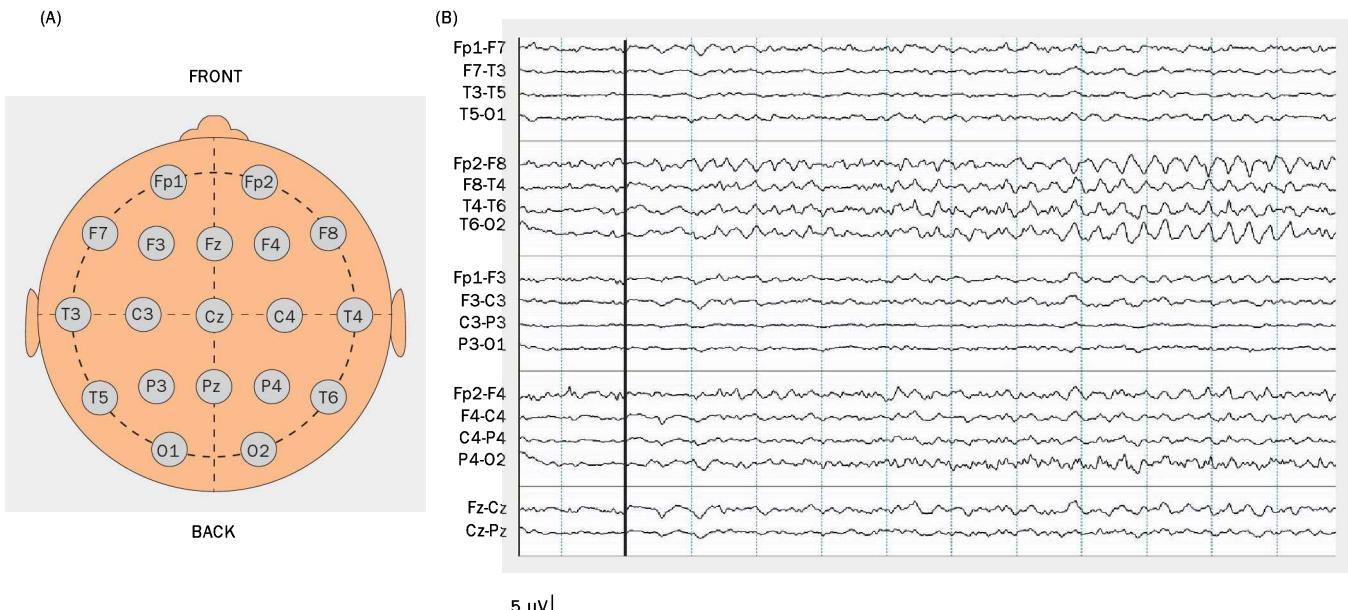
We have encountered seizures and epilepsy many times in this book. With the proper framework of studying brain disorders established, we are now ready to discuss the symptoms, causes, and treatment strategies of seizures and epilepsy. A **seizure** is an episode involving abnormal synchronous firing of large groups of neurons; about 1 in 20 people has at least one seizure in the lifetime. **Epilepsy** is a chronic condition characterized by recurrent seizures, which affects about 1% of the human population. When cortical neurons are engaged in abnormal synchronous firing, the activities can often be detected on **electroencephalograms (EEGs)**; EEGs record electrical potential differences between surface electrodes placed on specific locations of the scalp, which report the collective electrical activities of many nearby cortical neurons underneath the surface electrodes (Figure 11–47).

Seizures are typically categorized into either focal (partial) or generalized. **Focal seizures** are defined by clinical symptoms or EEG changes that indicate an initial activation of neurons in a relatively small, discrete region of the brain. Depending on the brain region, the symptoms can be a temporary loss of sensation, an odd sensory experience, a temporary loss of movement control, or confusion. **Generalized seizures** affect multiple, bilateral regions of the brain. In primary generalized seizures, the entire cortex seems to be activated at the same time, whereas a secondary generalized seizure results when a focal seizure spreads to larger areas of the brain. A generalized **absence seizure** (formerly called petit mal) is characterized by a brief lapse of consciousness (about 10 seconds or less) and a cessation of motor activities without loss of posture. A generalized **tonic-clonic seizure** (previously called grand mal) is associated with loss of consciousness and a predictable sequence of motor activity:

patients first stiffen and extend all extremities (tonic phase), then undergo full-body spasms during which muscles alternately flex and relax (clonic phase).

As with many brain disorders discussed in this chapter, epilepsy has diverse causes, including head injury, infection, strokes, brain cancers, and brain surgery. Epilepsy can also result from inherited or *de novo* mutations in several dozen identified genes, some of which appear monogenic whereas others confer risks. Other brain disorders, notably neurodevelopmental disorders, such as Rett syndrome, can have epilepsy as a symptom. Despite the diverse causes, epilepsy shares a common phenotype: an abnormal balance between the actions of excitatory and inhibitory neurons (the **E-I balance**) results in hyperactivation of excitatory neurons that spread the abnormal excitation across the network. This notion is best illustrated by examining epilepsies caused by defective ion channels (**channelopathies**; Table 11–3).

Given the key roles of voltage- and ligand-gated ion channels in regulating neuronal excitability that we have learned in Chapters 2 and 3, it is not surprising that mutations disrupting ion channels can cause abnormal neuronal firing. For instance, voltage-gated K⁺ channels are key for repolarization of neurons after excitation; a reduction of their function can cause abnormal excitation of mutant neurons. Likewise, reduction of GABA_A receptor function can cause epilepsy because neurons do not receive proper inhibitory signals. Let's examine a specific example in more detail: loss of one copy of the gene encoding a voltage-gated Na⁺ channel, Na_v1.1. This human condition, called Dravet syndrome or severe myoclonic epilepsy of infancy (Table 11–3), has been recapitulated in the mouse model—mice heterozygous for a knockout allele of Na_v1.1 exhibit spontaneous seizures and sporadic death. Voltage-gated Na⁺ channels are generally

Box 11–4: Epilepsy is a disorder of neuronal network excitability (Continued)

Figure 11–47 Detecting seizure onset by electroencephalograms.

(A) Schematic of surface electrode placement on the scalp. The electrode positions are named according to cortical regions (F, frontal; T, temporal; P, parietal; O, occipital; C: central, that is, closer to the vertex of the head). **(B)** EEG record of a patient suffering from focal epilepsy presumed to originate from the right temporal lobe. Each row is the electrical potential difference between two designated electrodes according to panel A. The solid vertical line indicates the onset of seizure. Before the onset,

EEGs are small in amplitude and mostly asynchronous, reflecting brain activity during normal awaking period. After seizure onset, EEG records between multiple pairs of electrodes on the right hemisphere show a large-amplitude synchronous pattern. Note that the exact time of onset or the location of epileptic activity cannot be determined with scalp recordings; for this, intracranial electrodes need to be implanted in the presumed area of seizure activity. (Courtesy of Dr. Josef Parvizi, Stanford University Medical Center.)

responsible for producing action potentials, and therefore their reduction should inhibit rather than promote excitability. However, whereas most excitatory neurons express multiple genes encoding voltage-gated Na^+ channels, $\text{Na}_v1.1$ is highly expressed in GABAergic inhibitory neurons. Thus, a reduction of $\text{Na}_v1.1$ activity preferentially reduces the excitability of inhibitory neurons and thereby increases the network excitability. This example illustrates why mutations in a given ion channel cause specific types of epilepsy; this is likely because the neuronal types that uniquely express a particular ion channel (and therefore can least compensate for the loss) differ for each gene implicated in epilepsy.

Furthermore, the phenotypic severity of the same mutation often differs in individual patients or in mice with different genetic backgrounds, highlighting that even monogenic mutations may be subjected to complex interactions with other factors.

Channelopathies can reveal some of the mechanisms that produce seizures, but these mutations account for only a small fraction of epilepsy cases. Although the root causes in other cases are less clear, perturbation of excitation-inhibition balance may also be the culprit. For example, the post-injury neuronal process sprouting and synapse

Table 11–3: Representative examples of ion channel mutations that cause epilepsy

Affected protein	Disorder
α_4 subunit of nicotinic ACh receptor	autosomal dominant nocturnal frontal lobe epilepsy ¹
$K_v7.2$ or $K_v7.3$ (voltage-gated K^+ channels)	benign familial neonatal seizures ¹
α_1 subunit of GABA_A receptor	juvenile myoclonic epilepsy ²
$\text{Ca}_{v}2.1$ (voltage-gated Ca^{2+} channel)	absence epilepsy and episodic ataxia ²
$\text{Na}_v1.1$ (voltage-gated Na^+ channel)	severe myoclonic epilepsy of infancy ²

This is only a partial list of known mutations in ion channels that cause focal (denoted by ¹) or generalized (denoted by ²) seizures. All mutations above are autosomal dominant, and epilepsy in most cases is a result of loss-of-function effect (reducing the dose) of the gene product. Data from Lerche et al. (2013) *J Physiol* 591:753.

(Continued)

Box 11–4: Epilepsy is a disorder of neuronal network excitability

formation that results from physical injuries to the brain, such as strokes and surgical removal of brain tissues, may differ for excitatory and inhibitory neurons, thereby perturbing the delicate E-I balance. Another important factor that may contribute to recurrent seizures is the act of seizure itself: many neurons firing in synchrony can cause significant changes in the involved circuits according to the plasticity rules discussed in Chapter 10. These activity-dependent changes may in turn decrease the threshold for future seizures. Indeed, excess excitation of glutamatergic neurons, with abnormally high glutamate release and NMDA receptor activation, can result in excessive elevation of intracellular Ca^{2+} concentration of their postsynaptic target neurons, which can trigger **excitotoxicity** and neuronal death.

About two-thirds of epilepsy patients can be effectively treated by medication, thanks to the common phenotype of excessive network excitability. The most widely

used medications include GABA_A receptor agonists such as benzodiazepines (see Section 11.17) to boost network inhibition, drugs that enhance voltage-gated Na^+ channel inactivation to curb excitation, and drugs that inhibit voltage-gated Ca^{2+} channels to reduce synaptic transmission efficacy. About one-third of epilepsy patients suffer from intractable seizures that are not responsive to current medications. A fraction of these patients that suffer from focal seizures can be treated with brain surgery. The identification of seizure focus is the key, which is usually achieved by intracranial recording and stimulation during surgery. (Indeed, we have learned a great deal about the functions of individual human neurons as a result of this procedure; see Section 1.10.) If the seizure focus regulates non-vital functions and ideally is located in non-dominant hemisphere, then surgical removal of the affected brain tissues or severing their connections can be an effective treatment.

SUMMARY

Defined by a specific set of symptoms, each brain disorder has a unique pattern of genetic (and sometimes environmental) contributions. Huntington's disease (HD), Rett syndrome, and fragile-X syndrome are each caused by disruption of single genes. Mutations may follow Mendelian inheritance as in HD, or may be produced *de novo* as in Rett syndrome. More complex disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), intellectual disability (ID), and epilepsy are heterogeneous in their origin. Only a fraction of these disorders are caused by mutations in specific genes that follow Mendelian inheritance; most cases are sporadic and have incompletely defined causes that include genetic risk factors, *de novo* mutations, and environmental factors. Even more complex disorders, including all of the psychiatric disorders we discussed and the non-syndromic autism spectrum disorders (ASD), are mostly sporadic in the sense that genetic causes with full penetrance have not been identified. Whereas schizophrenia, bipolar disorder, and ASD have strong genetic contributions, environmental factors may play a more significant role in drug addiction, depression, and anxiety disorders. Future studies must carefully define the contributions of and interactions between genetic and environmental factors in the context of specific brain disorders.

A common pathological feature of neurodegenerative diseases is alteration in protein conformation, interactions, and homeostasis. AD is characterized by extracellular $\text{A}\beta$ deposition and intracellular tau aggregation. Most PD cases involve aggregated α -synuclein. Multiple ALS-causing mutations result in the aggregation of distinct mutant proteins, with TDP-43 aggregation occurring in most sporadic cases. HD and spinocerebellar ataxia are caused by toxic gain-of-function effects associated with aggregation of polyglutamine repeats in distinct proteins. Prion diseases are caused by propagation of pathogenic PrP^{Sc} , which converts nonpathogenic PrP^{C} to PrP^{Sc} aggregates. The ultimate symptoms for different neurodegenerative diseases reflect the distinct neuronal types affected. AD and prion diseases affect a broad range of neuronal types, whereas PD symptoms are primarily caused by death of substantia nigra dopamine neurons, and ALS preferentially affects motor neurons.

The causal roles of mutant genes in monogenic diseases are well established, and efforts to understand diseases with more complex genetic contributions can

benefit from investigating the subset caused by Mendelian mutations. For example, genetic alterations that increase APP expression, A β production, or propensity for A β oligomerization are sufficient to cause AD in humans and AD-like pathology in mouse models, suggesting that A β and its oligomers play a causal role in AD pathological process. Disease-causing PD mutations and drug-induced PD symptoms both implicate the importance of mitochondrial function in maintaining dopamine neuron health. While most neurodegenerative diseases do not have effective treatments, PD symptoms can be alleviated at least temporarily by L-dopa injection and deep brain stimulation, and may potentially benefit from cell-replacement therapy.

Our current understanding of psychiatric disorders has benefited from studying the actions of serendipitously discovered drugs that have therapeutic effects. For example, most antipsychotic drugs that reduce the positive symptoms of schizophrenic patients act as antagonists of the dopamine D₂ receptor. The most effective antidepressants block the action of serotonin reuptake into the presynaptic terminals. Enhancing GABAergic inhibition mediated by specific GABA_A receptors is effective in reducing anxiety. Studies on the cellular effects of addictive drugs and reward-based learning have suggested that addictive drugs act by hijacking the dopamine-based reward system. As these neurotransmitter systems have broad actions in diverse brain areas, investigating specific neural circuits that mediate these drug actions and that are abnormal in psychiatric disorders are likely key for generating better treatments in the future.

Animal models that recapitulate certain disease symptoms can be used to investigate disease mechanisms and potential therapeutic strategies. This approach has elucidated the causes of syndromic neurodevelopmental disorders such as Rett syndrome and fragile-X syndrome. Rett syndrome is caused by disruption of MeCP2, a global regulator of gene expression that is particularly important in post-mitotic neurons. MeCP2 is required both during postnatal development and in adults, and reactivation of MeCP2 in adult mice can ameliorate defects caused by developmental disruption of MeCP2. Fragile-X syndrome is caused by disruption of FMRP, an RNA-binding protein involved in translational regulation. FMRP's substrates include many ASD-associated genes. Recent human genetic studies have identified increasing numbers of genes associated with psychiatric and neurodevelopmental disorders. These studies suggest that despite their diverse symptoms, many disorders share synaptic dysfunction as a common cellular mechanism and potential target for further research and treatment efforts.

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