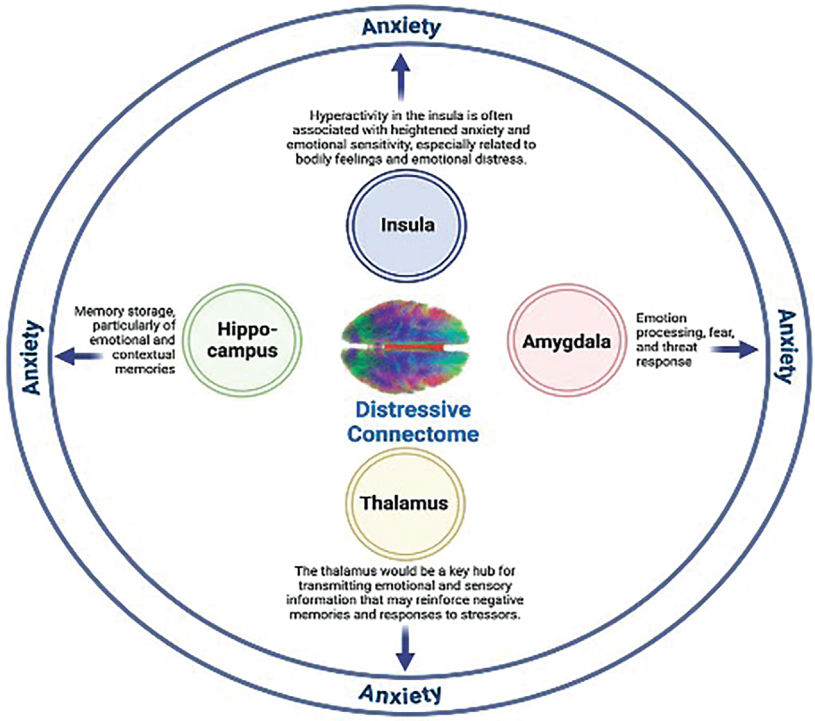
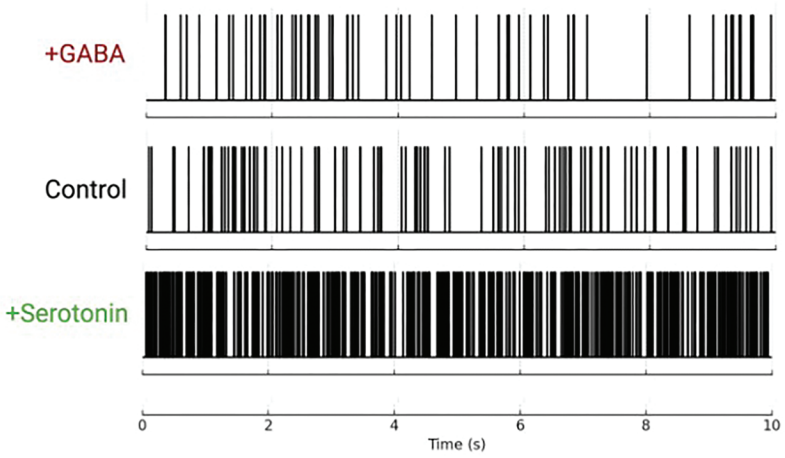
CHAPTER 2

Figure 2.1: The "Hyperactive Distressive Connectome" Hypothesis



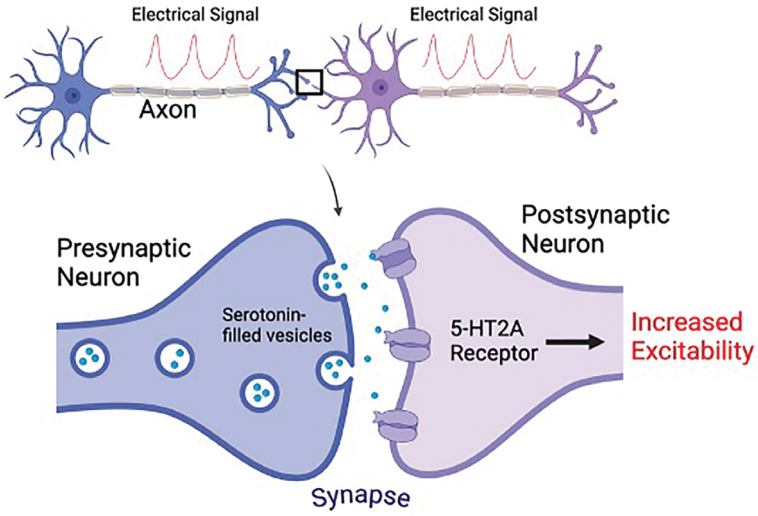
Note: As illustrated, this Connectome involves key brain hubs, including the **insula**, **hippocampus**, **amygdala**, *and* **thalamus**. *When hyperactivity is present in this network, it can perpetuate a cycle of emotional distress, leading to chronic anxiety.*

Figure 2.2: Simulated Neuronal Spiking Activity in the Presence of GABA & Serotonin



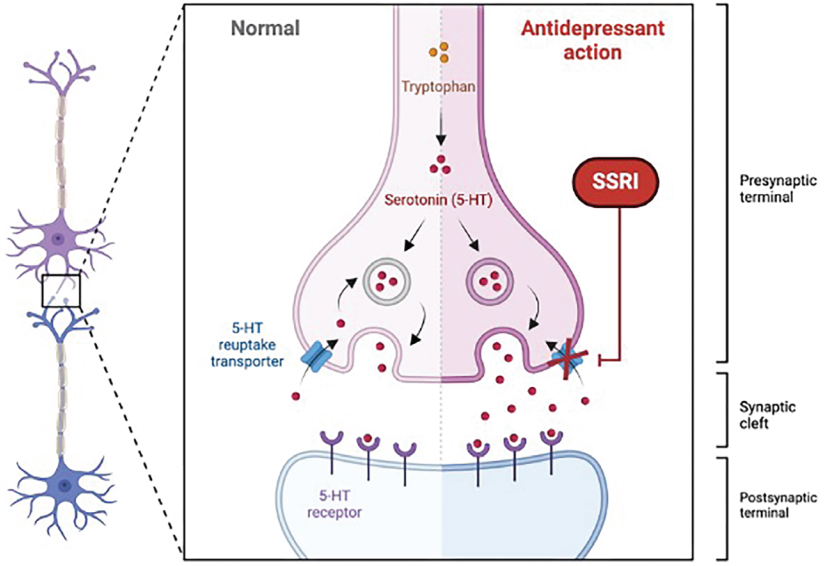
Note: These data do not represent actual data and are for illustration purposes only. The top panel shows neuronal spiking activity with the application of GABA, which acts as an inhibitory neurotransmitter, reducing the frequency of action potentials, the main form of electrical signals used to communicate within the nervous system. The middle panel represents control conditions, demonstrating typical neuronal spiking. The bottom panel shows the effect of serotonin application, which is known to have excitatory effects and lead to an increased frequency of action potentials. This contrast between GABA's inhibitory and serotonin's excitatory actions highlights these neurotransmitters' different modulatory roles in regulating neuronal activity.

Figure 2.3: The Serotonin Synapse



Note: The synapse is a narrow gap between the presynaptic and postsynaptic terminals of adjacent neurons at a synapse. It serves as a physical barrier for transmitting electrical signals between neurons. Neurotransmitters are chemicals that bridge that gap and allow the electrical signal to be converted into a chemical signal. In this case, serotonin is released by the presynaptic neuron into the synapse, diffusing across the gap to bind with, in this example, 5-HT2A receptors on the postsynaptic neuron, thereby facilitating the transfer of information between neurons. The result is an increased excitability (that is, firing rate) of the postsynaptic neuron.

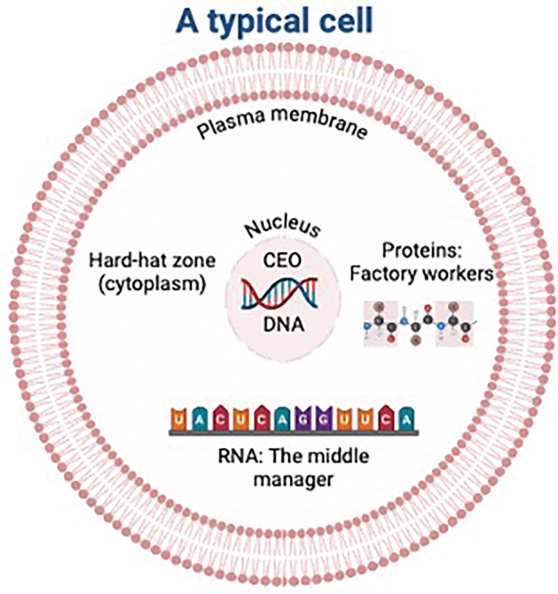
Figure 2.4: Selective Serotonin Reuptake Inhibitor (SSRI) Action



Note: This diagram illustrates the normal function of serotonin (5-HT) in the synaptic cleft and the effect of SSRIs on serotonin reuptake. In a typical synapse, serotonin is released from the presynaptic terminal and binds to 5-HT receptors on the postsynaptic neuron. Usually, serotonin is reabsorbed by the presynaptic neuron via 5-HT reuptake transporters, thus limiting its availability in the synaptic cleft. SSRIs inhibit the 5-HT reuptake transporters, preventing serotonin from being reabsorbed, which increases its concentration in the synaptic cleft and enhances its binding to postsynaptic receptors, resulting in an antidepressant effect.

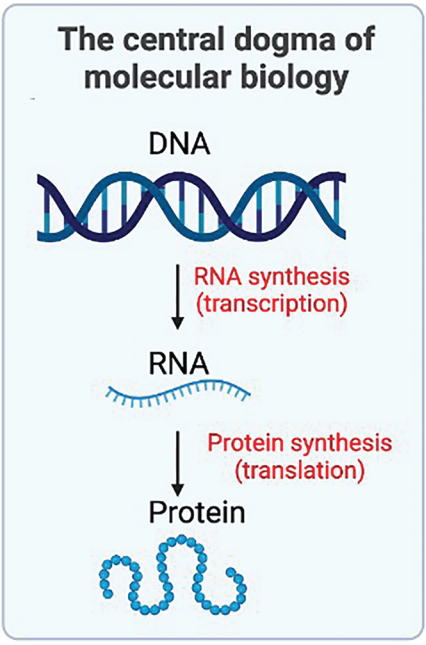
CHAPTER 3

Figure 3.1: Standard Features of All Cells



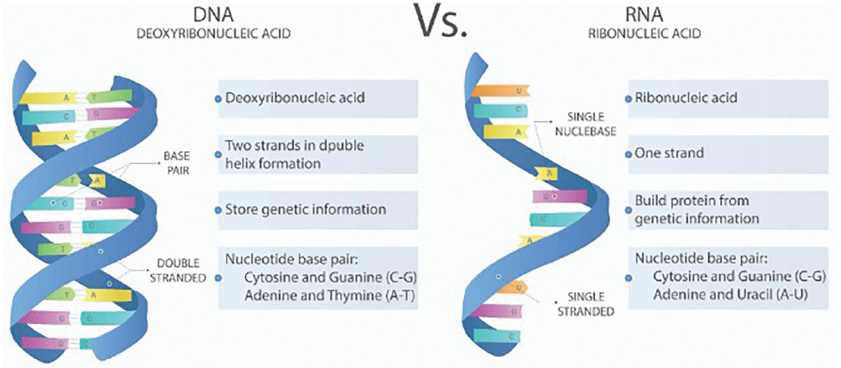
Note: The plasma membrane acts as a chain-linked fence, allowing tiny things to enter and exit, but larger hydrophilic substances cannot. The "CEO's office," where the DNA, the "top manager," resides, is in the nucleus, and entry and exit are highly restrictive. The hard-hat zone is the cytoplasm where basic metabolism happens, and protein synthesis occurs under the "middle manager": RNA.

Figure 3.2: Gene Expression in All Cells



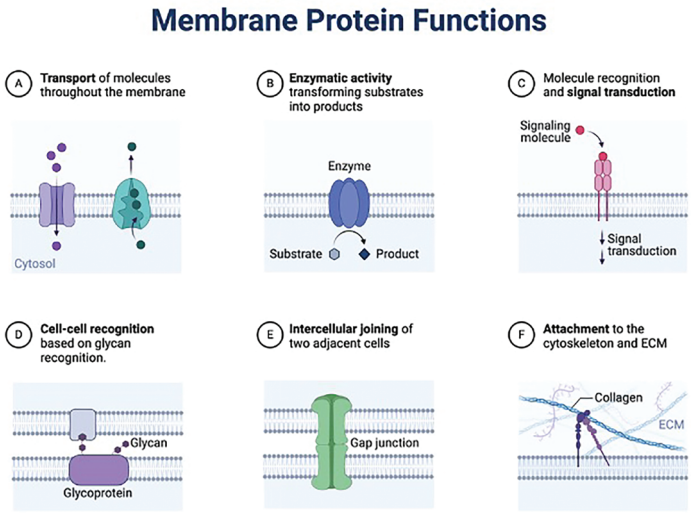
Note: The instructions in the DNA are read out (transcribed) and converted into mRNA. The message carried by the RNA molecule is converted (translated) into a protein molecule by linking amino acids.

Figure 3.3: Key Differences Between DNA & RNA



Note: DNA is double-stranded, forming a twisted helix structure, and its central role is to store genetic information. It uses the nucleotide base pairs adenine (A) with thymine (T) and cytosine (C) with guanine (G). In contrast, RNA is single stranded and helps in protein synthesis by translating genetic information from DNA into proteins. RNA's nucleotide base pairs include adenine (A) with uracil (U) and cytosine (C) with guanine (G). Adapted from Shutterstock.com.

Figure 3.4: Six Key Roles that Proteins Play in the Cell Membrane

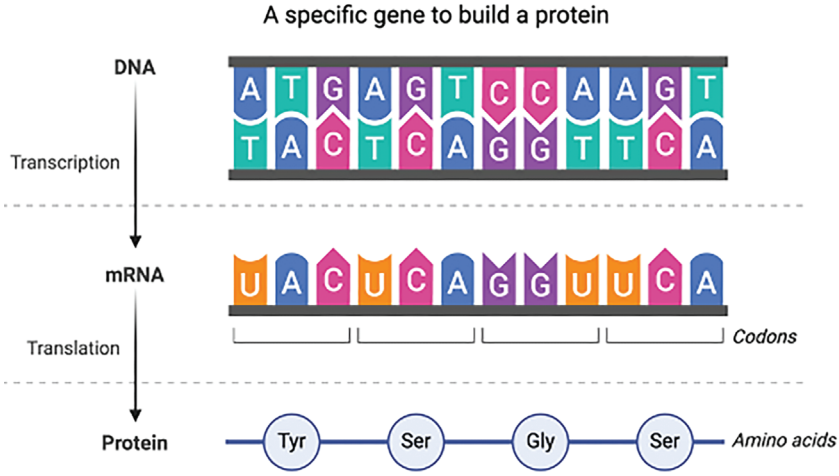


The six key roles depicted in Figure 3.4 are:

1. **Transport:** Proteins help move molecules like nutrients or ions into and out of the cell.
2. **Enzymatic activity:** Some proteins act as enzymes, speeding up chemical reactions inside or outside the cell.
3. **Signal recognition and transduction:** Membrane proteins can detect signals outside the cell and trigger responses inside.
4. **Cell to cell recognition:** Proteins help cells recognize each other, which is essential for communication and immune responses.
5. **Intercellular joining:** Proteins allow cells to connect and work together as a unit.
6. **Attachment to the cytoskeleton and extracellular matrix:** Some proteins anchor the cell to its surroundings and help maintain its shape.

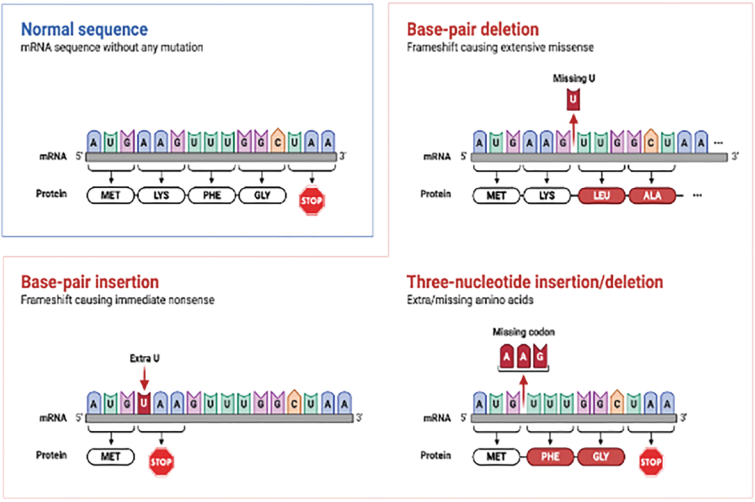
CHAPTER 4

Figure 4.1: Using DNA to Create Proteins



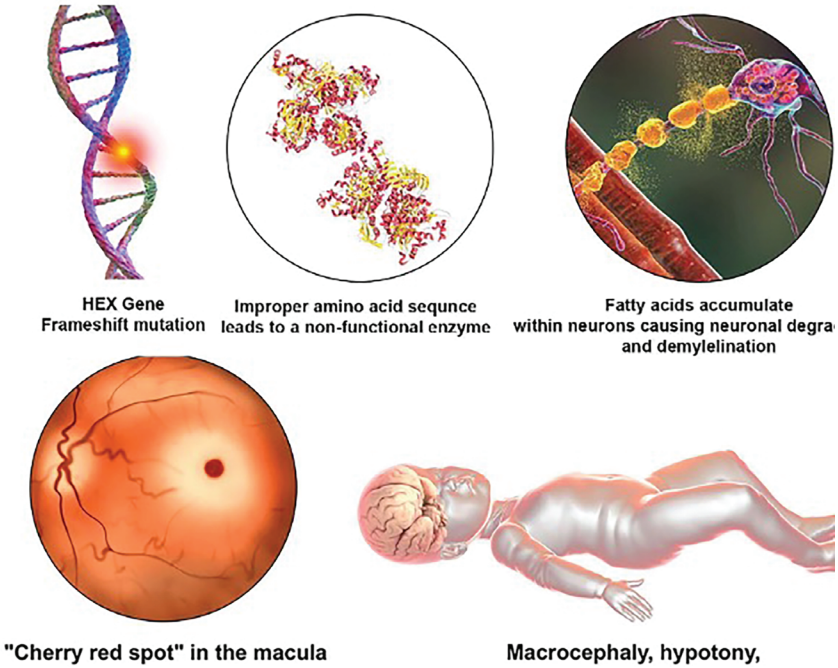
Note: This schematic illustrates the process of using DNA to create proteins. First, the information in DNA is copied into mRNA during a process called transcription. The next step is translation, whereby the mRNA is read in groups of three nucleotides, known as codons, to form a protein. Each codon represents an amino acid.

Figure 4.2: DNA Mutations Can Affect the mRNA Sequence



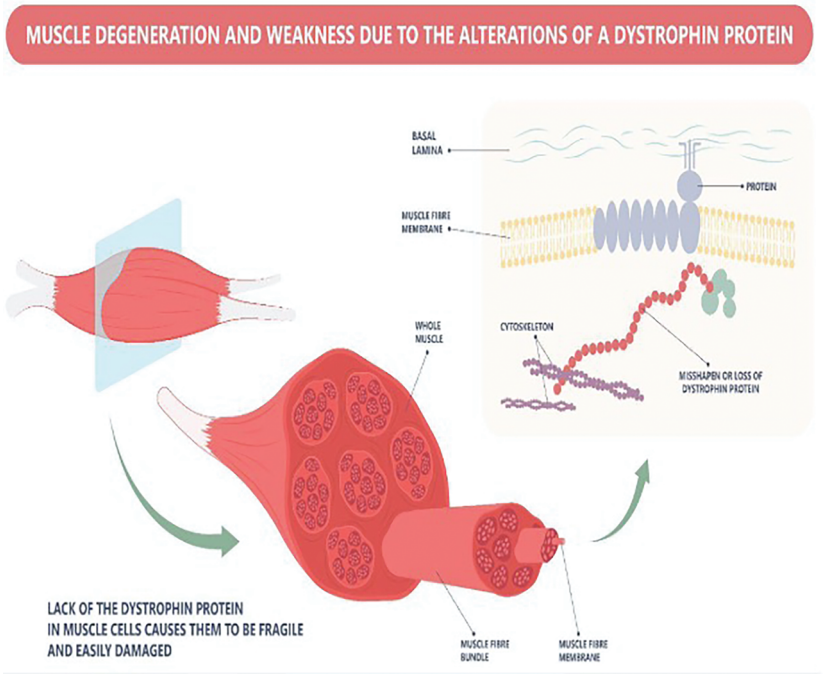
Note: This figure illustrates how different DNA mutations can affect the mRNA sequence and the resulting protein. The normal sequence in the top-left panel shows an mRNA sequence with no mutations, producing a functional protein. In the top-right panel, a base-pair deletion removes a single base/nucleotide, causing a "frameshift mutation." This mutation alters the reading frame and changes the resulting amino acids. The bottom-left panel shows a base-pair insertion, where an extra nucleotide causes an immediate stop codon, leading to a shortened, nonfunctional protein. Finally, the bottom-right panel shows a three-nucleotide insertion/deletion, which removes or adds a whole codon, leading to the loss or addition of an amino acid without shifting the reading frame. However, the missing codon still affects the protein structure.

Figure 4.3: A Frameshift Mutation Leads to Tay-Sachs Disease



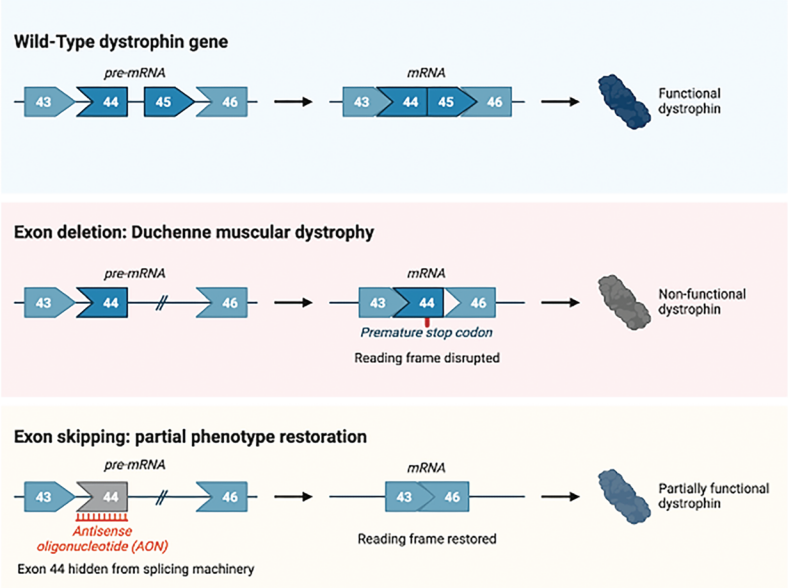
Note: Mutations in the HEXA gene lead to a nonfunctional enzyme (hexosaminidase A) that is quickly degraded by the neuronal cells. Because this enzyme is supposed to break down certain fatty substances called gangliosides, accumulating these fats leads to eventual neuronal death, loss of motor skills and vision, seizures, and eventually other problems. Unfortunately, children with Tay-Sachs do not live beyond early childhood. Adapted from Kateryna Kon/Shutterstock.com.

Figure 4.4: Duchenne's Muscular Dystrophy (DMD)



Note: The dystrophin protein plays a role in upholding the integrity of muscle cells. When it is absent or malfunctioning, as depicted, muscle fibers become delicate and prone to damage. Insufficient functional dystrophin causes muscle cells to struggle with the high mechanical demands of activities, resulting in a breakdown of muscle tissue over time.

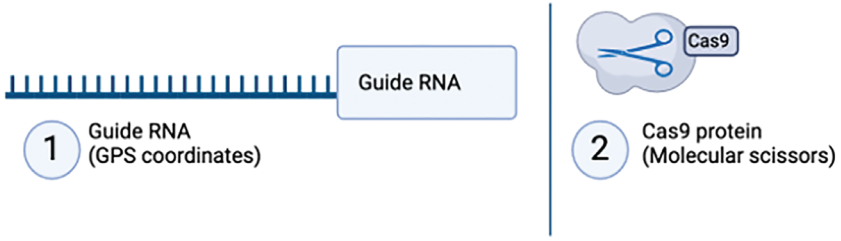
Figure 4.5: Impact of Alterations in the Dystrophin Gene



Note: Exons 43–46 are accurately connected to form the functional dystrophin protein. In DMD, a deletion results in the loss of exon 45 within the gene sequence, which disrupts the gene's reading frame, triggering an early stop signal and producing a truncated non-functional protein. Exon-skipping therapy involves a method where exon 44 is concealed from the genes reading mechanism, which allows exons 43 and 46 to join, skipping the faulty exon and restoring part of the reading frame. Consequently, a shorter yet partly operable dystrophin protein is manufactured. This process is the basis for the FDA-approved drug Casimersen (brand name Amondys 45), currently used as a new treatment for DMD.

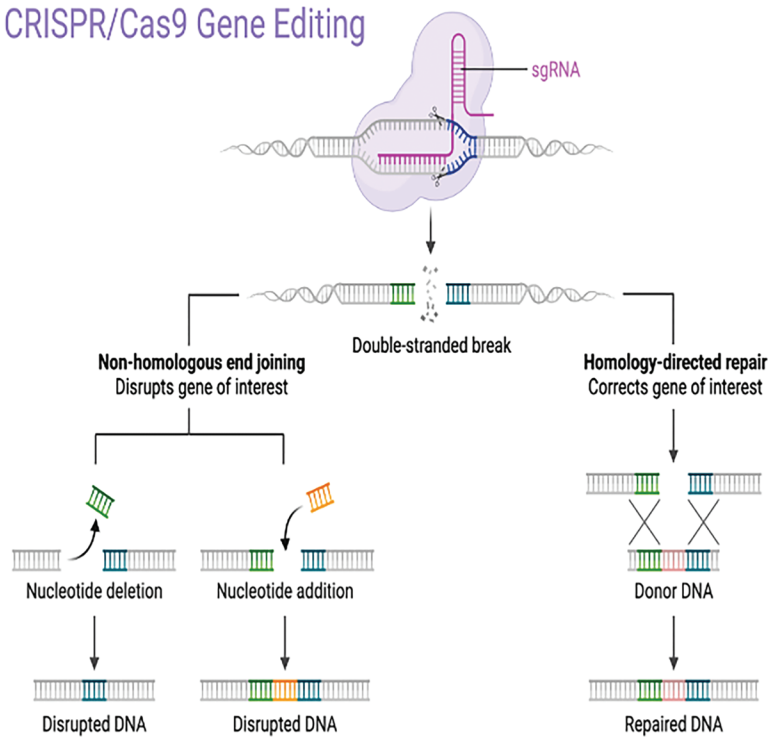
CHAPTER 5

Figure 5.1: Two Key Components of CRISPR



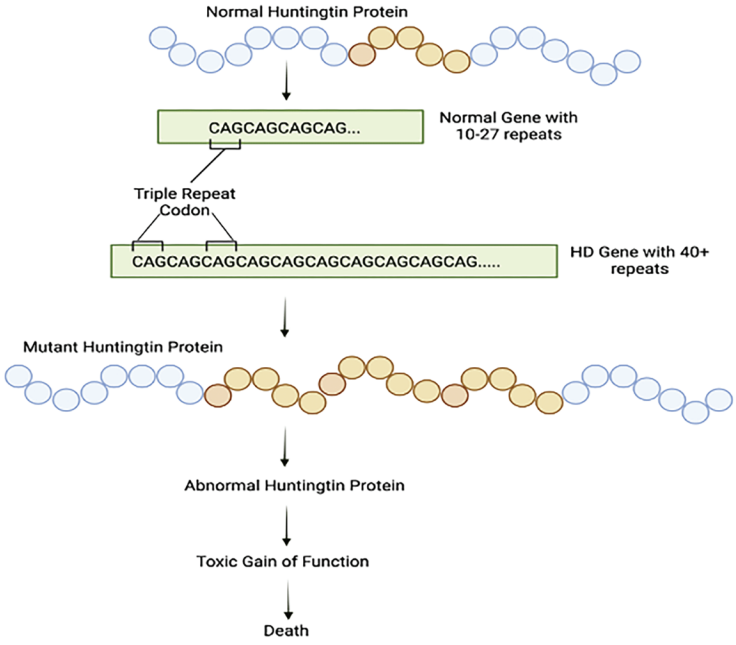
Note: The guide RNA acts like a set of GPS coordinates, directing the Cas9 protein to the precise location in the DNA that needs to be altered. Once at the target, the Cas9 protein functions as molecular scissors, cutting both DNA strands of the helix to enable the desired changes.

Figure 5.2: Two Methods to Mend Double-Stranded Breaks (DSBs)



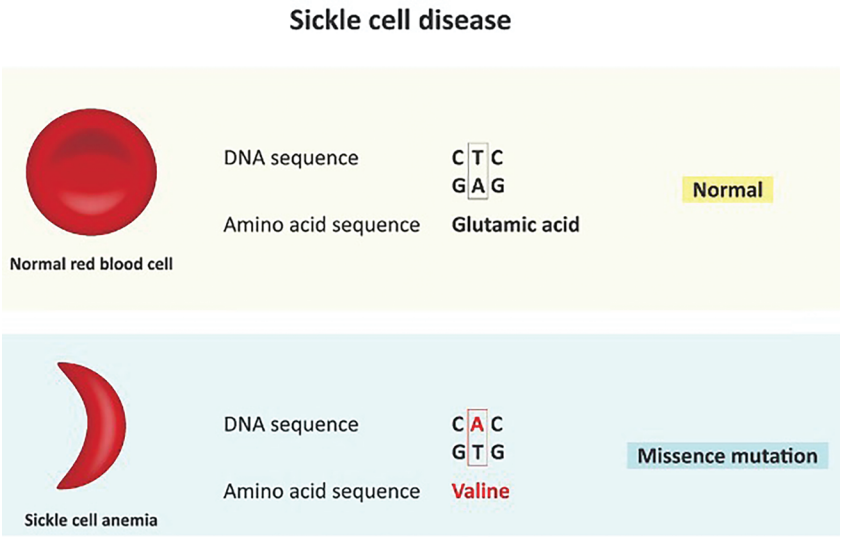
Note: Following cleavage by CRISPR/Cas9, two methods can mend the breaks. On the left is non-homologous end joining (NHEJ), a faster but less accurate technique than HDR. The right side depicts homology-directed repair (HDR), which mends the break precisely. From Dragt, Esmée (Creator), & Ngai, Louis (2017). Using CRISPR in your experiments. In CRISPR 101: A Desktop Resource (pp. 30-93). Addgene.

Figure 5.3: HD's Altered Versions of the Huntington Protein



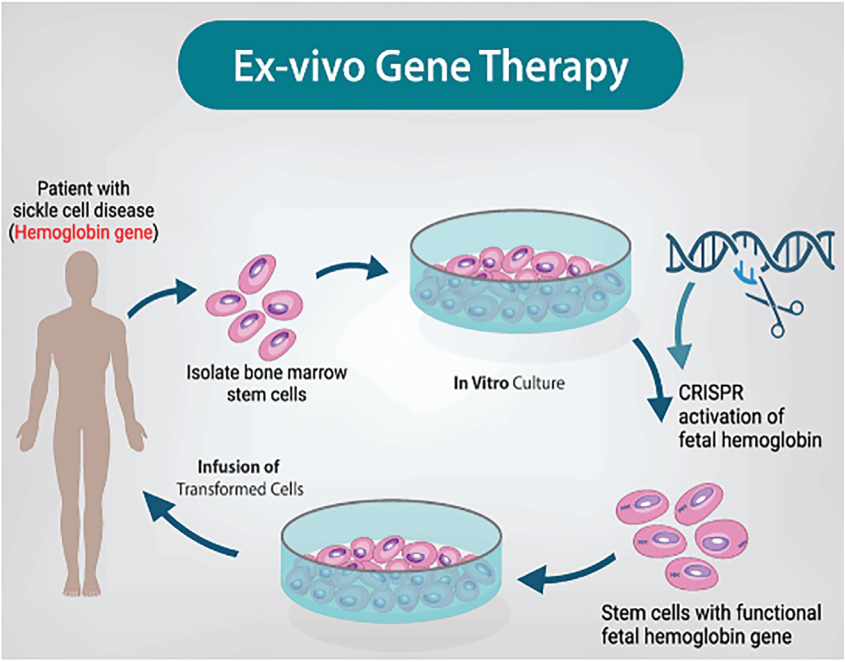
Note: The upper section displays the normal gene, with 10 to 27 CAG trinucleotide. In contrast, the lower section illustrates the HD gene, with 40 or more CAG repeats leading to a mutated form of the protein. The lower part shows that the altered protein acquires a toxic gain of function and eventual neuron death, leading to the symptoms of HD.

Figure 5.4: A Slight Alteration in DNA Results in Sickle Cell Disease



Note: In red blood cells (top), the genetic codon displays "CTC," which codes for the amino acid glutamic acid and maintains the round and pliable shape of the red blood cells. In sickle cell disease (bottom), a genetic mutation changes the DNA sequence to "CAC," resulting in valine being inserted as the amino acid. This small change alters the entire red blood cell structure into rigid red blood cells with a sickle-like shape; these cells can obstruct blood flow and lead to health issues.

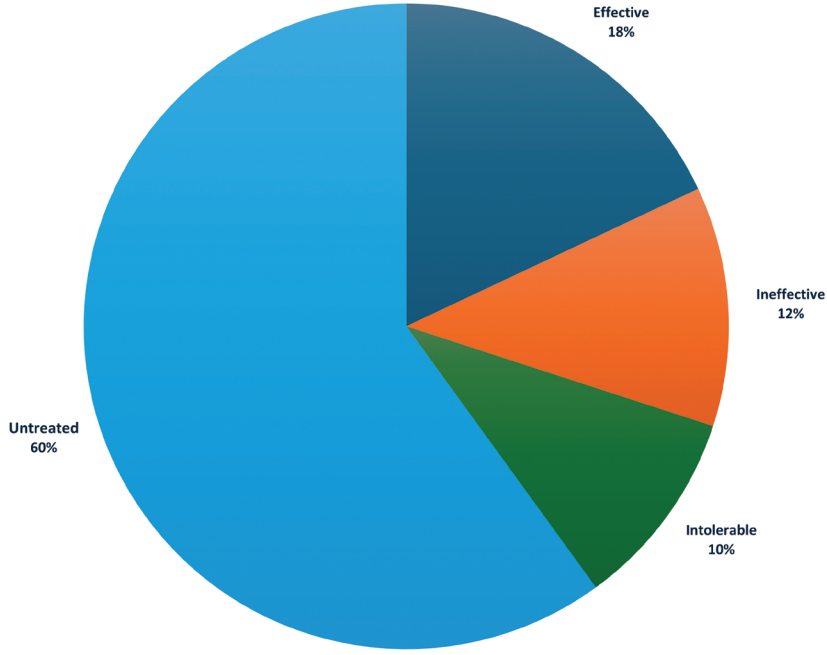
Figure 5.5: Gene Therapy for Sickle Cell Disease



Note: Stem cells from a sickle cell disease patient are extracted and cultivated in a lab. Next, CRISPR technology alters the DNA by activating the fetal hemoglobin gene. Finally, these modified stem cells are reintroduced into the patient's body with a working version of the fetal hemoglobin gene. These altered cells have the potential to support the generation of healthy red blood cells that have proven effective in affected patients.

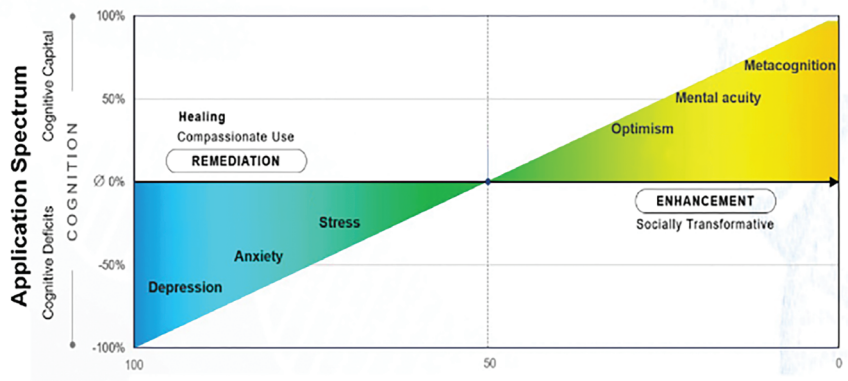
CHAPTER 6

Figure 6.1: The Overall Health Outcomes for Patients Diagnosed With an Anxiety Disorder



Note: A high proportion of the 60 percent did not receive any treatment, highlighting an issue in access to and provision of care. Of those who underwent treatment, 18 percent found it successful, 12 percent deemed it ineffective, and 10 percent encountered side effects.

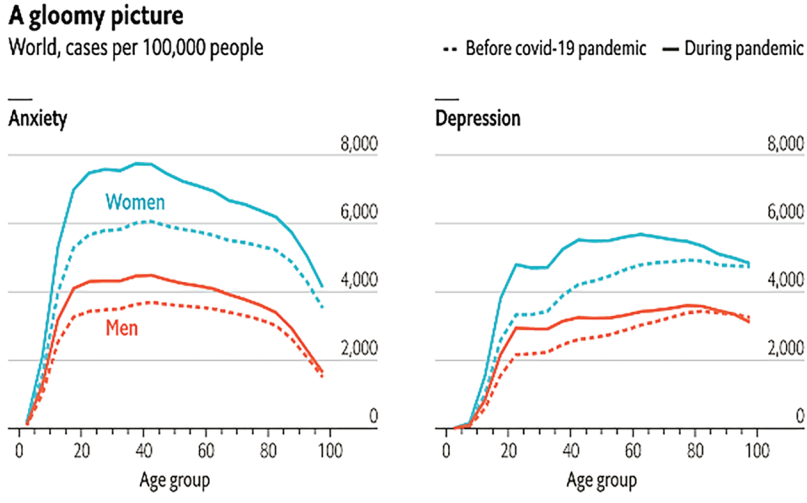
Figure 6.2: Applications of Gene Therapy: From Cognitive Deficits to Enhancing Abilities in Healthy Individuals



Note: On the left, therapies are used to manage conditions like depression, anxiety, and stress, falling under "compassionate use" for healing. Moving along the spectrum, the focus shifts to improving functions such as hopefulness and mental sharpness, which could impact society by enhancing cognitive reserve. The spectrum showcases both methods for therapy and potential advancements for cognitive enhancement.

CHAPTER 7

Figure 7.1: Global Prevalence of Anxiety and Depression Before and During the COVID-19 Pandemic, Across Different Age Groups

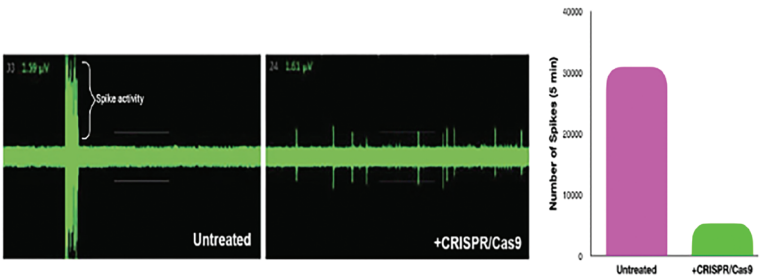


Note: Anxiety (left panel) and depression (right panel) show an increase in cases per 100,000 people during the pandemic (solid lines) compared to pre-pandemic levels (dashed lines). Women (blue lines) are more affected than men (red lines) in both disorders, particularly in younger age groups (Santomauro et al., 2021).

Figure 7.2: Using a CRISPR/Cas9 Gene-Editing Tool to Deactivate the HTR2A Gene

Note: The HTR2A gene is found on mouse chromosome 14, producing one particular type of protein: the 5-HT2A receptor. The black line represents the guide RNA (gRNA) and tells the Cas9 enzyme where to cut the DNA. The Cas9 enzyme will cut the DNA between two specific letters, T and C, which are part of the genetic code (represented by the red arrow). This gRNA is predicted to be very specific, meaning it is not expected to accidentally cut other parts of the DNA (off-target sites). After the cut, there is an 88.6 percent chance that repairing this cut causes a frameshift and introduces a premature stop codon and non-functional protein (see Chapter 5 for more detail on this protein production).

Figure 7.3: Using CRISPR/Cas9 Technology to Target and Disrupt the HTR2A Gene in Mouse Brain Neurons



Note: The left panel (Untreated) displays the electrical spikes recorded from untreated neurons, showing strong spike activity. The right panel (+CRISPR/

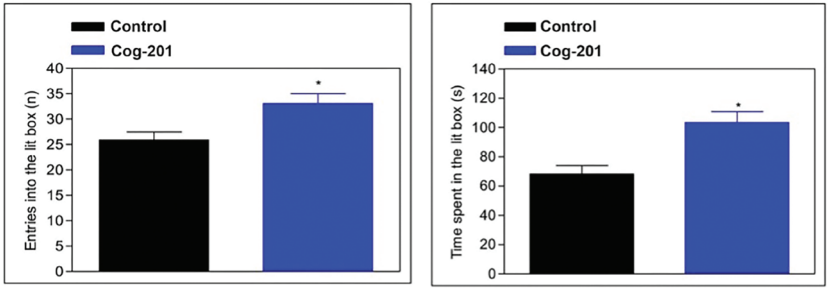
Cas9) shows that neurons treated with CRISPR/Cas9 to deactivate the HTR2A gene had much less electrical activity. The graph on the right quantifies this effect, revealing a significant drop in spikes (electrical signals) after the treatment (green bar), suggesting that knocking out this gene affects neuron firing.

Figure 7.4: Comparison of the Anti-Anxiety Effects of Gene-Editing Therapy (CRISPR/Cas9) and the Standard Anti-Anxiety Medication Diazepam (Valium)

Summary: We devised a sophisticated way of delivering gene therapy, which carried CRISPR/Cas9 and a genetic map (guide RNA) to target and turn off 5-HT2A receptors in particular regions of the brain associated with anxiety.

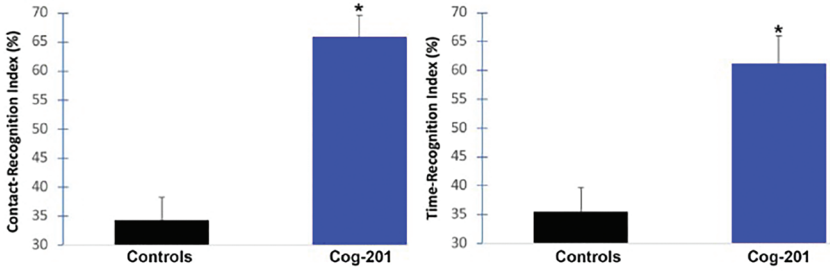
CHAPTER 8

Figure 8.1: The Impact of Two Treatments on Mice's Anxiety Levels



Note: Data from a light-dark box method test, where the black bars indicate mice that did not receive treatment (control), and the blue bars depict mice that received Cog-201. The graph on the left shows how frequently the mice went into the illuminated box area.

Figure 8.2: The Performance of Two Groups of Rats, One Treated With Cog-201 and One Left Untreated

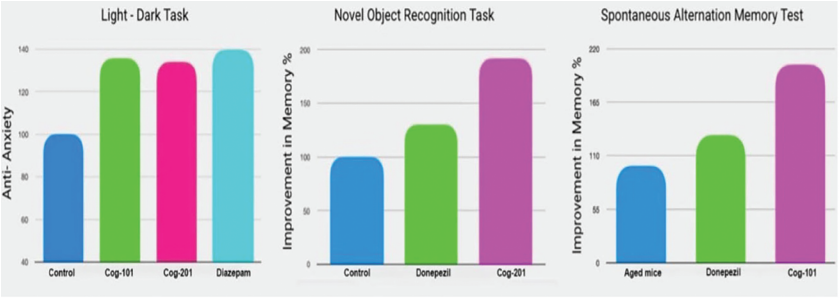


Note: One group was given gene therapy (represented by blue bars), and the other served as a control group (depicted by black bars). It illustrates their

performance in a memory assessment called the object recognition test. The graph on the left shows that the rats treated with gene therapy displayed significantly higher contact recognition index values than the control group represented by the black bars. This indicates that the rats treated with gene therapy tended to recognize and engage with the novel object more frequently than those in the control group. Treated rats also spent more time identifying the novel object than the control group, as indicated by the time recognition index in the right graph. These findings imply that Cog-201 enhanced memory and recognition in the treated rats.

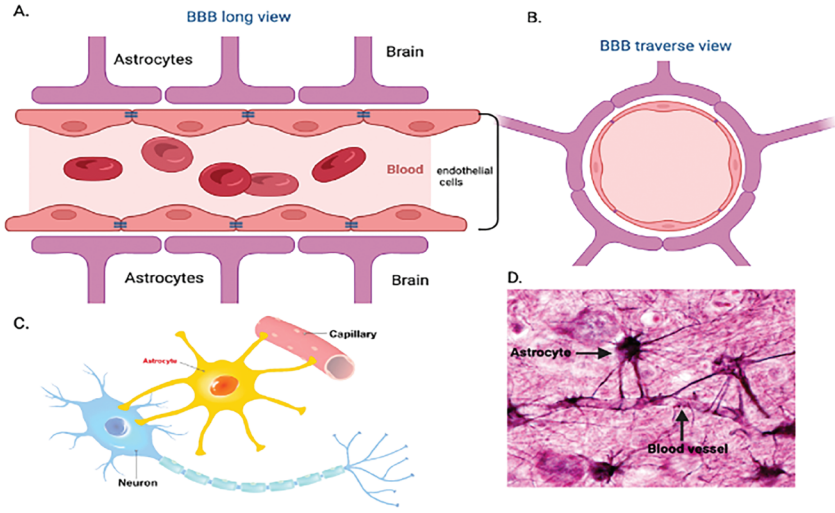
These findings were another "eureka" moment: not only did we have a potential therapeutic benefit on anxiety, but the improved memory could help millions of people who struggle with Alzheimer's disease, for which current medications are hardly effective. Therefore, precisely targeting the HTR2A gene presents a novel therapeutic approach for treating chronic anxiety and age-related cognitive decline.

Figure 8.3: Treatment Effects of Medication vs. Cog-101 or Cog-201



CHAPTER 9

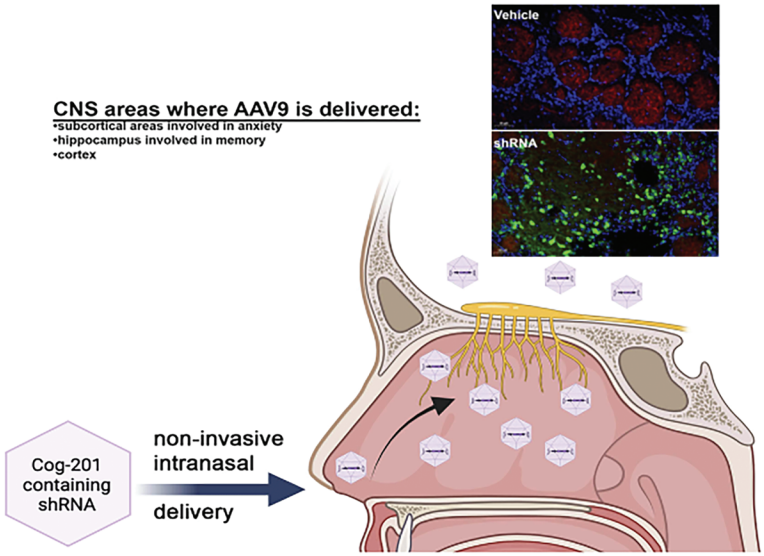
Figure 9.1: The Arrangement and Vital Elements of the BBB



Note: The BBB safeguards the brain from substances in the bloodstream while permitting nutrients to enter smoothly. Panel A: A long view of the BBB showcases how blood circulates through the capillaries (blood vessels). The barrier is constructed by packed cells lining these capillaries' walls. Surrounding these capillaries are astrocyte cells (in purple), which uphold the integrity of the BBB and sustain brain function. Panel B illustrates a cross-sectional view of the blood-brain barrier, with a circular depiction of a blood vessel surrounded by astrocytes. Astrocytes extend their "feet" to enfold the vessels and provide support while regulating the flow of substances into the brain. Panel C depicts the connection between neurons (blue), astrocytes (yellow), and blood vessels, highlighting how astrocytes facilitate communication between capillaries and brain cells to maintain brain function. Panel

D is an actual stained microscopic image in which dark-colored astrocytes envelop a blood vessel with their visible long extensions, interacting to uphold this protective barrier effectively and ensure the smooth passage of crucial nutrients and signals to the brain.

Figure 9.2: Nose-To-Brain Delivery of Gene Therapy Using AAV9 Vectors



Note: The top right panel depicts the mouse brain image indicating delivery of Cog-201 into the brain as illustrated by the green labeling of neurons in the olfactory bulb.

Figure 9.3: Nose-To-Brain Delivery of AAV9 Gene Therapy Via the Olfactory Pathway

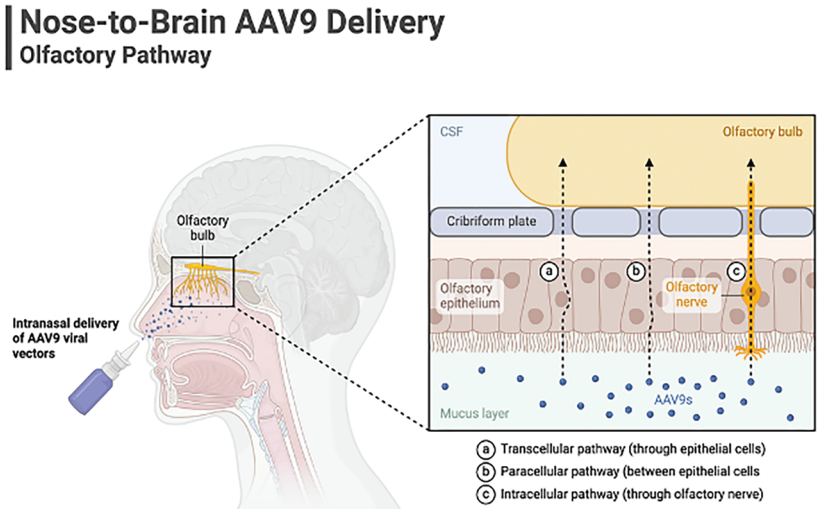
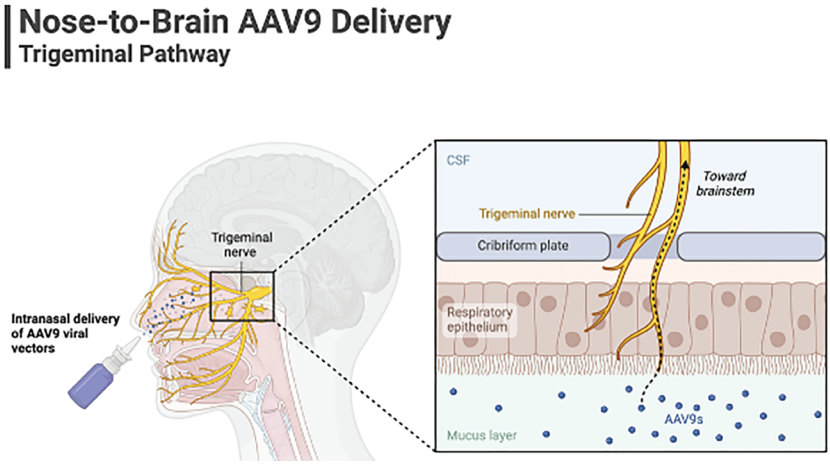


Figure 9.3 shows how AAV9 viral vectors, which carry shRNA, are delivered intranasally (through the nose) and can reach the brain. After being sprayed into the nose, the viral particles pass through the nasal cavity, where they come into contact with the olfactory epithelium, a tissue responsible for detecting smells. From here, the viral vectors can take different routes: they may pass directly through the cells, between the cells, or even travel along the olfactory nerve to reach the olfactory bulb, a brain region. This non-invasive delivery method targets the brain through natural pathways, providing a potential therapeutic approach for brain conditions.

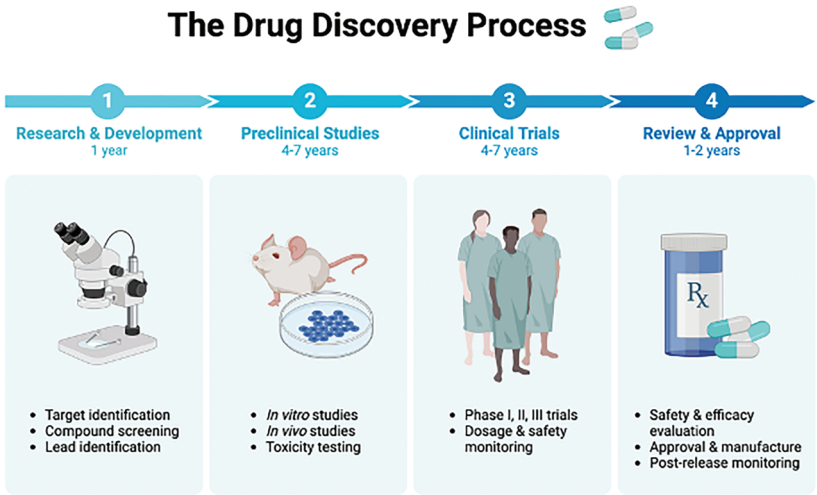
Figure 9.4: Nose-To-Brain Biomolecule Delivery Via the Trigeminal Pathway



Note: This image shows how AAV9 viral vectors can be delivered to the brain through the trigeminal nerve pathway. Adapted from Jeong et al., 2023.

CHAPTER 10

Figure 10.1: Drug Development in Four Phases



Note: The four phases are research and development (R&D), preclinical studies, human clinical trials to assess the effectiveness and proper dosage levels, and review for official approval before being available to the public market.