1 Introduction to Behavioral Endocrinology

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In this first chapter we review the basic principles of how hormones and the nervous system work. That means that this information will be packed rather densely. But students should have been exposed to most of the information before, and virtually all of these topics will be dealt with in specific examples in later chapters. This chapter is intended to be used as a reminder and a reference source for the basic concepts needed to appreciate the information given in the chapters that follow.

Some of the questions you should focus on while reading this chapter include: What are hormones, what are the different kinds of hormones, and how do they change biological processes? How does the brain control hormones, and how do hormones affect the brain? How do we measure behavior and hormones?

The Study of Behavioral Endocrinology

Behavioral endocrinology is the study of how hormones influence an animal's behavior. A. A. Berthold is credited with conducting the first formal experiments in behavioral endocrinology in 1849. We have learned a great deal since Berthold's time, but the basic logic and experimental design used for demonstrating a causal relationship between hormones and behavior is the same now as it was then.

Berthold removed the testes of a rooster and observed the animal's behavior. He found that the rooster no longer crowed or engaged in sexual or aggressive behavior. When Berthold reimplanted one testis in the body cavity, the castrated rooster once again began crowing and also exhibited normal sexual behavior and aggression. The reimplanted testis did not reestablish nerve connections, so Berthold concluded that some chemical, produced by the testes and released into the general circulatory system, influenced the rooster's behavior.

We now know that the effect produced by the grafted testis can be mimicked by administering the hormone **testosterone** to a castrated rooster. Testosterone is the main hormone synthesized and released by the testes of male vertebrates. A causal relationship between the presence of a certain hormone (testosterone) in the circulatory system and behavior (crowing, sexual behavior, aggressive behavior) is established by conducting experiments to show that when the hormone is present, certain behaviors are more likely to occur.

The same kinds of experiments are conducted in behavioral endocrinology today. In order to show that a particular hormone influences a particular

behavior, the scientist must demonstrate that the frequency of the behavior changes when the endocrine gland producing the hormone is removed. Then the scientist must show that the frequency of the behavior can be returned to normal by providing the animal with the missing hormone.

Berthold's experiment also shows that the study of behavioral endocrinology is the study of two related systems. First, we must learn about the endocrine system, represented by the testes in this case. Of course, neither the testes nor the testosterone these glands released into the bloodstream completely determined the rooster's behavior. Other factors such as the time of day and the presence of a hen or a rival rooster also made a difference. But the testosterone released from the testes reached the brain. There the hormone acted on nerve cells (neurons) in various brain regions and changed the likelihood that those behaviors would appear under the appropriate circumstances. Therefore, to understand how the hormone influenced behavior, we must study a second system, the nervous system.

Inherent in our discussion of how hormones can influence an animal's behavior is the idea that if hormones produce a change in an animal's behavior, there has been some sort of change in the animal, probably in its brain. We can try to find out which parts of the brain have been changed, how they have been changed, and how those modifications have caused an alteration in behavior. Conversely, if hormones cause alterations in the brain, there should be a corresponding change in the behavior or the physiology of the animal that we may be clever enough to detect.

Behavioral Methods: Field versus Laboratory Studies

Experiments in behavioral endocrinology obviously require the assessment and measurement of behavior. Most people assume that this is the easy part: Sit and watch. However, there are many ways to measure behavior, including ways that may not be valuable. To discuss the issues involved in methodology, we must first consider two very different types of research—laboratory and field. Surely the best way to understand behavioral endocrinology is to observe the creatures of interest in their normal habitat: deer in the woods, mice in the fields, and monkeys in the jungle. But research in the field is a great challenge. Conditions are not very comfortable for humans. Many important variables are beyond inspection or control. Unexpected turns of events are common. Therefore, it is difficult to observe the exact same phenomena twice. Consequently, two researchers trying to study the same things may observe and report very different field results. The difficult task of field researchers is to achieve consistent, repeatable results. Only then can they feel confident that they understand the phenomena.

The consistency field researchers strive for is, in fact, the great advantage of laboratory research because the investigator in the laboratory can control many variables and keep them constant. Temperature, humidity, food and water availability, and even, in some cases, the genetic make-up of the subjects, can all be controlled. If laboratory rats of strain X are kept in conditions

Y and given hormone Z, then they should display the same behaviors each time, and to a great extent they do. As will become obvious at various places in this book, however, there are plenty of individual differences even in a laboratory setting. But as more and more conditions are standardized to achieve consistency in laboratory results, you run a risk that what you are learning is applicable only to the laboratory.

This demonstrates an important strength of field research—validity. If you do learn something in the laboratory and can repeat this observation in the field, you know it is valid for the world-as-it-is. Ideally, laboratory workers read the field reports to help them judge which phenomena may be relevant outside the laboratory and therefore valid. In turn, if field researchers suspect, for example, that hormones are affecting a particular behavior, they may try to test that notion under the more controlled conditions of the laboratory.

To obtain results in the laboratory that approximate conditions in the field, the scientist must approach the experimental question from the perspective of the animal. If your subject is a rat, you need to ''think like a rat.'' In other words, you should design your behavioral tests to take advantage of the normal behavior of the animal in the wild. I. Q. Whishaw et al. described in exacting detail the methods that can be used to analyze the behavior of a laboratory rat. Some of these ideas are summarized in table 1.1.

This approach can be applied to the study of other species in the laboratory. The exact details of the behavioral tests will vary with the species, but the methods used to observe, analyze, and challenge the neurological functions of an animal can be generalized to any species. These authors argue that with the careful observation and description of an animal's appearance and behavior, ''meaningful generalizations about the organization of the nervous system [can] later be made'' (Whishaw, Kolb, and Sutherland 1983). This is also true for the behavioral endocrinologist who wants to make meaningful generalizations about the influence of hormones on the nervous system.

Experimental Design

The challenge of obtaining meaningful behavioral results in the laboratory can also be addressed by careful construction of the experimental procedures. There are a number of important things to consider when designing an experiment. For example, let's say that you have decided to investigate the effect of estrogen on sexual behavior in female rats. What groups will you compare? In order to control circulating estrogens in your animals, you may want to begin with animals that have had their own source of estrogen (the ovaries) removed (i.e., **ovariectomized** animals).

One way to test the effect of estrogen on sexual behavior in these ovariectomized rats would be to test all the animals first without estrogen. Then you could give the animals estrogen and repeat the behavioral test (this is known as a sequential design, repeated measures, or a within-animal comparison). One thing you need to be aware of, however, is that sometimes experimental procedures themselves can change an animal's behavior. For example,

Table 1.1 Behavioral Assessment of Neurological Function in Animals: Summary of Features of Behavior and Appearance That Can Be Examined

MEASURE	SPECIFIC FEATURE
Appearance	Body weight, core body temperature, eyes, feces, condition, genitals, muscle tone, pupils, responsiveness, saliva, teeth, toenails, vocalizations
Sensory and sensorimotor behavior	Response to auditory, olfactory, somatosensory, taste, vestibular, and visual stimuli presented both in home cage and open field
Posture and immobility	Behavior when spontaneously immobile; posture and muscle tone when immobile; tonic immobility or animal hypnosis; environmental influences on immobility
Movement	General activity, movement initiation, turning, climbing, walking, swimming, righting responses, limb movements, mouth and tongue movements
Species-typical behaviors	All species-typical behaviors including grooming, food hoarding, foraging, taste aversion, sleep, maternal behavior, sexual behavior, play, nest building, and burying
Learning	Classical conditioning, instrumental conditioning, and learning sets, especially including measures of spatial learning, avoidance learning, and conditioned taste aversion

From Whishaw et al. 1983.

perhaps the experience of the first test would affect performance in the second test regardless of hormone treatment. So it is important to run a control group that is tested a second time without receiving the hormone.

Another way to conduct the experiment is to use a simultaneous design (between-animal comparison). With this experimental design, each ovariectomized rat would be tested only once. One group would receive the hormone treatment—say, estrogen dissolved in peanut oil—while the control group would receive injections of the peanut oil vehicle without the estrogen. The behavior of the two groups of animals during the test would then be compared to see if the hormone has affected the behavior.

The advantage of the sequential design is that usually fewer animals are needed. The behavior of each animal during the two tests can be compared, and this tends to reduce variability in the data. On the other hand, if your procedures are causing changes in the animals' behavior for reasons other than the hormone treatment, a simultaneous design may be the best way to test your hypothesis. There are more complex experimental designs, but they are essentially variations and combinations of these two basic protocols.

Behavioral Observations

Next, you must decide how you will define and quantify the behavior. You must also choose your testing environment and procedures. Most behavioral

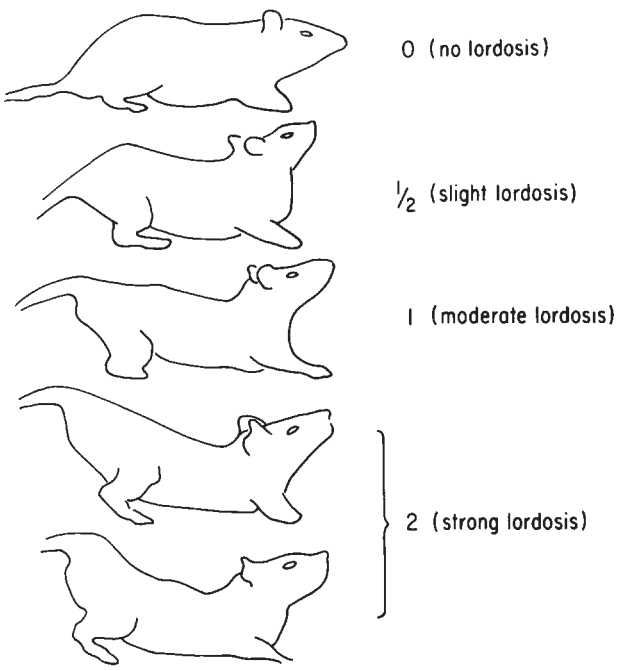

Figure 1.1 Lordosis in the female rat. These drawing were traced from single frames of films of rat mating encounters. Note the arching of the back with the elevation of the head and rump. (From Brink et al., 1978.)

experiments involve procedures that require an observer to make decisions about the animals' behavior. For example, before she is mounted by a male, a female rat exhibits a number of behaviors that have been termed ''proceptive'' behaviors: She will hop and dart and display ear wiggling in response to a male. You must decide if you are going to record these behaviors; if so, you must define them so that they are always scored in the same way. When a male rat mounts a receptive female rat, she will arch her back (head and rump are elevated) and deflect her tail to one side. This is a reflexive behavior known as **lordosis** which permits the male to achieve intromission (i.e., place his penis in her vagina). You must also decide how to quantify this behavior. You could count the number of times the rat displays lordosis in proportion to the number of mounts, measure the amount of time the rat is in lordosis, judge the completeness of the lordosis (figure 1.1), or make-up some combination of these. For each behavior you must come up with a written definition that you and the other observers agree on.

Because behavioral observation often requires making subtle judgments, it is also important that the observer be unaware of the specific treatment that an animal has received (this is known as being ''blind'' to the experimental conditions). This is usually achieved by coding the animals or treatments to hide their identity from the observer in order to prevent the observer's expectations from coloring his or her judgment. It is also a good idea to have more than one observer score the behavior to test the reliability of the data

across observers. In other words, do both observers score the behavior in the same way? This knowledge is important for several reasons. First, you want to be sure that the data have internal consistency, i.e., that all animals were scored in the same way. Second, you want to be sure that your definitions are precise enough to allow two observers using these definitions to obtain the same results. If so, then other investigators will be able to repeat your methods and replicate your results.

Other Considerations

Other things that need to be considered include when to make your behavioral observations and what dose or treatment regimen to use for the hormone administration. When in doubt, it is best to be guided by endogenous, physiological concentrations of the hormone (if they are known) and by what is already known about the temporal relations between the endogenous hormone fluctuations and the behavior under consideration.

When considering the dose, some investigators might be tempted to begin with high doses of a hormone just to be sure that they see an effect. Results from high doses, however, can be very misleading. A bell-shaped curve showing the relationship between a hormone and a behavior is frequently found in behavioral endocrinology. This means that within a physiological range of doses, the higher the dose, the more effective the hormone at producing a change in a behavior. On the other hand, higher doses of the hormone might have no effect or even an opposite effect on the behavior.

Alternatively, when a behavior is altered *only* by a large dose, it is possible that the extremely high dose has made the animal sick or disoriented and thereby has had a crude effect on behavior that is unrelated to the normal actions of the hormone. This latter concern is especially important when a large dose of a drug or hormone *decreases* a behavior, because the animal may display the behavior only when it is feeling comfortable and relaxed. Similarly, you must always be concerned when a treatment alters behavior in the same way that general stress might alter it; perhaps the treatment distressed the animal and thereby changed the behavior, which reflects nothing about the normal influence of that hormone on that behavior.

There are many ways to administer hormones. Steroid hormones are not very soluble in water, so they are usually dissolved in oil and injected just under the skin (subcutaneously). Another way to administer hormones is to pack solid hormone into a piece of silicone rubber (the brand name is Silastic) tubing and seal the ends. This Silastic implant is placed under the animal's skin. The hormone will slowly diffuse out of the tubing. By varying the thickness and length of the tubing, the amount of hormone to be delivered and the duration of hormone delivery can be adjusted. This method has the advantage that a hormone can be delivered for long periods of time without the repeated stress of frequent injections. The disadvantages are that you do not always know how much hormone you are delivering, and, as we will learn, endogenous hormones are not released in such a constant fashion.

It may be desirable in some experiments to deliver hormone directly to specific areas of the brain in order to localize the brain area mediating a particular behavior. In order to do this, a small cannula (a hollow piece of stainless steel tubing) is tamped full of hormone at one end. The end of the cannula containing the hormone is then introduced into an animal's brain in a specific location. If the behavior is changed by this local application of hormone, you need to make sure that the effect is specific to the hormone and that it acted in that particular area of the brain. Maybe the cannula damaged the brain, causing the observed effect, or perhaps the hormone diffused to another area of the brain a few millimeters away. Therefore, to be sure of your result, you need to find out if cannulae containing control hormones (i.e., biologically inactive versions of the same hormone) and cannulae containing the active hormone placed in other brain regions produce the same effect.

Thus, the study of hormones and behavior in the laboratory requires a design that will accurately measure and evaluate the effect of experimental manipulation on the behavior. In order to produce results that can be generalized to animals outside the laboratory, it is a good idea to use behavioral tests that reflect naturally occurring behaviors and hormone treatments that closely approximate endogenous hormone secretions.

Relating Behavior to Brain: Highlights of Chapters to Follow

Using procedures such as these and others, scientists have investigated how hormones affect behavior, and that is the topic of this book. Investigators have studied the way hormones affect neuronal function as well as how the brain is organized. For example, we know that specific areas of the brain are responsible for sexual behavior and that the areas of the brain important for sexual behavior are different for males and females. In chapter 3 you will learn how the hormones produced by the fetus are responsible for the sexual differentiation of the brain. In fact, some hormones determine whether certain neurons live or die (chapters 3 and 11). You will learn how this exciting field of research has demonstrated that sex differences in both reproductive behavior and brain morphology are determined by the hormonal milieu in which neurons develop.

In the second section of the book, the consequences of hormonal influences on brain development for sexual behavior, courtship, and parental behavior will be explored in greater detail. In chapter 4 you will learn about the intricate hormonal cycle that coordinates the simultaneous production of eggs with the onset of sexually receptive behavior in many female mammals. You will also learn that more than one strategy has evolved to maximize the possibility that fertilization of the egg will take place. In chapter 5 you will learn, among other things, how scientists have been able to determine that different areas of the brain are important for sexual arousal and sexual motivation in males. Then, in chapter 6, you will be asked to consider how the data from nonhuman animals relates to what we know about hormonal influences on sexual behavior in humans.

In chapter 7 you will learn more about the diversity of hormone-behavior relations that have evolved in various species. You will see that even though the hormone-behavior relations may appear to be very different, the same neuroendocrine systems are involved in controlling reproductive behaviors. In some species getting together in order to reproduce is more challenging than for others. These species have evolved elaborate courtship behaviors. Frogs, birds, and fish have evolved different mechanisms to convey to members of their own species information about their reproductive status and fitness. Hormonal influences on sensory receptors, muscle development, and the electrical activity and morphology of neurons in the brain all play a role in ensuring that males and females of a species find and select their mates. But there is a rich diversity among species in where and when hormones produce their effects. Part II concludes with a discussion in chapter 9 of how the hormones of pregnancy also facilitate the initiation of parental behavior. Since the main goal of reproduction is to produce viable young, parental behavior is an additional way that hormones act in the brain to ensure survival of a species.

In Part III you will examine the interactions among hormones and regulatory functions. For example, you will learn in chapter 10 that the immune system is quite sensitive to neuroendocrine signals. Conversely, chronic stress has a profound impact on the secretion of hormones by the adrenal gland, and in chapter 11 you will learn that these hormones can have dire consequences for an animal, affecting energy availability, growth, reproduction, and the immune system. Then, in chapter 12, you will learn how endogenous biological rhythms coordinate the many endocrine systems, serving as one of the major organizing forces for hormone-behavior relations.

In Part IV you will learn that hormones can act on specific areas of the brain to affect sensorimotor and cognitive function. Basic studies, described in chapters 13 and 14, have investigated some of the possible underlying neural processes involved in these functions for nonhuman animals, and correlative experiments, discussed in chapter 15, have suggested that hormones have similar effects on cognitive function in humans.

In the final part of the book, you will learn how intimately hormone-behavior relations are related. Even though hormones have profound effects on brain and behavior, the environment in which an animal lives and the behavior it engages in impact its hormones. All in all, it is a delicate and fascinating arrangement wherein our hormones and our day-to-day experiences interact—hormones influencing behavior and behavior influencing hormones. For example, in chapter 16 you will learn that hormones can affect eating and drinking and that these behaviors affect the secretion of many hormones. Chapter 17 describes the research on invertebrate systems that has demonstrated the ways in which various behaviors triggered by different hormones interact to ensure successful metamorphosis. We will see many examples of the ways in which secreted hormones alter behavior, but conversely, exposure to stimuli and even the execution of behavior itself can affect hormonal

secretions in turn. Chapter 18 describes many of the various ways in which behavior and experience can affect hormones. Input from each of our senses affects our neuroendocrine systems and serve to coordinate neuroendocrinology and behavior.

Each chapter in this book describes what is known about how hormones affect behavior and the underlying neural mechanisms mediating these hormone-behavior relations. This means that in addition to understanding how behavior is studied, we must also have a basic understanding of both the nervous system and the endocrine system. In every chapter, whenever possible, we have included some of the latest information from modern molecular biology. In chapter 2 we will spend some time telling you about those methods. But first, let's review the basics.

Basic Concepts in Cell Biology

Experiments similar to those performed by Berthold are still conducted today to establish hormone-behavior relations. Of course, today's behavioral endocrinologist can investigate the underlying neural mechanisms mediating hormone-behavior relations, the subcellular events that occur in response to hormones, and even the molecular biology of those subcellular responses. Therefore, today's student of behavioral endocrinology must become familiar with the basic mechanisms of neural and cellular function to understand hormone-brain interactions.

Before we discuss how and where hormones act in the brain to influence various components of an animal's behavior, we will first review some basic facts and principles of biology, endocrinology, and biopsychology. As mentioned earlier, these ideas will be important for an understanding of the topics to be discussed later in the book. This discussion is not intended to be a comprehensive review or ''everything you ever wanted to know'' about the brain and endocrine systems. Instead, it is a brief review of some basic ideas, which can be referred to when reading the rest of the book if questions arise. For more details and further explanations of the subject material, additional readings are suggested at the end of this chapter.

The Biology of Eucaryotic Cells

All complex biological organisms are made up of individual eucaryotic cells that contain a spherical-shaped nucleus, in which the genetic material for the organism is stored, and a surrounding cytoplasm, which contains many subcellular organelles that perform diverse functions. While the number of cells in an organism and the complexity of relations between cells varies considerably across species, most of the cellular processes that eucaryotic cells carry out are found in all species. In particular, the ways in which genes store or encode the information needed to produce an organism is a fascinating story that seems to change little across species from worms and flies to people. The modern behavioral endocrinologist takes advantage of the principles and methods of the genetic code and its regulation when asking questions

about hormones and behavior. Because many chapters in this book discuss the effects of hormones that produce their effects by activating specific genes, a brief overview of how the instructions contained in the genome are carried out will help you understand how hormones produce their effects.

THE GENETIC CODE

The genetic information for each cell is stored in fiberlike structures called chromosomes. The chromosomes are actually long, twisted molecules of **deoxyribonucleic acid** (or **DNA**) that remain in the cell's nucleus. DNA is composed of four different molecules called **nucleotides** (because they were originally isolated from cell nuclei). The nucleotides form a long chain with a sugar (deoxyribose), linking them together. Two of these DNA chains wind around each other in the famous double helix. DNA codes for the sequence of amino acids that go into individual proteins. Some of these proteins are packaged by the cell for export; others are enzymes that allow the cell to make other molecules or perform certain functions. Amazing as it may seem, all genetic instructions come down to which particular proteins are to be made in different cells at different times.

A particular piece of DNA that contains the instructions for making a protein is known as a gene. In order for DNA to produce a protein, the gene must first be **transcribed** (figure 1.2). During this process of transcription, a single strand of DNA serves as a template for the assembly of a string of nucleotides forming **ribonucleic acid** (**RNA**). Each of the four different DNA nucleotides have a tendency to pair with only one particular RNA nucleotide. There are, therefore, four different RNA nucleotides, each complementary to a DNA nucleotide. The newly transcribed string of RNA nucleotides forms a molecule known variously as the ''message,'' the ''transcript,'' or the messenger RNA (**mRNA**).

Since each DNA nucleotide is transcribed as a specific RNA nucleotide, the code for an amino acid sequence is now contained within the series of RNA nucleotides. The mRNA leaves the nucleus for the cytoplasm, where protein synthesis occurs when the genetic instructions encoded in the mRNA transcript are **translated** into a chain of amino acids. A series of three nucleotides constitutes the name (also referred to as the **codon**, or code) for which of the 20 or so different amino acids will be added to the chain. A long series of such triplets codes for a long series of amino acids, and that is what constitutes a protein. This flow of information from the gene to a protein is the foundation upon which modern molecular biology is built (figure 1.2).

SUBCELLULAR ORGANELLES

Outside the nucleus but within the cytoplasm of the cell are a number of subcellular organelles that serve different specific functions. For example, **mitochondria** provide energy to the cell. **Ribosomes** (themselves made up of special ribosomal RNA) are the site of protein synthesis, i.e., translation. The **endoplasmic reticulum** (literally, a network of membranes) and **Golgi appa-**

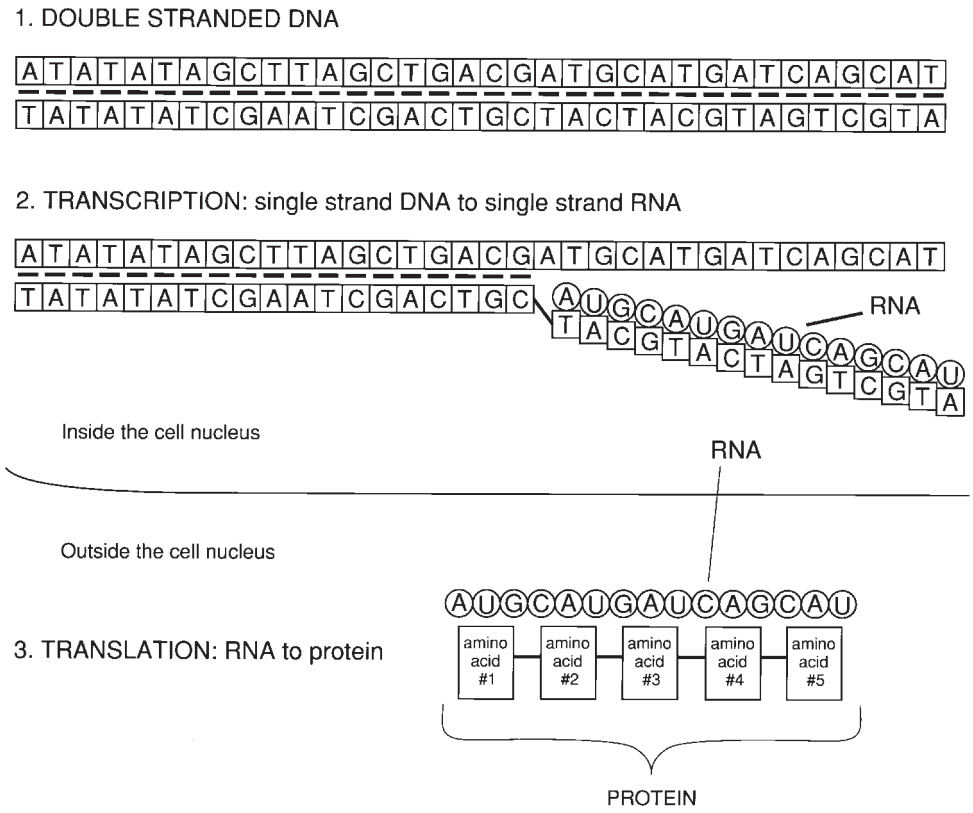

Figure 1.2 The four nucleotides that make up each DNA molecule (adenine, thymine, guanine, and cytosine) are represented schematically in this figure by boxes with the letters A, T, G, and C, respectively. The four nucleotides in RNA (adenine, uracil, guanine, and cytosine) are represented by circles with the four letters A, U, G, and C.

1. In forming the double-stranded DNA molecule, each nucleotide is always paired with only one of the other base pairs: C with G, T with A. Normally the two strands are tightly twisted around each other in a "double helix."

2. In order for transcription to occur, the two strands of the DNA molecule must first unwind. The single strands of DNA can then serve as templates for synthesis of single strands of RNA. Each of the four DNA nucleotides always codes for only one of the four RNA nucleotides (C for G, G for C, T for A, and A for U). The newly synthesized RNA can then leave the nucleus.

3. Translation of the sequence of amino acids to make up a protein occurs outside the nucleus. Each series of three RNA nucleotides is the code for one particular amino acid to be added to the chain. A short series of amino acids is called a peptide; a long series of amino acids is a protein.

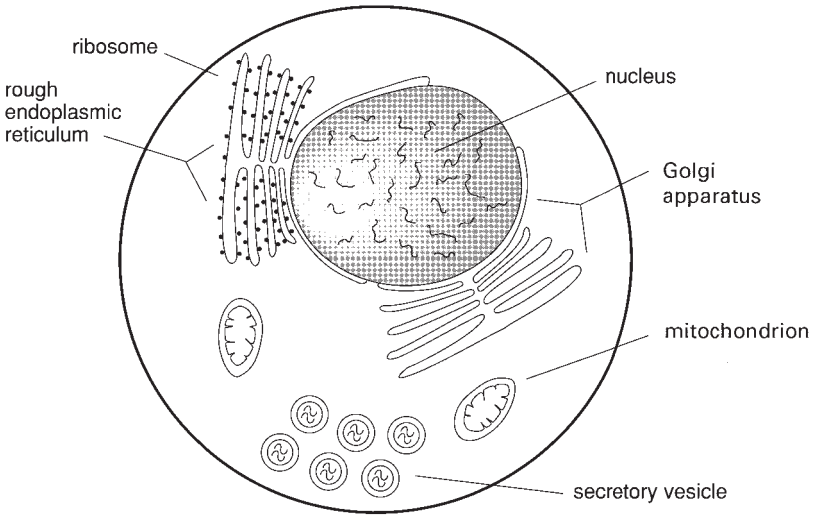

Figure 1.3 Schematic drawing of some of the subcellular organelles found in eucaryotic cells. The genetic material (large strands of DNA coiled into chromosomes) is sequestered within the nucleus of the cell (depicted as fibers within the nucleus). Protein synthesis occurs on the ribosomes in the rough endoplasmic reticulum. Additional processing takes place in the Golgi apparatus where proteins that were synthesized for export are sequestered in secretory vesicles. Mitochondria provide energy for cellular metabolism.

ratus assist with protein synthesis and transport within the cell. Proteins that are destined for secretion from the cell or insertion into the cell's own membrane are synthesized on ribosomes found within the endoplasmic reticulum. Together, the ribosomes and endoplasmic reticulum are referred to as the **rough endoplasmic reticulum** because in electron micrographs the ribosomes appear as bumps on the membranes of the endoplasmic reticulum. This is illustrated schematically in figure 1.3.

THE SECRETORY PROCESS

All cells in an organism engage in protein synthesis. However, some cells use proteins or other chemicals produced by enzymes (which are themselves proteins) for communication between cells. We will be interested in three different types of these intercellular chemical messengers: (1) **hormones**, produced by endocrine cells and released into the circulatory system, (2) **neurotransmitters**, produced by neurons and released at the synapse, and (3) **neurohormones**, produced by specialized neurons known as neurosecretory cells. The three types of chemical messengers differ in where they are produced, where they are released, and the distance the chemical has to travel to produce an effect. These methods of communication differ, therefore, in the speed of the message and the type of information they convey. Nevertheless, cells using these different methods produce, package, and secrete their different chemical messengers in much the same way. With this idea in mind,

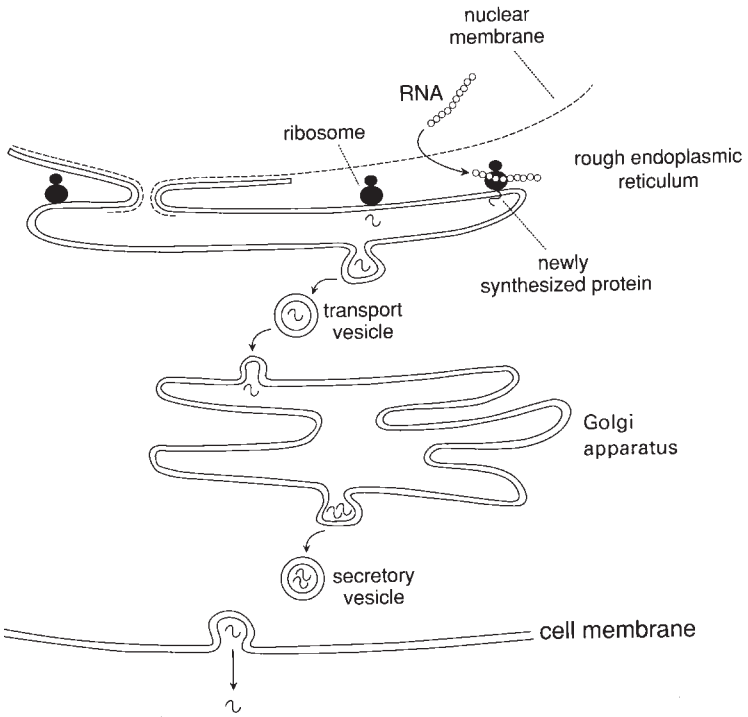

Figure 1.4 Schematic close-up of the subcellular organelles and the synthesis and packaging of protein into secretory vesicles for release by exocytosis. mRNA leaves the nucleus and protein synthesis occurs on the ribosomes in the rough endoplasmic reticulum. Newly synthesized proteins undergo additional processing in the rough endoplasmic reticulum and are then transported to the smooth endoplasmic reticulum where they are packaged into secretory vesicles for release by exocytosis. The movement of the protein from where it is synthesized, to where it is packaged, and then released by exocytosis is indicated by the arrows.

we shall examine the mechanisms involved in a generic model of the secretory process in all cells.

The general model for the secretory process is based on the now-classic work of George Palade. Investigating the synthesis and secretion of proteins by the pancreas, Palade followed the fate of the radioactively labeled amino acids as they were incorporated into newly synthesized proteins. He determined that amino acids were first incorporated into proteins as they were produced by the ribosomes of the rough endoplasmic reticulum (Palade and Farquar 1981). The radioactive protein was later found packaged into transport **vesicles** (sphere-shaped sacks). Still later, these radioactivity-filled vesicles were found in the Golgi complex.

In the Golgi complex, the protein was packaged into **secretory vesicles** and was released from the pancreas by a process known as **exocytosis** (figure 1.4). Exocytosis is the fusion of a secretory vesicle with the extracellular membrane and the subsequent discharge of the vesicle contents outside the cell. Exocytosis is an active process that requires both energy and the presence of

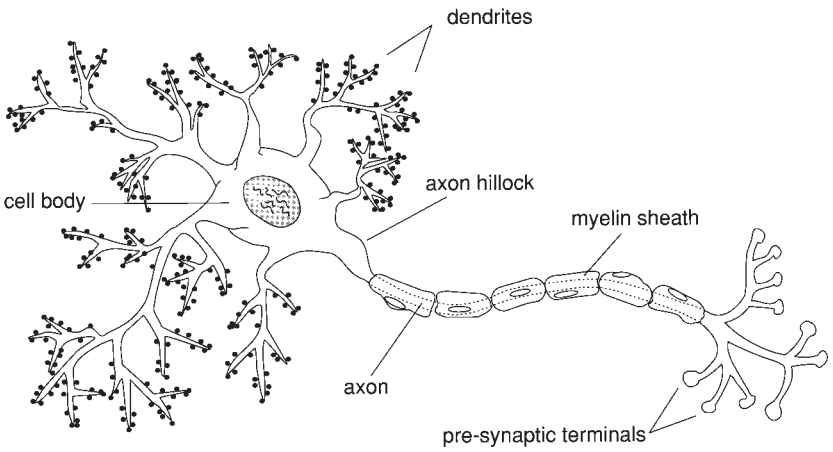

Figure 1.5 In neurons, as in other eucaryotic cells, the nucleus is sequestered in the cell body or soma. Dendrites project in treelike formation from the soma and receive information from other neurons. A single axon projects from the cell body. When the membrane potential at the axon hillock reaches a certain threshold, an action potential is generated and transmitted down the axon to the presynaptic terminal where the neurotransmitter is released by exocytosis, to transmit information to other neurons.

free calcium ions. Endocrine cells, neurons, and neuroendocrine cells all use exocytosis to release secretory products.

In the synthesis of some hormones and neurotransmitters, the secretory product and its packaging into vesicles differ from this model. In fact, steroid hormones are probably not packaged into vesicles at all. But in all other cases, the Golgi apparatus (or an analogous structure) is involved in the formation of the secretory vesicles, the secretory product is packaged into secretory vesicles, and release occurs by exocytosis.

These are the general processes mediating protein synthesis and secretion. In order to understand the ideas discussed in the following chapters, you need to be familiar with these basic relations between DNA, RNA, and proteins. (A more detailed look at these processes is the topic of chapter 2.) Now we are ready to consider the details of the nervous and endocrine systems.

Basic Concepts in Neurobiology

The adult human brain contains from 30 to 100 billion neurons, whereas in the tobacco hornworm moth (*Manduca sexta*) it is estimated that there are 30 to 100 thousand neurons. But in both moths and humans neurons behave very much alike—they share characteristics that allow them to receive, process, and transmit information.

Parts of the Neuron

Most neurons have three structural components: the **cell body**, the **dendrites**, and the **axon** (figure 1.5). Neurons have an outer membrane made of a double

layer of fatlike molecules (lipids) with proteins that float within the lipid membrane. As in almost all other cells, the neuronal cell body (or soma) has a nucleus as well as the various subcellular organelles typical of eucaryotic cells.

Extending from the cell body are two different types of projections, axons and dendrites (figure 1.5). Dendrites are usually widely branching fibers that receive information from other neurons. Axons are single fibers that extend from the cell body and transmit information to other neurons. The site where the axon leaves the cell body appears to be slightly swollen and is therefore called the **axon hillock**. The axon usually branches many times at its terminal end, so that multiple contacts can be made with many different target neurons. At the end of each axon branch is a swelling known as the **presynaptic terminal**. The site where the presynaptic terminal contacts another neuron is known as a **synapse**.

The Membrane Potential and Action Potentials

Neurons can receive and transmit information because, unlike most other types of cells, their membranes are electrically excitable. Because there are more negatively charged ions inside neurons than outside, the cells have a **polarization** or electrical charge across the membrane, called the **resting potential**. All cells have a resting potential that they actively defend, but it is a change in the membrane potential that is the result and cause of the transmission of information between neurons.

If an electrical stimulus is applied to the neuronal membrane to make the potential across the membrane *less* negative (i.e., the inside becomes less negative than the outside), then we say that the membrane has become **depolarized**. If an electrical stimulus makes the potential across the membrane *more* negative (i.e., the inside becomes even more negative than the outside), then the membrane is said to be **hyperpolarized**. Such changes in membrane polarization can be caused by an experimenter using electrical stimulation, but in the normally functioning nervous system, changes in membrane polarization are brought about by different means, as we shall soon see.

However the membrane polarization is altered, and whether the alteration is a depolarization or a hyperpolarization, the membrane will **passively conduct** this change in potential along the membrane away from the site that was stimulated. This type of conduction is called **decremental conduction** because the change in membrane potential is smaller and farther from the site of stimulation. The analogy frequently used is that a wave created by dropping a pebble into a pool of calm water becomes smaller as the distance from the site of impact increases. The change in membrane potential created is also referred to as a **graded potential**. This is because the size of the change in membrane potential is proportional to the size of the stimulus. As our analogy illustrates, if you drop a large rock into the pool of calm water, you will produce bigger waves than you would with a pebble.

The events that normally produce changes in neuronal membrane potential occur at the synapse. A **presynaptic** neuron releases its **neurotransmitter**, and this produces changes in the membrane potential of the **postsynaptic** neuron. The postsynaptic response in the next neuron is passively conducted by decremental conduction. The response is also a graded potential, so it is proportional to the amount of neurotransmitter released at a synapse. Throughout the dendrites and cell body, any stimulation of the neuron results in a graded potential and decremental conduction of that potential away from the many sites of stimulation. Because it is literally covered with synapses, each neuron receives input from thousands of other neurons. The neuronintegrates the many depolarizations and hyperpolarizations received by the dendrites and cell body. But it is at the axon hillock that the *net* polarization determines whether this neuron has become sufficiently depolarized to pass on the information to other neurons.

If the net depolarization of the membrane potential at the axon hillock reaches a certain threshold, the axon becomes depolarized, an **action potential** is generated, and the neuron is said to ''fire.'' This action potential is an all-or-none phenomenon: Once an action potential has been generated in the axon hillock, it cannot be stopped, and the strength of the signal does not diminish as it travels down the axon at some 10 to 100 miles per hour (mph). If, as is usually the case, the axon branches, the action potential travels down each branch, and available neurotransmitter is released at each synapse. Action potentials are relatively easy to measure by a variety of electrophysiological techniques.

Synaptic Transmission

The process of communication between two neurons relies on the release of a chemical messenger, the **neurotransmitter**. When the action potential arrives at the presynaptic terminal, the terminal releases a neurotransmitter. The released neurotransmitter crosses the synapse and is detected by postsynaptic receptors. Release of a neurotransmitter and its reception by the postsynaptic neuron is a process known as **synaptic transmission**. This term reflects two important aspects of this process—*synaptic*, because it occurs only at a synapse, and *transmission*, because information is communicated or transmitted between two cells. Synaptic transmission relies on (1) the availability of the neurotransmitter, (2) the release of the neurotransmitter by exocytosis, (3) the binding of the postsynaptic receptor by the neurotransmitter, (4) the response of the postsynaptic cell, and (5) the subsequent removal or deactivation of the neurotransmitter.

AVAILABILITY OF NEUROTRANSMITTER: SYNTHESIS AND PACKAGING INTO VESICLES

Most neurotransmitters are small molecules, about the size of a single amino acid, but neurotransmitters can also be larger proteins or peptides. Some of the substances that are currently thought to be neurotransmitters are listed in

Table 1.2 Partial List of Putative Neurotransmitters and Releasing Factors

Monoamines	***Peptides***
Acetylcholine	Dynorphin
Catecholamines	Enkaphalin
Dopamine (DA)	Substance P
Norepinephrine (NE)	Neurotensin
Epinephrine (EPI)	Bombesin
Indoleamines	Somatostatin
Serotonin (5-hydroxytryptamine or 5-HT)	β-Endorphin
istamine	Neuropeptide Y
Amino acids	Cholecystokinin (CCK)
Glutamate	Oxytocin
Gamma-aminobutyric acid (GABA)	Vasopressin
Glycine	Angiotensin II
Aspartate	Vasoactive intestinal polypeptide (VIP)
Purines	Corticotropin releasing hormone (CRH)
Adenosine	Growth hormone releasing factor (GRF)
	Thyrotropin releasing hormone (TRH)
	Gonadotropin releasing hormone (GnRH)

table 1.2. The synthesis of a neurotransmitter varies according to the size of the molecule. Protein and peptide neurotransmitters are synthesized as described above, in the cell body, and then packaged into secretory vesicles (which in neurons are called **synaptic vesicles**). Once the proteins or peptides have been manufactured and packaged into vesicles in the cell body, they are transported down the axon to the presynaptic terminal for release. If the neurotransmitter is a small molecule, then synthesis does not occur only in the cell body. The enzymes for neurotransmitter synthesis are assembled in the cell body and transported to the presynaptic terminal. The neurotransmitter is then synthesized and packaged into synaptic vesicles in the presynaptic terminal, close to where it will be needed.

EXOCYTOSIS

When a neuron fires, the arrival of the action potential at the presynaptic terminal causes calcium influx into the terminal. The availability of free calcium results in the release of the neurotransmitter from the presynaptic terminal by exocytosis. The neurotransmitter diffuses across the synaptic cleft, where it contacts postsynaptic receptors—large protein molecules or multiprotein complexes that recognize and bind to the neurotransmitter (figure 1.6).

RECEPTOR BINDING

When the neurotransmitter contacts the postsynaptic receptor, the two molecules are thought to fit together in such a way that for a short time they are bound to each other. Because a particular neurotransmitter will fit into only some receptors, such **receptor binding** is usually conceptualized as a ''lock and key'' association between the two molecules. But the binding is

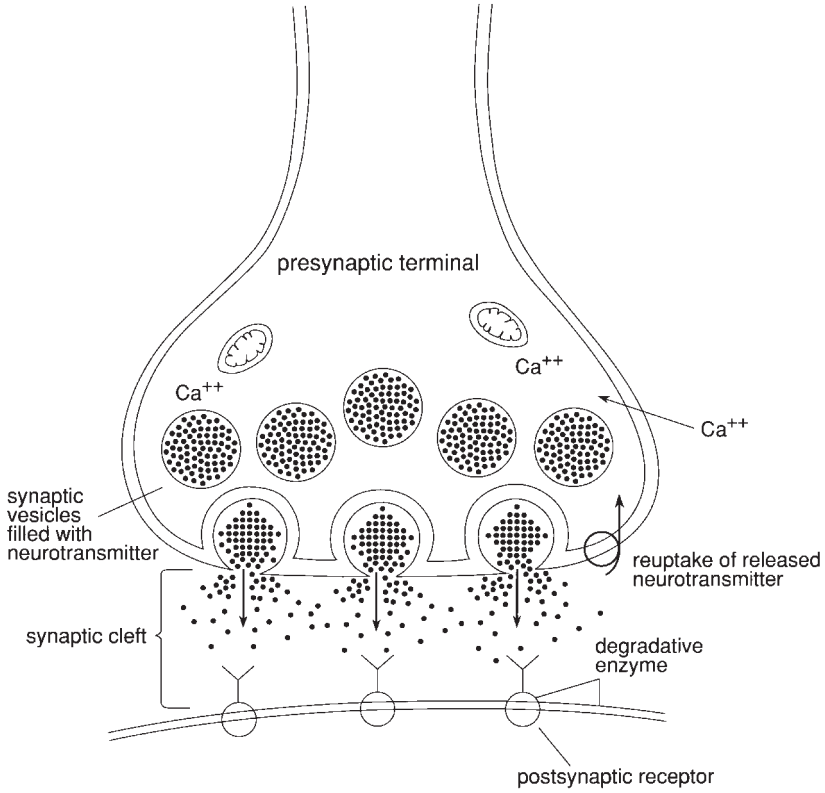

Figure 1.6 Synaptic transmission occurs with the arrival of the action potential at the presynaptic terminal. This causes an increase in calcium (Ca^{++}) influx and results in movement of the synaptic vesicles filled with neurotransmitter to the presynaptic terminal membrane where the membranes fuse and neurotransmitter is released by exocytosis. The neurotransmitter diffuses across the synaptic cleft to bind to the postsynaptic receptors where a graded potential is generated in the postsynaptic cell. (The synaptic vesicles are depicted with only one membrane, but their membranes are also lipid bilayers as depicted for the extracellular membrane.)

like Velcro—when two complementary strips come in contact, they stick together, but they can also be pulled apart with no change in the shape or usefulness of either. Thus, when the two are attached, they are bound to each other, but this is not a permanent attachment. Similarly, a neurotransmitter has an affinity for its receptor, so it sticks to the receptor for a brief time and then is easily released.

The binding of the neurotransmitter at the postsynaptic receptor induces changes in the electrical potential of the postsynaptic neuronal membrane. The change in membrane potential is proportional to the amount of neurotransmitter released. A neurotransmitter can produce either inhibition (by hyperpolarization) or excitation (by depolarization) of the membrane of the postsynaptic cell. The particular neurotransmitter and the particular postsynaptic receptor determine the direction of the response. If the postsynaptic response is hyperpolarization, the chance of depolarization occurring at the

axon hillock is ultimately reduced, and therefore the cell is inhibited from firing. Conversely, if the postsynaptic response is depolarization, the cell is excited. In either case, the information from this synapse is summated with all the other information arriving at this postsynaptic neuron, and when the **threshold** is reached at the axon hillock, this neuron will fire.

DEACTIVATION OF THE NEUROTRANSMITTER

A neurotransmitter is available only briefly to bind to the postsynaptic receptors and then it is rapidly deactivated. Deactivation occurs through either degradative enzymes present in the synaptic cleft or removal of the neurotransmitter by reuptake into the presynaptic terminal. Nevertheless, a brief exposure of the receptor to the neurotransmitter is quite effective at producing a postsynaptic response.

Some Simple Pharmacology

To investigate how specific neural systems interact with the endocrine system, it is often helpful to be able to manipulate neuronal activity. Because pharmacological manipulations are frequently used, it is a good idea to become familiar with the general principles and terminology used to describe the ways in which drugs can influence neuronal activity.

Drugs that act on the brain to influence an animal's behavior usually do so by altering neurotransmitter activity, and there are a number of different ways that drugs can produce their effects. Many drugs act on specific neurotransmitter systems and therefore alter activity in specific populations of neurons. For example, **neurotransmitter synthesis** can be prevented or decreased by drugs that affect the *synthetic enzyme for a neurotransmitter*. When neurotransmitter synthesis is blocked, the amount of neurotransmitter available for release is decreased, so there is decreased neurotransmitter activity. Alternatively, **storage** in synaptic vesicles can be prevented with drugs that make *synaptic vesicle membranes leaky*. This results in depletion of neurotransmitter stores and also decreases neurotransmitter activity.

Other drugs block or stimulate the **release** of specific neurotransmitters. Still other drugs act by **blocking receptors**, so that the real neurotransmitter cannot bind to the receptors. Drugs that *prevent a neurotransmitter from binding* to its receptor are called receptor **antagonists**. For example, drugs used to treat patients with schizophrenia such as haloperidol, chlorpromazine, and clozapine (i.e., neuroleptics or antipsychotic drugs), are antagonists at receptors in the brain for the neurotransmitter dopamine. Other drugs act by binding to a receptor and *mimicking the normal neurotransmitter*. Such drugs are called receptor **agonists**. An example of a receptor agonist is Valium, a benzodiazepine that mimics the effect of the endogenous neurotransmitter gamma-aminobutyric acid (GABA) to decrease anxiety. Other drugs interfere with the **deactivation** of a neurotransmitter after it has been released, thereby prolonging the action of a neurotransmitter. This can be done by blocking reuptake (as cocaine does for catecholamines) or inhibiting

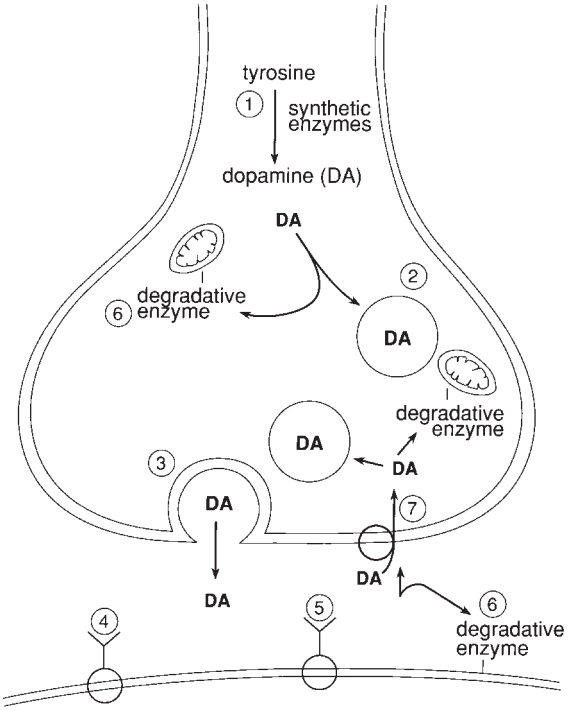

Figure 1.7 Possible sites of drug action in a dopamine (DA) neuron. These same sites can be affected by drugs in neurons that use other neurotransmitters. Drugs can also influence the endocrine system by acting at analogous sites: 1. synthesis; 2. storage; 3. release; 4. receptor-agonist; 5. receptor-antagonist; 6. degradative enzymes; 7. reuptake. (Adapted from Cooper, Bloom, and Roth 1996.)

degradative enzymes. Finally, drugs that **prevent an action potential** from occurring can *block neuronal activity* throughout the brain and the peripheral nervous system. Drugs such as tetrodotoxin (pufferfish poison) and others that block neural activity are usually lethal. The various ways in which drugs can affect neuronal activity are illustrated schematically in figure 1.7 for a neuron that uses dopamine (DA) as its neurotransmitter.

Basic Concepts in Neuroendocrinology

In vertebrates, the central nervous system develops from a long hollow tube of cells called the neural tube. As the brain develops, the neural tube forms three outpouchings. The adult brain is divided into subdivisions based on these embryological formations of forebrain, midbrain, and hindbrain (table 1.3). In mammals, the cerebral hemispheres of the forebrain comprise the largest portion of the brain. These include the cerebral cortex, the basal ganglia, and the limbic system. The cortex can be subdivided into four lobes: frontal, parietal, occipital, and temporal (figure 1.8). However, behavioral endocrinologists, are most interested in a relatively small region at the base of the forebrain, a part of the diencephalon called the hypothalamus. As we

Table 1.3 Major Subdivisions of the Vertebrate Brain

Forebrain	Telencephalon	Olfactory bulbs Cerebral hemispheres Basal ganglia
	Diencephalon	Epithalamus Thalamus Hypothalamus
Midbrain	Mesencephalon	Tectum Tegmentum
Hindbrain	Metencephalon	Cerebellum Pons
	Myelencephalon	Medulla oblongota

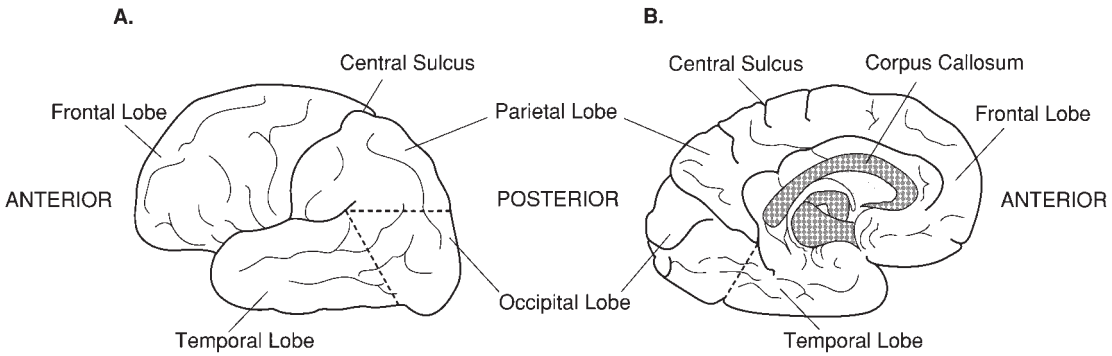

Figure 1.8 Diagram illustrating the cortical regions in the human brain. (*A*) View of the lateral surface of the cerebral hemispheres. (*B*) View of the medial surface of the cerebral hemispheres. The cerebral cortex is divided into four main lobes: the frontal lobe, the parietal lobe, the temporal lobe, and the occipital lobe.

will see, the hypothalamus warrants this attention because it exerts a profound influence on the secretion of nearly every known hormone (figure 1.9)

Hypothalamic-Pituitary Relations in Vertebrates

By definition, hormones are substances produced by a gland and carried by the circulatory system to a distant target organ to cause an effect. The brain is a target organ for many of these hormones and in turn the brain regulates hormone secretion. Although the relations between the brain and the endocrine system in vertebrates are conceptually simple, as with most biological systems the details can be quite complex.

In vertebrates, the hypothalamus is the neural control center for all endocrine systems. Although it is relatively small, it contains a number of specialized nuclei (i.e., functional groups of neurons) (figure 1.10). These nuclei are involved in the regulation and integration of endocrine and physiological functions and behaviors. For example, the suprachiasmatic nucleus (named

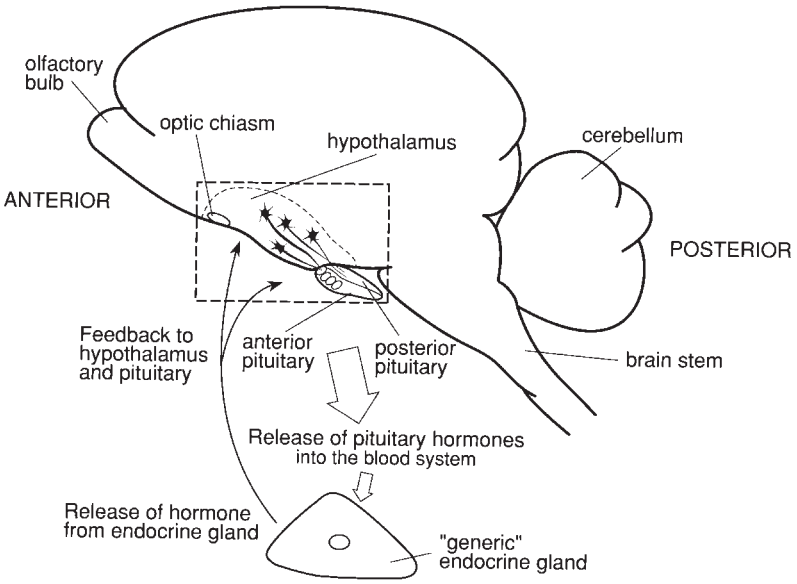

Figure 1.9 The hypothalamic-pituitary-endocrine gland feedback system. A ''generic'' case. In this figure, the brain is viewed from the side. There are two different classes of neurosecretory cells in the hypothalamus, as illustrated schematically in this figure. The first class of hypothalamic neurosecretory cells send their axons to the median emminence (see figure 1.10). There the cells deliver releasing factors onto blood vessels in the hypophysial portal system. The releasing factors travel to the anterior pituitary to stimulate or inhibit the release of anterior pituitary hormones. The second class send their axons down the pituitary stalk and terminate near blood vessels in the posterior pituitary. When these neurons fire, they release neurohormones (either oxytocin or vasopressin) directly into the circulatory system. Hormones from the anterior pituitary or neurohormones from the posterior pituitary reach their target glands and induce hormone release. Hormones released from the endocrine gland feed back to both the hypothalamus and pituitary. Stippled box indicates region of close-up in figure 1.10.

after its location above the optic chiasm) is involved in the maintenance and coordination of biological rhythms, and the medial preoptic area and ventromedial hypothalamus are involved in sexual behavior (among other functions). In the chapters that follow, you will learn much more about the various hypothalamic nuclei and their many functions.

Specialized types of neurons that allow the brain to influence the secretions of the endocrine system have evolved in the hypothalamus. These neurons, which communicate directly with endocrine systems, are called **neurosecretory cells** because they release their products not into a synapse but into blood vessels that carry the neurotransmitter to other organs to produce its effect. Such neurotransmitters can be thought of, therefore, as **neurohormones**.

The hypothalamus is directly above a very important endocrine gland, the **pituitary**. The pituitary gland (sometimes called the **hypophysis**) is sandwiched between the roof of the mouth and the hypothalamus. Cradled in

a small nook in the cranium, the pituitary is actually attached to the hypothalamus by a slender stalk. As we will see, the hypothalamus receives information about external conditions from other brain regions and, using this information, controls secretions from the pituitary. However, the hypothalamic neurosecretory cells exert their control over the pituitary in two quite different ways.

As illustrated schematically in figure 1.9, neurosecretory cells in the hypothalamus are associated with the both the anterior and posterior lobes of the pituitary gland (there is also a slim intermediate zone in the pituitary, but it will not concern us). One type of neurosecretory cell releases its neurohormones onto specialized blood vessels that supply the *anterior* pituitary to stimulate the release of hormones. These neurohormones are also called **releasing factors** because they stimulate or inhibit the release of hormones by cells in the anterior pituitary gland. The other type of hypothalamic neurosecretory cell sends axons into the *posterior* pituitary to release its neurohormones directly into the general circulatory system.

HYPOTHALAMIC CONTROL OF THE ANTERIOR PITUITARY

It was widely accepted for some time that the hypothalamus produced releasing factors that acted on the anterior pituitary to stimulate or inhibit pituitary hormone secretion. However, isolation of a releasing factor did not occur until the early 1970s. The convention now is to call a substance a ''releasing factor'' until it has been chemically isolated and characterized. Once its chemical structure is known, it is called a **releasing hormone**. We will use the term **releasing factor** as a generic term to refer to both.

Releasing factors are secreted into a specialized system of blood vessels that runs between the base of the hypothalamus and the anterior pituitary lobe (or **adenohypophysis**) rather than the posterior pituitary lobe (or **neurohypophysis**). The hypothalamic-pituitary portal system is actually a profuse tangle of small blood vessels (i.e., capillaries) that use blood flow to deliver the hypothalamic-releasing factors to the pituitary. There are many different types of cells in the anterior pituitary: Some release growth hormone (GH) and are called somatotrophs; other cells (lactotrophs) release prolactin (Prl), and so on (table 1.4). Each of these specialized pituitary cells is controlled by specialized hypothalamic releasing factors. The pituitary cells respond to the releasing factors by either increasing or decreasing their production and release of pituitary hormones (see figure 1.10).

The pituitary hormones are released into the bloodstream and subsequently act on peripheral endocrine glands to stimulate or inhibit *their* function and hormone release. The hormones produced by the various endocrine target glands then feed back to both the hypothalamus and the pituitary. In this way, the hormonal production of the endocrine glands can be continuously monitored and regulated by the brain (see figures 1.9 and 1.10).

For example, the hypothalamus manufactures and releases corticotropic-releasing hormone (CRH) into the portal blood system. CRH reaches the

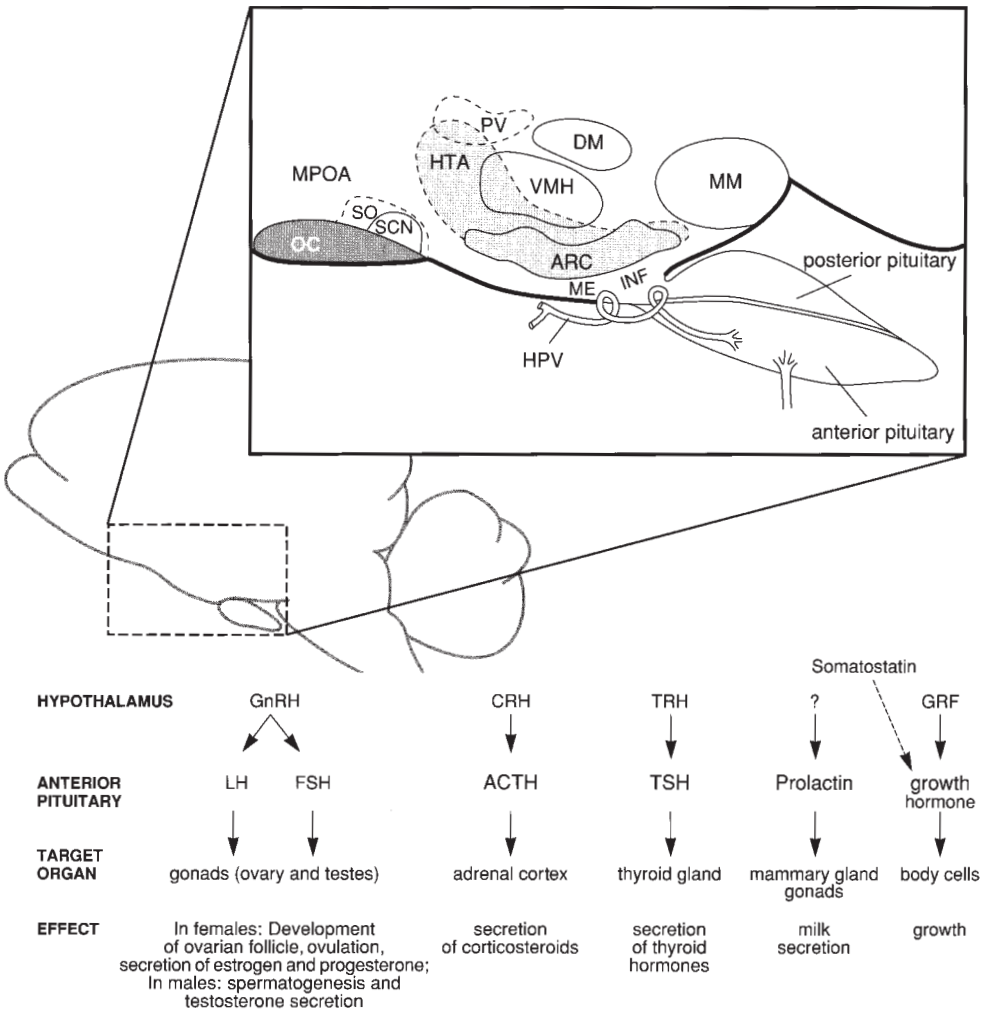

Figure 1.10 A close-up of the hypothalamus and pituitary region indicated by the stippled box, with a schematic representation of the relations between hypothalamic-releasing factors, pituitary hormones, and their target glands. A solid arrow indicates a stimulatory effect; a dashed arrow indicates an inhibitory effect. The releasing factors are released by neurons in the hypothalamus and diffuse through the hypophysial portal blood vessels (HPV) to the anterior pituitary.

1. Gonadotropin-releasing hormone (GnRH) stimulates the pituitary to release luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH and FSH in turn stimulate their primary target organs, the gonads, to promote spermatogenesis in the testes or the development of eggs in the ovary.
2. Corticotropic hormone (CRH) stimulates the pituritary to release adrenocorticotropic hormone (ACTH), which acts primarily on the adrenal cortex to induce the release of corticosteroids.
3. Thyrotropic hormone (TRH) stimulates the release of thyroid stimulating hormone (TSH) from the pituitary, which acts primarily on the thyroid gland to stimulate the release of thyroid hormones.
4. Scientists are still uncertain about the hypothalamic-releasing factor that induces prolactin release. At high concentrations, dopamine will inhibit prolactin release. Prolactin has a wide range of actions in the body and the brain. Prolactin gets its name from its effect on the mammary gland to promote milk secretion. Prolactin also acts in conjunction with LH to promote gonadal function.

pituitary and stimulates corticotrophs there to release adrenocorticotropic hormone (ACTH) into the general circulation. ACTH travels to the adrenal glands (just above the kidneys) which in turn release various corticosteroid hormones (such as cortisol) into the bloodstream. This pattern of control holds for all the anterior pituitary hormones: Neurosecretory cells in the hypothalamus release their releasing factors into the portal system. The releasing factors act on specific pituitary cells to stimulate or inhibit the release of their particular hormone. The anterior pituitary hormone travels through the general circulation to affect the hormonal secretions of the target organ(s).

We can use this same example to illustrate another basic feature of hypothalamic-pituitary interactions: negative feedback. The adrenal steroids, released in response to pituitary ACTH (itself released in response to hypothalamic CRH), travel through the bloodstream to reach the entire body, including the brain and the pituitary. At these two sites, the adrenal hormones act to stem the flow of CRH from the hypothalamus and inhibit the responsiveness of pituitary adrenotrophs. Both actions result in decreased ACTH release. Lowered ACTH levels then result in a decrease in any additional release of adrenal hormones. The negative feedback loop of information means that the release of adrenal cortex hormones is (normally) self-limiting. The adrenal cortex hormones turn off the stimulus that caused them to be secreted in the first place. In turn, as the blood concentration of adrenal cortex hormones falls, CRH and ACTH levels are released from inhibition, and adrenal cortex hormone levels rise again. Thus, the negative feedback aspect of this loop helps to maintain relatively constant levels of adrenal hormones. If they get too high, CRH and ACTH levels are inhibited. If blood concentrations of adrenal cortex hormones fall too low, CRH and ACTH levels rise to bring them up again.

This negative feedback effect is a general phenomenon characteristic of the regulation of the hypothalamus and the anterior pituitary. To cite another example, the hypothalamus also makes and releases thyrotropin-releasing hormone (TRH). TRH stimulates the pituitary to release thyroid-stimulating hormone (TSH). TSH stimulates the thyroid gland to release thyroxine and other thyroid hormones. These thyroid hormones then have a negative feedback effect, reducing hypothalamic release of TRH and pituitary release of TSH. When thyroid hormone levels fall below a certain level, TRH and TSH are released again to restore them.

Figure 1.10 (continued)
5. Somatostatin inhibits, and growth hormone relasing factor (GRF) stimulates, the release of growth hormone (GH) from the anterior pituitary. GH stimulates growth of cells; this includes bone growth as well as growth of other cells in the body. ARC, arcuate nucleus; DM, dorsomedial nucleus; HPV, hypophysial portal vessels; HTA, hypophysiotrophic area; INF, infundibular stalk; ME, median emminence; MM, mammilary nucleus; MPOA, medial preoptic area; OC, optic chiasm; PV, paraventricular nucleus; SCN, suprachiasmatic nucleus; SO, supraoptic nucleus; VMH, ventromedial hypothalamic nucleus. (Adapted from Shepard 1988.)

Table 1.4 Vertebrate Endocrine Glands and Some of the Hormones They Are Known to Secrete

ENDOCRINE GLAND	HORMONES SECRETED
Anterior pituitary	Growth hormone (GH) Prolactin (Prl) Melanaphore stimulating hormone (MSH) Adrenal corticotrophic hormone (ACTH) Luteinizing hormone (LH) Follicle stimulating hormone (FSH) Thyroid stimulating hormone (TSH)
Posterior pituitary	Arginine vasopressin (AVP), also known as antidiuretic hormone (ADH) Oxytocin
Thyroid gland	Thyrotoxin Calcitonin
Adrenal cortex	Glucocorticoids Corticosterone Cortisol Mineralicorticoids Aldosterone
Adrenal medulla	Epinephrine Norepinephrine Enkephalins Endorphins
Kidney	Renin
Liver	Preangiotensin
Pancreas	Insulin Glucagon
Stomach and intestines	Gastrin Secretin Cholecystokinin (CCK) Vasoactive intestinal peptide (VIP) Bombesin Somatostatin Leptin Orexin
Pineal	Melatonin
Gonads: ovary	Estrogens Estradiol (E2) Estriol Estrone Progesterone
Gonads: testes	Androgens Testosterone (T) Dihydrotestosterone (DHT) Androstendione

While the negative feedback loop tends to keep hormone concentrations in blood relatively constant, there are also conditions under which the feedback system results in regular cycles of varying hormone concentrations. This occurs during the reproductive cycle in females (chapter 4), during pregnancy (chapter 9), and with endogenous rhythms (chapter 12). For example, during the female reproductive cycle, estrogen exerts both negative and positive feedback effects on the hypothalamus. During initial development of an egg (or oocyte), a small amount of estrogen is produced and released by the ovary (see figure 4.1). This inhibits hypothalamic release of gonadotropins-releasing hormone (GnRH) and pituitary release of luteinizing hormone (LH) as a negative feedback effect. Then, as the oocyte becomes mature, there is a rapid increase in estrogen release from the ovary. Instead of inhibiting GnRH and LH release, however, this rapid increase in estrogen has the opposite effect. It *stimulates* a pulse of GnRH release from the hypothalamus. This GnRH pulse induces a pulse of LH release from the pituitary, which is crucial for the final maturation and release of the mature egg (see chapters 3 and 4).

HYPOTHALAMIC NEUROSECRETORY CELLS AND THE POSTERIOR PITUITARY

Oxytocin and vasopressin are hormones made by neurosecretory cells in the hypothalamus. Oxytocin and vasopressin are both neurohormones and neurotransmitters. Some of these cells send their axons down to the median eminence and into the posterior pituitary. When these neurons fire, oxytocin or vasopressin is released from the posterior pituitary directly into the bloodstream. You can think of oxytocin and vasopressin as hypothalamic neurohormones that affect target organs directly, without using pituitary cells or their hormones as intermediaries. However, not all neurons that make oxytocin or vasopressin project to the posterior pituitary. Some of the hypothalamic cells that make these hormones project to the brain and spinal cord, synaptically releasing their products upon neurons.

Oxytocin released into the bloodstream triggers milk ejection during nursing and uterine contractions for childbirth. In fact, hospitals often administer a synthetic version of oxytocin, called pitocin, to induce or speed delivery. Vasopressin is vital for fluid conservation. Additional functions of oxytocin and vasopressin will be discussed in chapters 4, 9, and 16. Oxytocin and vasopressin are ''sister'' hormones—their chemical structures are very similar. This structural similarity probably reflects a common evolutionary origin. Amphibians, for example, have only one such hormone, called vasotocin, which has actions reminiscent of both oxytocin and vasopressin.

Most vertebrates produce vasopressin and oxytocin or vasotocin, but there are species variations in the molecular structure of the actual hormones produced and in the functions of these hormones. Table 1.4 lists some of the endocrine glands and the hormones that they produce in most vertebrate animals (mammals, reptiles, amphibians, fishes, and birds). The relations among the hypothalamic-releasing factors, the anterior pituitary hormones,

and their target endocrine glands are illustrated schematically in figure 1.10. Each releasing factor stimulates the release of a specific hormone (or, in the case of GnRH, the release of two hormones). These hormones are released by the pituitary into the bloodstream and are then carried to their target organs, where they produce their specific effects, usually stimulating the target organs to release hormones of their own.

Mechanisms of Hormone Action

OVERVIEW: STEROIDS VERSUS PEPTIDE HORMONES

Hormones fall into two general classes based on their molecular structures. The first class comprises steroid hormones produced by the gonads and the adrenal cortex and the steroidlike hormones produced by the thyroid gland. The steroid hormones are all synthesized from the common precursor cholesterol (see figure 3.2). The thyroid hormones have a steroidlike three-dimensional structure that influences their chemical properties, making them behave like steroids. Because these hormones share many common properties they are usually discussed together. The second class of hormones comprises the glycoprotein hormones (LH and follicle-stimulating hormone [FSH]) and protein or peptide hormones. As we hinted earlier, these different hormones are synthesized and packaged for secretion in different ways.

The protein and peptide hormones are chains of amino acids and are processed like most proteins (see p. 15). The glycoprotein hormones undergo additional processing in the Golgi apparatus, where a sugar group (glycogen is the basic building block in sugars, hence the ''glyco'' prefix) is added to the protein prior to packaging into vesicles. Steroid hormones, on the other hand, are synthesized from precursors in the smooth endoplasmic reticulum, processed further in the mitochondria, and then returned to the smooth endoplasmic reticulum for final processing. The thyroid hormones are synthesized from amino acids that form a complex steroidlike structure. It is currently believed that, unlike protein hormones, steroid and steroidlike hormones are not stored in vesicles. It is thought that they simply diffuse out of cells after synthesis. Release of these hormones is, therefore, governed primarily by the rate at which they are synthesized.

The molecular structure of a hormone is also important because of the way it can act upon other cells. Protein, peptide, and glycoprotein hormones do not readily pass through cell membranes, so they usually act upon receptors found in the outer membrane of responsive cells. Only cells containing the appropriate receptor can respond to a particular hormone. Like a neurotransmitter and its receptor, the hormone and the receptor have complementary shapes that result in the binding of the hormone to the receptor. Protein, glycoprotein, and peptide hormones bind to specific receptor molecules that span the cell membrane, which triggers intracellular chemical reactions that can have a wide range of effects, as we will see.

In contrast, because steroid hormones are lipid soluble, they are thought to pass through the extracellular membrane. Inside the cell, steroid hormones bind to specific receptor proteins, and this steroid-receptor complex then binds to DNA. The binding of the hormone to DNA results in an increase or decrease in the synthesis of specific proteins, which can begin a wide range of effects.

PROTEIN HORMONES

Among the vertebrates, protein hormones include the hypothalamic-releasing factors as well as the pituitary hormones (see table 1.4). The posterior pituitary hormones oxytocin and vasopressin are both peptides composed of only nine amino acids. Insulin, another vital protein hormone, consists of about 100 amino acids and is released from the pancreas. Like other proteins, the molecular structure of these hormones is coded for by genes in DNA.

The binding of a protein hormone to its receptor sets into motion a chain of chemical events inside a cell. One of the first events is the activation of a second messenger system that results in a cascading series of reactions within the cell. The exact second messenger varies among the protein hormones, but all are thought to employ second messenger systems to induce a response in target cells. The initial response of the target cell is much like that of a neuron responding to a neurotransmitter, i.e., a change in membrane potential. The particular chain of subsequent events depends upon the type of receptor that was activated and the state of intracellular events that existed before the hormone arrived. Figure 1.11 is a diagram of the rather complicated cascade of chemical reactions triggered by the arrival of a glucagon molecule at a target cell.

The regulation of receptors for protein hormones is thought to be mediated by negative feedback mechanisms similar to those operating at neurotransmitter receptors. In general, the number and activity of these receptors are inversely regulated by the amount of hormone or neurotransmitter available. When neurotransmitter release or protein-hormone release is high, a ''down-regulation'' of receptors occurs, so that even more hormone is needed to induce a biological response. In contrast, when hormone release is low, the receptors' response can become supersensitive to stimulation by protein hormones, i.e., less hormone is required to produce the same biological response. This negative feedback system serves to maintain a relatively constant biological response even when the endocrine system is damaged or altered. For each hormone and receptor, however, there are also other factors that are important in the regulation of receptor activity, including the regulation of receptors by steroid hormones.

One example of the operation of the negative feedback mechanism carried to an extreme occurs in people with adult onset diabetes (also known as type II, or insulin-independent diabetes mellitus). This form of diabetes, as suggested by its name, develops late in life and is usually associated with

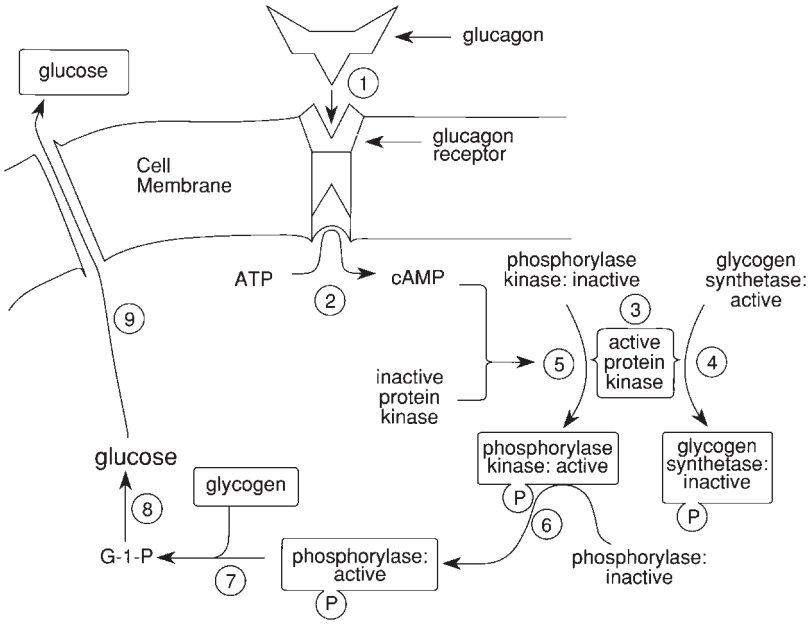

Figure 1.11 Cascade of events triggered by the arrival of a glucagon molecule at a target cell. Glucagon binds to its cell membrane receptor (1). This stimulates the activity of an effector enzyme that promotes the formation of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP) inside the cell (2). cAMP, known as the second messenger, activates one of a class of enzymes known as protein kinases (3). This particular protein kinase catalyzes the phosphorylation (i.e., the addition of phosphate groups (P)) to other specific enzymes (4), (5), and (6), which in turn catalyze the metabolism of glycogen to glucose-1-phosphate (G-1-P) (7) and then the generation of glucose (8) which is released from the cell (9). (Adapted from Norman and Litwack 1997.)

obesity. Unlike type I diabetes (in which individuals do not produce enough insulin), in type II diabetes insulin secretion is elevated and peripheral insulin receptors are reduced in number and insensitive to insulin. It is as though the elevated insulin secretion has desensitized the insulin receptors. Alternatively, it may be that the decreased receptor response has resulted in an increased insulin secretion. Frequently, after weight loss, these individuals regain normal insulin secretion and sensitivity (Norman and Litwack 1987). Why some individuals develop this abnormality in the negative feedback response to insulin is not known.

STEROID HORMONES

In vertebrates, steroid hormones are often referred to by the organ of their origin. For example, the hormones produced by the ovaries and testes (or gonads) are referred to as the gonadal steroids. The principal products of the testes are the androgens, testosterone and dihydrotestosterone. The ovaries primarily make two types of steroid hormones, estrogens and progestins. Examples of estrogens include estradiol, estriol, and estrone. Progesterone

is the principal progestin, so named because it promotes gestation or pregnancy. It should be noted, however, that testosterone is a precursor to estrogen and the ovaries also make testosterone. Conversely, estrogen is a metabolite of testosterone, so the testes also produce some estrogen.

The hormones secreted by the cortex of the adrenal gland are known as the adrenal steroids or corticosteroids. The corticosteroids include two classes of steroid hormones. These are the glucocorticoids and the mineralocorticoids. The glucocorticoids, in general, increase circulating glucose. Cortisol and corticosterone are examples of glucocorticoids. Cortisone is a synthetic version of cortisol that is used medicinally. Androgens (testosterone, dehydroepiandrosterone, and so on) are also produced by the adrenal cortex; they are released in response to ACTH, like the mineralocorticoids that regulate water balance (e.g., aldosterone). In fact, over 50 adrenal steroid hormones can be released in response to ACTH from the pituitary. As we will learn in chapter 11, scientists suspect that there are other hormones determining which steroids the adrenals will produce.

One word of caution may be needed here. Although the principal secretion of the testes in most male vertebrates is testosterone, the testes also secrete small amounts of estrogens, as noted earlier. Similarly, ovaries and adrenals also produce androgens. Thus, the idea that androgens are ''male'' hormones while estrogens are ''female'' hormones is overly simplistic; both sexes make both classes of hormone, albeit in quite different proportions (see chapter 3). The proportions and specific androgens or estrogens that are produced also vary with the species. For example, the stallion has exceptionally high concentrations of estradiol.

The third organ to release steroidlike hormones is the thyroid gland. As noted before, the thyroid hormones look and behave very much like steroids in that they are lipid soluble and not released by exocytosis. The principal thyroid hormones are triiodothyronine, abbreviated as T3, and thyroxine (also known as tetra-iodothyronine, abbreviated as T4).

Steroid hormones (and thyroid hormones) act quite differently from protein hormones. They are lipophilic and therefore readily cross the lipid cell membrane. Target cells are those that contain protein receptor molecules that recognize and bind to a particular steroid. Once the steroid binds to its receptor, the steroid-receptor complex binds to DNA and thereby manages to either increase or decrease the transcription of specific genes (probably by triggering the winding or unwinding of DNA coils), changing the production of a wide variety of proteins. Thus, steroid hormones can **regulate gene transcription** (or expression) and can therefore expert powerful influences upon the development and differentiation of cells.

Another difference between steroids and protein hormones occurs in the regulation of their receptors. Steroid hormone receptors are sometimes regulated by a positive feedback system. In other words, in the absence of testosterone, for example, the number of testosterone receptors decreases.

Therefore, it is sometimes necessary to treat an animal repeatedly with a steroid hormone to induce sufficient numbers of receptors to get a biological response. The number of receptors that can be induced by this mechanism is finite, however, so that if excess hormone is present for a prolonged period of time, eventually no receptors will be available to bind the hormone.

Selective gene activation or regulation is an important concept. Even though all the cells in an animal contain the same genetic material (about 100,000 genes in a human), the genes used, or **expressed**, by individual cells vary. Genes that are expressed in a cell are those genes that are transcribed to produce proteins. Most cells will express the genes that contain the information needed to produce the synthetic and housekeeping organelles, so some genes are expressed in most cells. Then there are some genes that are expressed only in a particular cell type. For example, it has been estimated that liver cells express about 30,000 genes and various neurons express about 50,000 genes, and that about 20,000 of these genes are common to both liver cells and neurons. It is also important for a cell to be able to adapt to the changing needs of an organism. So the expression of genes is regulated by information obtained from within the cell and by information from external sources. One way that the expression of genes can be regulated is through hormonal input. You will learn more about hormone receptors and how they work in chapter 2.

Endocrine Methods

BIOASSAYS

In the old days, the only way to measure the concentration of a hormone in blood or in an organ was to reconstruct in the laboratory some physiological system that normally responds to the hormone. For example, what if you wanted to measure the testosterone concentration in a sample of blood plasma from a bull? You could castrate adult male rats, thereby depriving them of their own gonadal steroid hormones; you would then notice that the prostate glands surrounding the urethra become smaller afterward and that injections of testosterone make them bigger again. The weight of these glands can serve as a biological assay, or **bioassay**, of androgen concentrations. If you now inject the rat with a solution containing hormones extracted from the plasma of a steer (i.e., a castrated bull), kill the rat, and then weigh its prostate, you would see little or no growth, whereas if you injected the rat with a solution of hormones extracted from the plasma of a bull, you should see considerable growth of the rat's prostate. Such bioassays are effective but not very convenient or sensitive. Furthermore, minor differences in the way the rats are treated or the glands are dissected can dramatically affect the bioassay results. This makes it sometimes difficult to compare the results of bioassays from different investigators because not all laboratories measure a substance in the same way.

RADIOIMMUNOASSAYS

These days the most common method for measuring hormone concentrations in blood is the **radioimmunoassay**, or RIA. The RIA was invented by Rosalyn Yalow and Seymour Berson, for which they won the Nobel prize. To measure the testosterone concentrations in bull plasma with an RIA, you need three things: (1) testosterone (T) of known purity that was either expressly manufactured (known as ''synthetic hormone'' because it was synthesized by humans in either a chemistry laboratory or by the enslavement of bacteria) or was ''purified'' from some animal product (testes perhaps) by the use of biochemical methods; (2) testosterone that has been radioactively labeled (T*). By detecting the radioactive particles given off by the radioactive isotopes, it is possible to quantify the total radioactivity and therefore the total number of so-called ''hot'' T* molecules. Nonradioactive T molecules may be referred to as ''cold'' T; and (3) an **antibody** that recognizes and attaches itself to testosterone. Antibodies are large, complex proteins made by an organism to attach to invaders and mark them for destruction (see chapter 10). We can inject a rabbit with the substance of interest and later withdraw some of the rabbit's plasma to harvest the antibodies it made to the substance of interest—in this case, testosterone.

With these three reagents (which can be purchased commercially for most hormones), it is possible to assay the concentrations of testosterone in the sample of bull plasma. We refer to the bull's own testosterone as endogenous testosterone because it was made inside the bull in contrast to exogenous testosterone, which was introduced in either the bull or the plasma sample by researchers. If we were to add the antibodies that recognize testosterone to the sample, they would bind the testosterone more or less irreversibly. Unfortunately, there are no simple ways to count the antibodies. On the other hand, if we added the antibodies to our exogenously supplied ''hot'' T*, then separated the leftover free T* from the T* bound by antibodies, we could use Geiger counter–like machines to detect the antibody-T* complexes by measuring the radioactive T*.

So what would happen if we added a known amount of hot T* to our plasma sample and then added the antibodies? After waiting awhile for the antibodies to bind to both T and T*, we would separate out the leftover, unbound T*. If there were no endogenous testosterone in the sample, we would get just as many antibody-T* complexes (measured by the amount of radioactivity) as we'd get if we added antibody and T* to water instead of plasma. But if there were some endogenous testosterone in the plasma sample, some of the antibodies would stick to that testosterone (which of course would not be ''hot''), and therefore fewer antibody-T* complexes would be made. In fact, if more endogenous testosterone is present, more hot molecules will be displaced and therefore fewer antibody-T* complexes will be detected when we measure the radioactivity. We can find out how much hot T* will be displaced by a given amount of testosterone by measuring

out different amounts of ''cold'' T of known purity (say 50, 100, 250, and 500 micrograms [μg] worth) and seeing exactly how much T* is displaced by each. From this information we could generate a standard curve that would allow us to determine exactly how much testosterone exists in each of our biological samples.

Summary

Behavioral endocrinology joins together the fields of neuroscience, endocrinology, and psychology to ask important and exciting questions about how changes in the endocrine system influence the brain and behavior. In this chapter we have reviewed some of the basic concepts important to the study of the nervous and endocrine systems. Some of the most important concepts are summarized below.

1. The methods used to measure and evaluate behavior must be just as precise and exacting as the methods used to quantify hormones. In many ways, the behavioral component of experiments is much more difficult than the biochemical methods used to quantify the behavior because it frequently depends on visual observation. Thus, a great deal of time is needed to conduct precise and accurate measures of behavior.
2. You will study three different types of intercellular messengers: neurotransmitters, neurohormones, and hormones. Most of these intercellular messengers are packaged into secretory vesicles for release by exocytosis. Differences among these messengers lie in where they are produced, where they are released, and the distance that must be traveled to produce an effect. Neurotransmitters are produced by neurons in the brain, released at the synaptic terminal, and diffuse across the synaptic cleft to produce a response at the postsynaptic membrane. Neurohormones are produced by neurons in the brain, released from synaptic terminals into the bloodstream, and produce their effects at target organs. Hormones are produced by endocrine cells, released into the bloodstream, and produce their effect at distant target organs.
3. Communication between neurons occurs through the release by exocytosis of chemical messengers known as neurotransmitters. The neurotransmitter is released at the synapse and binds to a postsynaptic receptor. This process is known as synaptic transmission. The binding of the neurotransmitter to the receptor results in a change in the membrane polarization of the postsynaptic cell. This influences whether or not the postsynaptic cell will fire an action potential. Drugs that produce changes in neuronal activity usually do so by influencing one or more components of synaptic transmission.
4. Neurohormones known as releasing factors are released by neurosecretory cells in the hypothalamus. These releasing factors travel through a specialized system of blood vessels to reach the anterior pituitary which controls the release of pituitary hormones. The pituitary hormones enter the bloodstream to regulate the release of hormones from the peripheral endocrine glands.

Negative and positive feedback from the endocrine glands to the brain and pituitary results in coordination of the neuroendocrine systems.

5. Hormones are classified according to their molecular structures as either steroid or protein hormones. Both kinds of hormones produce their effects on target tissues by binding to specific receptors. The process induced by the binding of a hormone to a receptor depends on whether the hormone is a protein or a steroid. Protein hormones induce their effects by binding to membrane-associated receptors and inducing intracellular changes by activation of a second messenger system. Steroid hormones bind to intracellular receptors, and the steroid-receptor complex alters gene expression.
6. Genetic information is coded by the DNA found in the nuclei of eucaryotic cells. Particular lengths of DNA known as genes are transcribed into a string of specific RNA nucleotides that leave the nucleus. In the cytoplasm of the cell, RNA is translated into a specific sequence of amino acids. A short sequence of amino acids is called a peptide; a long sequence is called a protein. The genetic code is important for our understanding of information in later chapters for two reasons. First, steroid hormones produce their effects by altering gene expression, and this can be shown to be related to the behavioral effects of steroid hormones. Second, releasing factors, neurotransmitters, and some hormones are proteins or are synthesized from proteins. One way to measure changes in production of these molecules is to quantify the production of the mRNA that codes for these proteins.

Study Questions

1. You are observing your favorite species in the wild, the gastric brooding frog, and you would like to be sure that your observations of the animal's mating behavior will be reproducible. What steps would you take to obtain reliable, reproducible data? How would you determine if hormones are involved in this behavior?
2. What are the differences among oxytocin, LH, and estrogen? Include a discussion of the chemical structure, how the hormone is released, where it is released from, and the receptors on which the hormones act.
3. What are the differences between positive feedback and negative feedback in the control of hormone secretion? Give examples of each.
4. How do psychoactive drugs produce their effects in the brain? Be specific.
5. What is selective gene expression? How would you determine if a hormone was altering gene expression?

Sources of Additional Information

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