1 Hormonal Mechanisms

Social behavior has been linked to many hormones. The best established connections are to steroid hormones, pituitary prolactin, and a few peptide neurohormones (table 1.1). What are these hormones that have been shown to be important for social behavior? How do they work? How are they regulated by the physical environment and the social world? This chapter presents some essential facts and concepts that will inform the rest of the book. It also shares exciting discoveries and new ideas with major implications for behavior.

Hormonal mechanisms, like everything else about organisms, are a fascinating mixture of old and new. Modern biology has revealed astounding similarity in genomes and biochemical signaling pathways across long-separated lineages of the animal kingdom differing in ecology, morphology, and behavior. Similarly, some hormones and their actions are highly conserved and taxonomically widespread. Such mechanisms are very old and could reflect the limited number of workable solutions to basic problems of living given the physicochemical realities of the earth. Along with these are hormonal mechanisms that are more recent, more derived, more taxon specific, and serve different functions in different kinds of animals. This chapter highlights the relatively conserved hormonal mechanisms, occasionally hinting at some of the diversity that is seen. Subsequent chapters will draw on both types. Chapter 7 will return to a focus on the hormonal mechanisms themselves to look at broad-scale evolutionary patterns and differences between major lineages.

Like most aspects of the living world, hormonal mechanisms resist easy categorization. Even the concept of a "hormone" has undergone significant change. Hormones used to be defined as the secretions of the ductless (endocrine) glands. Hormones were thought of as relatively long-distance internal messengers related to regulatory functions such as growth, metabolism, or reproduction. They were contrasted, for example, with neurotransmitters such as dopamine, serotonin (5-HT), or acetylcholine, chemicals for rapid communication across the tiny gaps between neurons and between neurons and muscles. A prototypical vertebrate example would be testosterone, which is carried by the circulation from the testes to target tissues such as the rooster's comb and the neurons and muscles that produce crowing. The discovery that hormones can be made in the brain itself upset this neat dichotomy. Some vertebrate neural tissue can release neurohormones into the circulation and most insect hormones are neurohormones (Nijhout 1994). If hormones define an endocrine organ, then the vertebrate brain is the biggest endocrine organ in the body! The boundaries between hormones, neurohormones, neuromodulators,

TABLE 1.1
Steroids with known links to social behavior, along with neuromodulators and neurohormones that are important for understanding hormone action in relationship to social behavior

Chemical	*Acronym*	*Type of chemical*	*Comments*
Estradiol (1,3,5[10]-estratrien-3,17β-diol)	E_2	Steroid	An estrogen
Progesterone (4-pregnen-3,20-dione)	P	Steroid	A progestogen
Testosterone (4-androsten-17β-ol-3-one)	T	Steroid	An androgen
Androstenedione (4-androsten-3,17-dione)	A4 (AE)	Steroid	An androgen
Dihydrotestosterone (5α-androstan-17β-ol-3-one)	DHT	Steroid	An androgen
11-Ketotestosterone (4-androsten-17β-ol-3,11-dione)	11-ketoT	Steroid	An androgen; teleost fishes only
Corticosterone (4-pregnen-11β,21-diol-3,20-dione)	CORT (B)	Steroid	A glucocorticoid
Cortisol (4-pregnen-11β,17,21-triol-3,20-dione)		Steroid	A glucocorticoid
20-Hydroxyecdysone		Ecdysteroid	Arthropods
Gonadotropin-releasing hormone	GnRH	Decapeptide	See also table 7.2
Corticotropin-releasing hormone	CRH (CRF)	Peptide	
Adrenocorticotrophic hormone	ACTH	Peptide	
Prolactin	PRL	Protein	
Vasopressin (arginine vasopressin)	AVP (VP)	Nonapeptide	See also table 7.1
Vasotocin (arginine vasotocin)	AVT (VT)	Nonapeptide	See also table 7.1
Oxytocin	OT (OXY)	Nonapeptide	See also table 7.1
Melatonin	MT (MEL)	Indoleamine	
Thyroxine (3,5,3′,5′-tetraiodothyronine)	T_4		
Triiodothyronine (3,5,3′-triiodothyronine)	T_3		
Prostaglandins	PGs	Eicosanoids	
Juvenile hormones	JHs	Terpenoids	Arthropods

Source. Sources include Nijhout (1994) and Norris (1996).

and neurotransmitters are now rather fuzzy in both vertebrates and invertebrates, and the requirement of long-distance communication to be a hormone no longer seems necessary or desirable. Both nervous and endocrine systems originally evolved from a system of cell–cell chemical messengers, which is why chemically they overlap so much and why table 1.1 includes a number of substances not considered to be "classic" hormones.

As with other signaling systems, we can ask about the source of the signal (where the hormone is produced), the nature of the signal (the hormone), and the receiver for the signal (the target organ, tissue, or cell), which for behavior often means the nervous system and neurons. The brain is both a source and a target. Targets can even include other individuals in the case of those pheromones derived from hormones.

Why Does Social Behavior Need Hormonal Regulation?

Before delving into hormonal nuts and bolts, it is only fair to first provide some clues as to how hormones help animals solve real-world problems and achieve fitness. In general, hormones are coordinators: of reproduction, of suites of physiological and behavioral components, of different parts of the brain, of brain with body. On both short- and long-term (life history) scales, they coordinate behavioral and physiological sequences over time, establish the duration of events and sequences by regulating onset and offset, and modify the nervous system appropriately (Truman 1994). They help adjust behavior to circumstances and contexts: physical, social, and developmental.

Mating behavior illustrates well the merits of this functional approach. Mating has significant costs, such as increased risk of predation and communicable disease. The obvious benefit of mating is the achievement of reproductive success, but this benefit is possible only if there are mature gametes (eggs and sperm) ready for fertilization. Gamete maturation is hormonally regulated, and so one reason that mating behavior is hormonally regulated is to ensure that the behavior is coordinated with the presence of fertilizable gametes. Vertebrates achieve this by having the gonads produce both gametes and hormones, with the same hormones regulating gametogenesis and mating behavior. Insects achieve this by having the hormones from elsewhere that stimulate the gonads also stimulate sexual behavior. One of the major achievements of animal behavior research has been to show how other social behaviors such as territoriality and dominance also serve reproductive interests. To the extent that they should occur only when fertility is possible (their occurrence at other times would be too costly), they also need hormonal regulation.

Social behavior is often age related, and hormones are a mechanism that can ramp up the behavior at the appropriate age. The onset of adult reproductive behavior at puberty in mammals is a widely known example because of our

own personal familiarity with the phenomenon. In animals with indeterminate growth, size rather than age may be the trigger for the onset of adult social behavior, and hormones can be a messenger between size and behavior. In adulthood the behavior may need to ramp up and down seasonally, again requiring some kind of hormonal regulation.

Other reasons why hormonal regulation is needed are closely tied to the social nature of social behavior. Responding in appropriate ways to other individuals (to competitors or potential reproductive partners) is crucial for reproductive success. In many (not all) species, for mating to increase fitness, both the male and female have to be fertile at the same time. For a pair of birds to raise young together, both have to be in the parental mood at the same time. Hormones are a major mechanism ensuring coordination between different individuals. Another major achievement of animal behavior research has been to show how such "coordination" is often a mixture of conflict and cooperation. Hormones are likely to be responsive to this give and take.

Finally (to conclude this preview of coming attractions), social behavior is often different in quantity or quality between or even within the sexes. Animal behaviorists have devoted a great deal of attention to understanding why such differences have evolved. There need to be hormonal mechanisms to produce the behavioral phenotypes during development and cause the behavior to be expressed more in one sex or within-sex type in adulthood.

All these are compelling reasons for hormonal regulation of social behavior. Where none of these conditions apply, we would not expect the behavior to be hormonally regulated. If all individuals engage in the behavior regardless of age, sex, breeding condition, or social context, it wouldn't make sense to go looking for hormonal regulation.

The word "regulation" is appearing here for a reason. Unlike light switches turning light bulbs on and off, hormones don't make behavior happen in a deterministic manner. It would be a very poorly adapted male who would begin a mating sequence just because he had a lot of testosterone even if no female was present and he was surrounded by predators. Rather, hormones are one of several factors that go into the nervous system's decision. They may change the thresholds for other factors that enter into the decision (for example, thresholds for responding to stimuli from another animal) but are not normally the sole triggering agent. Thus, words like "regulate" or "prime" are often used, and "permit behavior" is usually preferred to "cause behavior." This important concept will be a recurring theme.

Steroids

The use of steroids for internal signaling is probably universal in metazoans (Wang et al. 2001). Like other chemical regulators, steroids are normally pres-

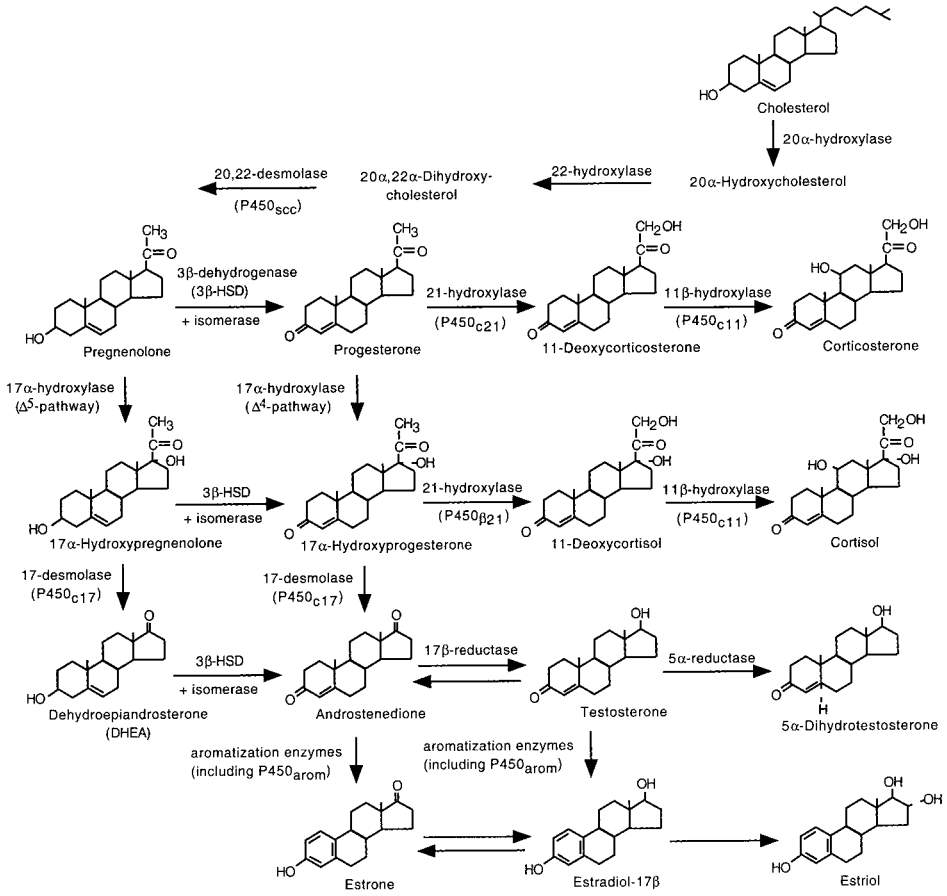

FIGURE 1.1. Pathways for the synthesis of sex steroids and glucocorticoids in gonadal and adrenal (interrenal) tissue. Note that most arrows are unidirectional (the reverse transformation does not occur) while others are bidirectional. The P450 enzymes are members of the cytochrome P oxidase family and have alternative names such as CYP19 (for $P450_{arom}$) or CYP11A (for $P450_{scc}$, where "scc" stands for "side chain cleavage"). Redrawn with minor modifications from Bentley (1998). © 1998 Cambridge University Press.

ent in minuscule amounts. Their mechanisms of action involve cascades of events that amplify these tiny signals. This property helps explain how a very dilute concentration of circulating steroid can dramatically change the behavior of a very large animal, as when estradiol induces estrus behavior in a lion.

Estradiol is a steroid. What are steroids? How and where are they made? In today's world the word "steroid" triggers images of bulked up super-athletes. Steroids are a much larger category than this, for a steroid is any molecule (and there are many) with the distinctive structure shown in fig. 1.1. Steroids

are small molecules; molecular weights are usually in the 250–350 range. The structure they all have in common is the four rings in the arrangement shown: three hexagons and one pentagon. Different steroids vary in what is attached to the carbons of these rings and whether the rings contain double bonds. Rings with three double bonds, like the A rings of the estrogens, are said to be aromatized. They get this way through an enzymatic process (aromatization, see next section) that is very important for behavior and will be referred to repeatedly. Names like "testosterone" or "corticosterone" then refer to specific steroid molecules, to the different variations on the basic structural theme. These specific steroids are identical across species. For example, testosterone, a potent androgen and the predominant circulating androgen in many vertebrates, is the exact same molecule in anchovies, axolotls, adders, antbirds, aardvarks, and apes. This makes steroids very different from peptide neurohormones and amino-acid-based hormones such as prolactin, which vary structurally among species.

These steroids are then lumped into categories according to chemical structure, bodily source, or physiological functions. One category is the gonadal steroids, also called sex steroids, those manufactured by vertebrate testes and ovaries. These are predominantly androgens ("masculinizing" steroids), estrogens ("feminizing" steroids), and progestogens. Another is the adrenal steroids, those manufactured by the adrenal cortex or interrenal tissue (in fishes and amphibians), including the glucocorticoids and the mineralocorticoids, which together constitute the corticosteroids. Another category is the ecdysteroids, which function in molting or ecdysis and are limited to insects. Vertebrate sex steroids have been found in invertebrates as well, but their functions, if any, are still unclear.

As is usually the case when humans try to carve up nature, the so-called gonadal sex steroids keep slipping the boundaries of their name in ways that will become very important for understanding their relationship to social behavior. Both androgens and estrogens are produced by cells located outside the gonads, for example, by the adrenal cortex (interrenal tissue), by fat, and by the brain. This means that removal of the gonads does not always eliminate or even reduce circulating androgens or estrogens.

The association of androgens with masculine traits and estrogens with feminine traits is also a poor fit with nature's ways. No sex-specific steroids (or other hormones, for that matter) have been discovered in vertebrates. Furthermore, androgens can have feminizing actions and estrogens can have masculinizing actions. It is common to find sex differences in circulating steroid levels (including corticosteroids), especially at times when animals are breeding, but this is not always the case. Male pigs and horses have as much estradiol in the blood as estrous females. Some female fish, lizards, and amphibians produce as much or more testosterone than males do (Borg 1994; Staub and DeBeer 1997). Furthermore, even with "conventional" sex differences (higher andro-

gens in males, higher estrogens in females) females often have more androgen than estrogen in absolute terms (numbers of molecules per unit volume).

Steroid Synthesis and Metabolism

Vertebrate gonadal and adrenocortical (interrenal) tissues do not produce the exact same steroids, although there is some overlap. Gonads usually release more androgens, estrogens, and progestogens, whereas adrenals usually release more glucocorticoids and mineralocorticoids. In addition, not all species produce the exact same steroids. For example, in primates such as humans, and in teleost fish such as salmon, cortisol is the predominant glucocorticoid, whereas birds and most rodents produce corticosterone. In many nonprimate mammals the weakly androgenic steroid dehydroepiandrosterone (DHEA) comes mainly from the gonads but in primates it comes mainly from the adrenals. What is it about the manufacture of steroids that accounts for these species and tissue variations? Steroids are produced in steps by a series of synthesizing enzymes. Which steroids are found where is a function of which enzymes are produced there. This in turn is a function of whether the animal has the gene for the enzyme, in which tissues the gene is being transcribed into mRNA for the enzyme, and in which tissues the mRNA is being translated.

The synthesizing pathway for the production of androgens and estrogens by vertebrate gonads and for the production of glucocorticoids by adrenal tissue has been well established (fig. 1.1). Both pathways begin with the abundant sterol cholesterol. Steroid synthesizing tissue contains a store (depot) of cholesterol obtained from plasma lipoproteins that is mobilized for steroid synthesis by steroidogenic acute regulatory protein (StAR). The enzyme that converts cholesterol to pregnenolone and thus is essential for all steroid production is cytochrome P450scc. Its name indicates that it is a member of the large and very ancient cytochrome P450 family of enzymes and that this particular member performs the side-chain cleavage (scc) step in steroidogenesis. Steroid secretion and concentration in circulation is more a matter of synthesis than storage (little is stored), so synthesis needs to happen fast, as does putting the brake on it. Conversion of cholesterol to pregnenolone by P450scc is the rate-limiting step, with StAR rapidly mobilizing cholesterol for synthesis.

Different enzymes then turn pregnenolone into progesterone and other progestogens and turn progestogens into either corticosteroids or androgens. The aromatizable androgens such as testosterone and androstenedione are the substrate for the production of estrogens (which have a fully aromatized A ring), including estradiol. This feat is carried out by another member of the cytochrome P450 family, P450arom, also called aromatase or estrogen synthase. Regulation of the gene for aromatase determines how much aromatizable androgen is turned into estrogen, that is, sets the ratio of those androgens to

estrogens (Lephart 1997). Aromatization is a one-way street; estrogens cannot be turned back into androgens. The synthesis of estrogens from androgens will be important for understanding steroids and social behavior, and is one of several facts that flies in the face of the common assumption that androgens are strictly male hormones and estrogens are strictly female hormones. Not all androgens can be aromatized; 5α-dihydrotestosterone (5α-DHT) cannot, nor can the teleost fish androgen 11-ketotestosterone.

The gonads and adrenals are not the only parts of the body that express these enzymes and can carry out these conversions. The liver, fat tissue, and the mammalian placenta can carry out one or more of the steps. Steroid targets, including the brain, also have this capability, as when a rooster's comb converts testosterone to the more potent 5α-DHT or the hypothalamus of the brain, a region rich in aromatase, converts testosterone to estradiol. This phenomenon in which a steroid precursor or prohormone made elsewhere is turned at the target into a locally more potent steroid is important for understanding steroid action.

Steroids are small lipophilic molecules that can easily move between cells and tissues. They can easily pass from the circulation to the brain or from the brain to the circulation (Schlinger and Arnold 1991). The yolky (lipid) eggs of nonmammalian vertebrates invariably contain steroids, often in the same amounts as in the maternal circulation (Dickhoff et al. 1990; Schwabl 1993; Adkins-Regan et al. 1995).

Regardless of where the steroid originates and is transported, the eventual fate of most circulating steroid molecules is metabolism to other forms by the liver, which then pass out of the body in the urine and other excretory products. Endocrinologists describe these forms as "weak" or "inactive" on the basis of their minimal biological activity in the producer, but these molecules can be highly informative to conspecifics (chapter 2).

More recently, it has been proposed that the brain not only can convert steroids made elsewhere, but also can manufacture steroids such as estrogens de novo ("from scratch") from cholesterol (Baulieu and Robel 1990; Tsutsui and Schlinger 2001; Mellon and Griffin 2002). Evidently, neurons or glia (the "other" brain cells) express the necessary steroid-synthesizing enzymes. These "neurosteroids" are thought to differ from other steroids found in the brain (those made from gonadally or adrenally produced precursors) in three important ways. First, they can be made in parts of the brain that are not conventional targets for circulating steroids, for example, in the mammalian isocortex ("neocortex") or avian caudal nidopallium. Second, it is suspected that parts of the steroidogenic pathways might differ from those in fig. 1.1. Estrogens might be made from nonandrogenic precursors, and the different steps in the synthesis sequence might take place in different cell groups rather than all in the same cells. Third, these neurosteroids are thought to act locally, possibly on the same cells where the final synthesis step occurs. These exciting discoveries have seldom been directly linked to ecologically relevant behavior, but

they are inspiring new hypotheses relating steroids to brain function that promise to make the connection soon.

Steroid Measurement and Dynamics

Many experimental studies of hormones and social behavior measure steroid levels. As in any kind of science, it helps to know something about the assumptions and limitations of the methods used to do this. What are the experimenters up against in trying to figure out what the numbers mean? What should an informed critical reader know?

Steroid levels that are reported are most often amounts of the steroid in plasma separated from whole blood drawn from the systemic circulation, and are most often expressed as weight per unit volume. For example, plasma testosterone in male amphibians ranges from less than 1 to over 200 nanograms per milliliter (ng/mL) and estradiol in female lizards ranges from less than 1 to over 200 picograms per milliliter (pg/mL) (Norris 1996).

Getting a blood sample from a wild animal presents special problems. Imagine trying to study hormonal responses to intermale competition in elephants. Or it might be important on research or conservation grounds to avoid disturbing the animal. For these reasons, there is increasing interest in being able to measure steroids noninvasively. Fecal or (in humans) saliva samples are viable alternatives provided they are stored properly and carefully validated (as they must be) (Goymann et al. 2002; Lynch et al. 2003; Buchanan and Goldsmith 2004). In some digestive systems the hormonal signal has been integrated over a substantial time period, which is either an advantage or a disadvantage depending on the question asked.

The most commonly used method for measuring steroids in samples is some type of radioimmunoassay (RIA), a competitive protein-binding assay in which radioactively labeled steroid added to the sample competes with the animal's own steroid for an antibody (the binding protein). The four criteria for a valid RIA are well established (Midgley et al. 1969). They include (1) specificity (is it measuring what you are trying to measure instead of picking up some other steroid or blood constituent?); (2) accuracy (on average, does it give values that correspond to the actual amount in standard samples?); (3) precision (how variable are the numbers that the assay yields? if the same sample is assayed multiple times, do the numbers agree?); and (4) sensitivity (can it detect the steroid at the levels present in the samples?). It is always reassuring to see these criteria addressed in the methods section of a paper, especially when an assay is being used in a new species where its performance has not already been established. For example, it is wise to look hard at sensitivity whenever samples are reported as below the limit of detectability. This does not always mean that the levels are biologically low. An assay that is

sensitive enough for one species' normal range of values may not be for another's. Where steroids have been extracted from the sample prior to assay, an additional consideration is recovery, that is, what percentage of the steroid actually present was successfully extracted? The RIA values have to be corrected for this recovery percentage in arriving at a final number. Assays are not perfect, and the final results are estimates of true but unknown values.

More recently, enzyme immunoassays (EIA), another type of competitive assay, have been developed that have sufficient sensitivity for a range of animal sizes. These have the important virtues of being faster than an RIA and not requiring radioactive substances or licenses. As with any assay, they must be validated first.

Assuming that the assay is valid, what has been measured? Some of the steroid molecules in the blood are attached to binding proteins, including nonspecifically binding albumins, which are much larger molecules than the steroids themselves. For example, glucocorticoids in the blood of many vertebrates are bound to corticosteroid-binding globulins (CBGs), which in spite of their name bind some other steroids as well. Because the function of these binding proteins has been a bit of a mystery, they are often ignored, but this threatens to be a perilous strategy (Breuner and Orchinik 2002).

Furthermore, what has been measured is the amount of steroid in the blood at the one point in time when the sample was taken. However, steroids, like other hormones, are subject to a variety of biological rhythms over timescales of minutes to months. Their levels are dynamic, not static. Some are secreted and released in a pulsatile manner, so that blood levels rise suddenly and then fall gradually. This phenomenon is well known in mammals but is also seen when birds are cannulated so that blood samples can be taken frequently (Ottinger 1983; Bacon 2001). The rate of decay of the pulse and the half-life of the steroid in the blood reflect clearance through binding to receptors at the targets and metabolism by the liver. Steroid half-lives are on the order of minutes to an hour or more, and vary depending on the steroid and the animal's metabolic rate.

Several steroids tend to show pronounced daily rhythms. Glucocorticoids in mammals and in some birds peak around the time of the onset of the daily period of activity (awakening from sleep), while in other birds they peak during the night when the birds are inactive (Carsia and Harvey 2000). Testosterone in male mammals, including humans, shows a similar rhythm, that is, peaks at awakening, contrary to our cultural notions that night is the sexy time. Given this dynamism, how can one sample adequately capture an animal's steroid level? In some sense it can't. The practical solution to this problem used by many researchers is to take all the samples from all the animals at the same time of day, usually the time when the steroid level is highest or in the middle of its daily range, so that results of experimental manipulations are not confounded by daily hormone rhythms.

Finally, what is important for the production of behavior is the amount of steroid that the brain and other targets "see," not necessarily the amount in the general circulation. The steroid receptors in the brain and elsewhere usually have a higher affinity for steroids than any binding proteins in the blood. Furthermore, even if the target and blood amounts are positively correlated, the target's active role in converting the steroid to another form means that we almost never know how much of the locally active steroid the target cells actually saw.

Neuropeptides and Prolactin

Peptides are amino-acid-based molecules and thus very different from steroids both chemically and biologically. Along with other amino-acid-based molecules, but unlike steroids, their structure (exact sequence of amino acids) differs among species. The version of the peptide found in one species won't necessarily work when given to another, especially if the two are unrelated. Even if it does work, the effect (the function thus altered) will not necessarily be similar, because similar molecules can be employed for different purposes in different taxa. The good news is that peptides are closely linked both to interesting social behavior and to genes, and hold great promise for aiding the search for molecular mechanisms of such behavior. Each amino acid in the peptide sequence is coded by a triplet of DNA nucleotides. Peptides are in a profound sense close to the genome.

A large number of peptides function as chemical messengers and regulators in both the nonneural periphery and the nervous system of metazoans. The same exact peptide molecule can be both a neuropeptide (the category of primary interest here) and a peripheral hormone. Like many other chemical regulators of neural activity, neuropeptides are produced by discrete brain cell groups. The brain is a source of peptides as well as a target, but because peptides do not readily cross the blood–brain barrier, some brain region targets "see" only those peptides that were produced in the brain, not those in the peripheral circulation. This is why research on peptides and social behavior seldom measures peptide levels in the general circulation or administers peptides systemically, but instead relies on manipulations and measurements of peptides in the brain itself.

Table 1.1 includes some of the large and ever-increasing number of neuropeptides that have been discovered. Many of these fall into families, that is, groups of peptides that have high but not complete overall amino-acid sequence resemblance. The families that will appear the most frequently in this book are the oxytocin family of nonapeptides (a relatively conserved family of peptides containing nine amino acids, named for a mammalian version), the gonadotropin-releasing hormone (GnRH, formerly called luteinizing-releasing

hormone or LHRH) family of decapeptides (containing 10 amino acids), and the corticotropin-releasing factor (CRF, also called corticotropin-releasing hormone or CRH) family. These families are very widely distributed among animals, suggesting that they are quite old, possibly appearing early in metazoan evolution (Peter 1983; Sherwood and Parker 1990; Lovejoy and Balment 1999; Gorbman and Sower 2003). Functions related to reproductive behavior appear to be old as well, predating vertebrates. For example, lys-conopressin, a member of the oxytocin family, regulates sexual behavior in a snail (van Soest and Kits 2002).

Peptides of the GnRH family have an essential role in reproduction in all vertebrates. At least 14 forms have been found altogether, and each vertebrate lineage produces one or more of them. Molecules with GnRH-like activity also occur in invertebrates such as *Aplysia* (a mollusc) and cnidarians, and it will be interesting to see if they turn out to have reproductive functions in these animals as well (Tsai et al. 2003). The discovery of multiple GnRH forms has produced a confusing naming situation. Initially forms were named for the type of animal in which they were discovered, for example, "mammalian GnRH." They still have these names even though they have since been found in other kinds of animals; the names are historical accidents that no longer make sense (Fernald and White 1999). Chapter 7 will take another look at the GnRH family but until then "GnRH" will refer to those that regulate the gonadotrophic hormones of the vertebrate pituitary. In mammals GnRH is released in a pulsatile manner by a "pulse generator" in the hypothalamus. The GnRH message to the anterior pituitary lies in the frequency of the pulses, not their amplitude. The higher the frequency, the more gonadotropin is released into the circulation.

The neuronal cell groups that produce the neuropeptides linked to social behavior are located in characteristic brain regions. For example, in several mammals arginine vasopressin (AVP) is produced by cells in the supraoptic nuclei of the hypothalamus, the medial nucleus of the amygdaloid complex (MeA), the bed nucleus of the stria terminalis (BNST, the output from the MeA to the hypothalamus), and the lateral septal region (de Vries and Miller 1998). When different vertebrate lineages are compared, there is some conservation but also some diversity in the anatomical distribution of a neuropeptide such as AVP (Moore and Lowry 1998; Goodson and Bass 2001). The working hypothesis is that neuropeptides have different functions depending on where they are produced (in what neurons), where the projections from those neurons go, and whether the source or projection is steroid regulated. In a wide array of vertebrates, AVP neurons in the BNST and MeA and their projections to the lateral septum are steroid regulated.

Prolactin is an amino-acid-based hormone, but the number of amino acids is much greater than in neuropeptides such as GnRH or AVP. It is produced by the anterior pituitary and in the brain. Its name comes from its stimulating

effect on milk production in mammals. In spite of this sex- and taxon-specific name, it is produced by both sexes, and molecules resembling mammalian prolactin are found in other vertebrates. Because prolactin is a large molecule with an amino-acid sequence that varies significantly across taxa, it is difficult to measure and to manipulate. An RIA antibody that recognizes mammalian prolactin will not necessarily recognize avian or other prolactins. Prolactin that is taken from pituitaries of mammals such as sheep and given to birds or fish will not always mimic the biological effects of their own prolactins.

Where and How Do Steroids Act to Alter Behavior?

Steroids, like other hormones, have targets, that is, groups of cells that contain the machinery to respond. The brain is a key steroid target for understanding social behavior, because it contains a lot of steroid target cells and is responsible for making behavior responsive to the environment, purposeful, and intelligent (fitness enhancing). Other steroid targets also impact social behavior, such as deer antlers or sonic muscles of vocalizing fish. With the explosion of interest in neuroscience, the brain has taken center stage, but the periphery cannot be ignored. Among other reasons, hormone-dependent external morphological characters are clearly important signals to other animals.

The brain targets, like peripheral targets, express steroid-metabolizing enzymes that regulate what the active steroid is and how much of it the neurons see. Targets are dynamic, not passive, a key concept for understanding behavior. This "supply and demand" aspect of local steroid metabolism is essential for a signaling system. The molecular mechanisms of the regulation of the genes for these enzymes are a critical link between steroids and behavior. One consequence of the role of local steroid conversion in brain and other targets is that circulating steroids aren't necessarily the active steroids for behavior. This means that even steroids that are thought to be behaviorally irrelevant because they are only "weakly" androgenic or estrogenic should not be ignored, because they could be converted in the brain to behaviorally active steroids. This is why the abundant but supposedly "weak" androgen DHEA is receiving renewed attention (for example, Soma et al. 2002).

Studies of aromatase provide strong support for this principle that local conversion is critical for steroid action. It is present in all vertebrate brains, and its regional distribution (especially in the diencephalon and limbic system) is reasonably conserved (Callard 1984; Saldanha et al. 1998; Naftolin et al. 2001). Levels of aromatase activity are especially high in brains of birds and teleost fish. Aromatase is expressed in ovaries but not testes of these animals, but nonetheless males have circulating estradiol. All of that estradiol has most likely come from the brain. The initial discovery of aromatase activity in the brain led to the radically new hypothesis that some of testosterone's role in

the development and expression of maleness might actually be due to local conversion to estrogens (Naftolin and MacLusky 1984). The counterintuitive hypothesis that estrogens might be the "real" male sex hormones for some components of masculine behavior has received resounding experimental support for several species of birds and mammals (Balthazart and Ball 1998).

Steroid molecules can readily pass through the cell membranes of neurons and glia, but they can just as readily pass back out. To become concentrated in sufficient quantity and for long enough to do anything there needs to be some mechanism to grab and hold on to them. This is what intracellular steroid receptors do. Six types are thought to occur in most vertebrates: progesterone receptor (PR), androgen receptor (AR), glucocorticoid receptor (GR), mineralocorticoid receptor (MR), and two types of estrogen receptors, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) (de Kloet 1995; Carsia and Harvey 2000; Breuner and Orchinik 2001a,b; Sloman et al. 2001; Thornton 2001). In spite of their names, they are not always specific to a single steroid or steroid category. Androgen receptors often bind both testosterone and 5α-DHT and their role in behavior is well established. In mammals, and probably birds as well, MRs (type I corticosteroid receptors) have a high affinity for (bind strongly) glucocorticoids as well as mineralocorticoids such as aldosterone, and regulate basal (resting) levels and daily rhythms of glucocorticoids. There are lots of them in the brain, mainly in hippocampal and septal neurons. GRs (type II corticosteroid receptors) are also found widely in the brain, including the hippocampus, and in both neurons and glia. They have a lower affinity for glucocorticoids than type I receptors and appear to function primarily when glucocorticoids are elevated, for example, during a stress response. The role of ERα in responses of targets related to behavior is well established, and the term "estrogen receptors" usually refers to this receptor type. The behavioral or other functions of ERβ and of the recently discovered teleost ERγ are not yet clear, but research in these areas is being actively pursued (Hawkins et al. 2000; Ogawa and Pfaff 2000; Temple et al. 2001; Ábrahám et al. 2003).

The locations of the sex steroid target cells in the brain are somewhat but not entirely conserved in vertebrates. Forebrain targets for circulating steroids include specific nuclei or regions within the preoptic area, the hypothalamus, a portion of the amygdaloid complex and its homologs, the BNST and its homologs, and the septum (fig. 1.2). The gross distribution of sex steroid targets also overlaps the distribution of GnRH family peptides, especially in the septal and preoptic areas (Demski 1984). The steroid target areas in the brain shown in fig. 1.2 are targets because neurons (or glia [Jordan 1999]) there express these receptor proteins. The figure is oversimplified because the different kinds of receptors (and possibly receptor subtypes as well) don't have exactly the same anatomical distributions (Bernard et al. 1999). The receptors themselves are highly but not totally conserved proteins (Baker 1997). There

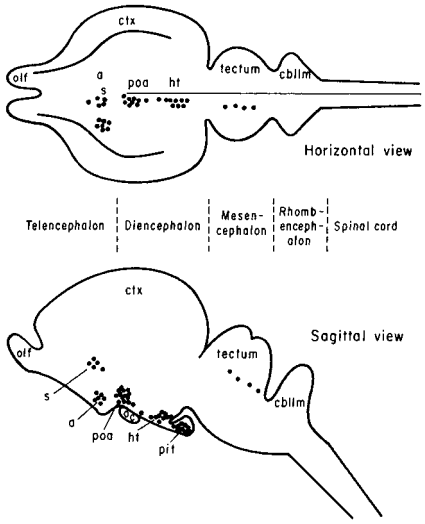

FIGURE 1.2. Conserved brain targets for sex steroids. This schematic of a generalized vertebrate brain shows where groups of labeled neurons (each group indicated by a black dot) have been found following injection with radioactively labeled testosterone or estradiol in most species studied. In some species additional target areas are seen using this or other methods (see, for example, fig. 2.11 showing the telencephalic steroid targets unique to songbirds). a, amygdala or its homolog; cbllm, cerebellum; ctx, cortex; ht, tuberal region of the hypothalamus; oc, optic chiasm; olf, olfactory bulb; pit, pituitary; POA, preoptic area; s, septum. Reprinted from Morrell and Pfaff (1981). © 1981 Plenum Press (Kluwer Academic/Plenum Publishers).

is some anatomical overlap between the expression of enzymes that produce steroids in the brain and the expression of the receptors for those steroids, although not as much as used to be assumed (Ball et al. 2002).

How can a steroid molecule bound to an intracellular receptor accomplish anything as powerful as making an antler grow, a bird sing, or a fish spawn? The answer is wonderful: steroids "tickle the genome." Steroid receptors are what are called ligand-dependent DNA-binding transcription factors. The steroid is the ligand. The steroid–receptor complex binds to hormone response elements located in the promoters of steroid regulated genes (fig. 1.3). In combination with co-activators and co-repressors they alter gene transcription and regulate the amount of mRNA transcript emanating from the target genes. Those transcripts are in turn translated into peptides and proteins. Exactly what genes are affected, and how the target tissue will respond, depends on the steroid receptor co-factors and downstream mechanisms (Charlier et al. 2002). At peripheral targets such as antlers of deer or clasping muscles of amphibians, these proteins might result in cell multiplication and tissue growth. In the brain, the protein products can include enzymes for steroid synthesis and metabolism, steroid receptors (steroids often regulate their own receptors), enzymes for

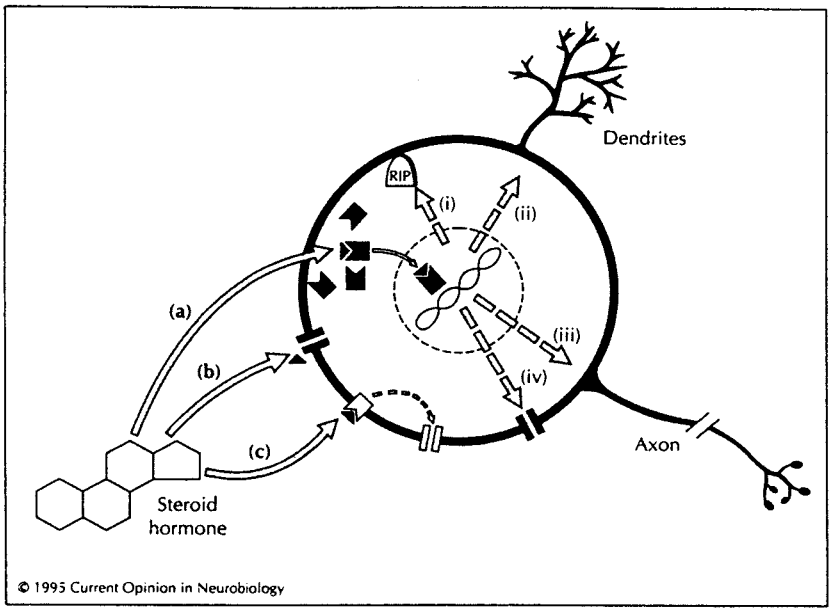

FIGURE 1.3. Mechanisms of steroid action on neurons. (a) Steroid hormones bind to intracellular receptors that alter gene expression, resulting in (i) programmed cell death (apoptosis), (ii) growth or regression of dendrites, (iii) changes in synaptic function, and (iv) synthesis or modulation of ion channels. Two classes of nongenomic effects are also shown: (b) direct binding of steroids to ion channels, and (c) binding of steroids to steroid receptors on the cell surface and modulation of ion channels via second messengers. The cell surface and intracellular receptors are structurally dissimilar. Reprinted from Weeks, J. C. and Levine, R. B. 1995. Steroid hormone effects on neurons subserving behavior. *Curr. Opin. Neurobiol.* 5: 809–815. © 2000 Elsevier Science.

production of neurotransmitters, receptors for neurotransmitters, neuropeptides and their receptors, ion channels, proteins for building and repairing axons, dendrites, and synapses, and substances that increase the number of newly proliferated neurons (Harding 1992; Saligaut et al. 1992; Micevych and Hammer 1995; Young and Crews 1995; Zakon 1998; Fowler et al. 2003). That's a lot of behavior-impacting gene products! Through their intracellular receptors steroids alter neural activity now and in the future, alter their own production and reception and that of other steroids, and regulate some of the other neural signaling systems important for social behavior.

The fact that steroids change gene activity puts them at the center of any effort to bridge the gap between genes and social behavior. It tells us that the connection between genes and hormonal mechanisms is bidirectional, not unidirectional. Furthermore, social interactions cause changes in steroid levels, which in turn cause changes in the gene activity of both participants, a point that will be developed in chapter 2.

Steroid receptors are not the only gene transcription factors and steroids are not the only cellular messengers that activate genes. Rather, they are part of one superfamily containing over 60 related transcription factors. This superfamily includes receptors for thyroid hormones and for vitamins such as retinoic acid, and a vast number of orphan receptors such as SF-1, whose ligands have not yet been discovered.

One of the hallmarks of this gene transcription mechanism of action is that the whole process, especially the DNA transcription and mRNA translation, takes significant time, on the order of hours. Tissue growth or neural remodeling would require gene transcription over an even more extended period. This time course of hours to days is consistent with many well-known behavioral effects of sex steroids. For example, if a castrated male quail is given testosterone, he begins to crow in 3–5 days and to mate in 5–7 days (Beach and Inman 1965; Adkins and Adler 1972).

Is this the only pathway by which steroids can act to alter behavior? There is a great deal of interest in multiple signaling (transduction) pathways for steroids. A particularly exciting prospect that could help solve several puzzles about social behavior is fast nongenomic mechanisms for altering neuronal activity, located in cell surface membranes (Zakon 1998; Moore and Evans 1999; Makara and Haller 2001). Possibilities include additional categories of steroid receptors, interactions between steroids and neurotransmitter receptors, and effects of steroids on the ion channels that control neuronal firing (fig. 1.3). The downstream pathway from such cell surface mechanisms would likely be a second messenger pathway instead of the gene transcription pathway of the intracellular steroid receptors.

There is already some evidence for rapid actions of these kinds on social behavior. Here "rapid" means a steroid effect seen in less than 15–20 minutes (Orchinik 1998). Corticosterone rapidly terminates mating behavior in newts through a membrane corticosteroid receptor (not yet isolated and identified) that constitutes a third category of corticosteroid receptor along with types I and II (Moore and Orchinik 1991). Corticosterone or cortisol treatment rapidly increases locomotor activity in mammals, birds, and turtles (Sandi et al. 1996; Breuner et al. 1998; Cash and Holberton 1999). Although membrane receptors for corticosterone are widespread in bird brains, it is not yet known if they are the mechanism of action for the rapid increase in activity. Rapid effects of steroids on the mating behavior of quail and rats and on investigation of conspecific chemicals by male rats have been obtained (Cross and Roselli 1999; Frye 2001; Balthazart et al. 2004). A membrane receptor for estrogens is suspected, but aside from its location it is not yet clear whether it constitutes a third estrogen receptor type along with ERα and ERβ. Goldfish and pigs have steroidal pheromones that result in rapid change in the behavior of conspecifics when detected by their chemosensory systems. This presumably means that

there are membrane receptors for these steroids in these systems (Signoret 1967; Melrose et al. 1971; Sorensen and Stacey 1999).

The discovery of steroidogenic enzymes and neurosteroids in parts of the brain that are not classical targets (that lack intracellular receptors) suggests that these neurosteroids might act mainly through rapid membrane-based mechanisms (Mellon and Griffin 2002). Furthermore, membrane-located mechanisms might have different steroid affinities. If so, then steroids (either neurosteroids or weak circulating steroids such as DHEA) that bind poorly to intracellular receptors could nonetheless have significant effects on neuronal activity. For example, allopregnanolone, a brain-produced metabolite of progesterone, is a potent ligand at the $GABA_A$ receptor (Majewska et al. 1986). Because $GABA_A$ is the principal inhibitory neurotransmitter in the brain, this discovery helps explain an old but previously puzzling phenomenon: progesterone in large dosages has anesthetic and barbiturate properties.

These newly discovered or hypothesized mechanisms of steroid action provide a potential means for rapid facilitation as well as inhibition of behavior, thus increasing its flexibility and adaptability. It will be important for research to establish connections between these mechanisms and naturally occurring social behavior of ecological significance, as Moore and Orchinik's work with newts has done.

Steroid Manipulation

What methods are available for studying steroid effects on behavior, and what are their limitations? The classic experimental design for showing that a behavior is steroid dependent is the "remove and replace" design of endocrinology. The source of the steroid is removed to see if the behavior disappears, and then the steroid is administered to see if the behavior reappears. Removing the source, for example, gonadectomizing an animal, has its problems, however. The surgery may be difficult or (in the case of adrenalectomy) cause illness or death. There may be another source for the steroid that cannot be removed at all, such as the estrogen-producing caudal telencephalon of songbirds. Fortunately, there is another experimental approach, using drugs developed for use in treating people with steroid-sensitive cancers. Some of these drugs work by inhibiting a key step in a steroidogenic pathway. Fadrozole, letrozole, and vorozole are aromatase (estrogen synthesis) inhibitors, and finasteride (now used by millions of men to slow down male pattern baldness) inhibits 5α-reductase, which converts testosterone to 5α-dihydrotestosterone, the baldness culprit. Others work not by reducing the amount of steroid, but as competitive antagonists ("blockers") at the intracellular receptors, thus preventing the steroid from acting at the target. For example, tamoxifen is an estrogen receptor antagonist, flutamide is an androgen receptor antagonist, and RU-486 is both

a progesterone and a glucocorticoid receptor antagonist. As this last example implies, these drugs are not always as specific as either clinicians or researchers would like. Also, they seldom completely inhibit or antagonize, and can even have mixed agonist/antagonist effects. For this reason, the fuzzier term "selective receptor modulator" is increasingly preferred to "antagonist" or "blocker," and even "selective" can be suspect. Furthermore, because of the negative feedback involved in regulation of steroid levels, these drugs may cause circulating steroid levels to rise. Also, membrane receptors won't necessarily be blocked by intracellular steroid receptor antagonists; they will be blocked only if the ligand binding site is similar (Schmidt et al. 2000). These are important considerations in interpreting results of experiments that use such drugs.

Replacing the steroid is often accomplished by regular injections to achieve good control over dosage, but administration by inserting an implant is usually preferred if disturbing the animal (or catching it, if it's free-living) is a problem. Peripheral administration works well because steroids readily cross the blood–brain barrier. In working with a new species it is desirable to determine the circulating level produced by the treatment to see if it is in the physiologically normal range. One of the future challenges in testing hypotheses about neurosteroids and ecologically relevant behavior is how to manipulate those steroids in the brains of free-living animals.

Mechanisms of Peptide Action

Because neuropeptides and protein hormones such as prolactin are such different molecules from steroids, we would expect their mechanisms and time course of action to differ as well. As a general rule they are relatively large hydrophilic molecules that do not readily cross a blood–brain barrier or enter into cells. For this reason their receptors are membrane (cell surface) receptors and not intracellular receptors. The receptor molecule amino acid sequences are not as conserved as those of the peptide ligands. Most neuropeptide receptors are members of the G-protein-coupled receptor superfamily (Darlison and Richter 1999). Peptide binding stimulates synthesis of cyclic AMP, a second messenger (the peptide is the first) that triggers the cascade of enzyme activity resulting in proteins that change the cell's physiology (Platt and Reynolds 1988; Norris 1996). A GTP-binding membrane protein (G-protein) mediates between the peptide receptor protein and the enzyme for cyclic AMP synthesis, adenylate cyclase. None of this takes a long time, and peptides can act fairly fast.

The peptides involved in social behavior each have different receptors and some, such as vasopressin, have more than one receptor subtype. There is diversity between receptor types and between taxa in the distributions of these receptors in the brain (Goodson and Bass 2001). That makes it difficult to summarize in any simple way where the principal peptide targets related to

social behavior are located, but a few regions stand out. The hippocampus is a major target for CRF. Oxytocin receptors are found in the medial and central nuclei of the amygdaloid complex and in the olfactory system and septum. In mammals $V1_a$ receptors (the type of AVP receptors linked to social behavior) are found in the paraventricular and supraoptic nuclei of the hypothalamus, in the medial nucleus of the amygdaloid complex and bed nucleus of the stria terminalis, and in some species in the septum and the diagonal band of Broca (de Vries and Miller 1998).

The mechanisms that regulate peptides and their receptors include steroids. Peptide and protein hormone actions sometimes require a background of steroid priming in which the steroids, acting through their intracellular receptors, stimulate transcription of the receptor genes, upregulating the peptide receptors (Norris 1996). In addition, peptides and their receptors are a key part of the downstream pathways for steroid action on the brain and behavior. This crosstalk between steroids and peptides and peptide receptors is why peptides must be included in thinking about steroids and social behavior (Witt 1997; Albers and Bamshad 1998).

Multiple Messengers, Multiple Behaviors

Chemical messenger regulatory systems interact with each other at several levels, such as when neurotransmitters and neuromodulators are regulated by steroids in some brain regions and peptide and protein hormone actions require prior steroid priming. These modulatory chemicals provide the flexibility and temporal and spatial coordination necessary for adaptive behavior sequences (Bicker and Menzel 1989). This makes it unlikely that any of the steroid or peptide chemical messengers in table 1.1 will have a specific (one-to-one) relationship to a given behavior. That is, any neurotransmitter, neuromodulator, or hormone functions in more than one type of behavior, and a single type of behavior is based on multiple chemical messengers, not one chemical regulator (Orchard et al. 1993; Weiger 1997). Each chemical regulator is functionally versatile. Structural genes code for some of the messenger molecules and for messenger receptors and so we would not expect any one-to-one correspondences between genes and behaviors either.

As ethologists and neuroscientists have long appreciated, inhibitory mechanisms are just as important as excitatory ones for behavior. Each act, however simple, results from a mix of excitatory and inhibitory inputs, each of which is modulated by multiple chemicals; the balance between them determines the output, allowing more precise regulation than a simple on–off switch would (Katz and Harris-Warrick 1999). Decisions between competing behaviors are outcomes of changing balances between excitatory and inhibitory mechanisms. A behavior can be increased not only by increasing excitatory tone but also by decreasing inhibitory tone. Copulation by male newts (*Taricha granulosa*)

illustrates this beautifully (Moore and Orchinik 1991). The peptides AVT and GnRH plus sex steroids (the excitatory mechanisms), glucocorticoids (which are inhibitory), and a host of other neuromodulators work together to produce an animal that mates in response to female stimuli but not if a predator threatens.

The importance of steroid priming for actions of other steroids and of peptides means that synergism occurs in experimental studies of hormones and behavior. "Synergism" in everyday language is often used incorrectly to refer to additive effects of two treatments. True synergism means that the effect when two hormones/peptides are given is greater than the sum of the effects of each given alone. The prototypical example is the combined effect of estrogen and progesterone on female rat and guinea pig lordosis. Neither a low dose of estradiol alone nor an injection of progesterone alone are particularly effective, but the combination given in the right sequence (first estrogen, then progesterone) brings on the behavior of a naturally estrous female. The synergism occurs because the estrogen priming increases gene transcription for the progesterone receptor protein, enabling the progesterone to act (Blaustein and Erskine 2002).

An old principle of comparative endocrinology is that the functions and effects of hormones have changed more in evolution than the hormones themselves. Current understanding of steroid and peptide actions has illuminated the mechanistic basis for diverse actions of the same hormone at different targets. We should look to those mechanisms (enzymes, receptors, and the cascades that they initiate) to see what has supported behavioral change over evolutionary time, a point that will be developed in chapter 5.

Hormones, Plasticity, and Development

Adult brains are like celestial objects or continents—more dynamic and plastic than most scientists used to imagine. No new neurons were born (it was believed), learning and memory happened somewhere somehow but only at the biochemical, not structural, level, and hormones somehow acted on neurons but without visibly altering their structure. It is fascinating to see how much this formerly canonical view of the adult nervous system has changed. Now it is clear that new neurons are formed and used throughout life in many vertebrates and invertebrates (Leonard et al. 1978; Easter et al. 1981; Gould et al. 1999; Harzsch et al. 1999). Now it is known that some forms of learning change the anatomy of neurons. Now it is clear that steroids can change the number, size, form, and dendritic structure of individual neurons and stimulate the formation of new connections between neurons (Luine and Harding 1994; Ball et al. 2002; Woolley and Cohen 2002). It seems quite likely that these steroid-induced structural changes are causally related to important real-world phenomena such as seasonal changes in behavior or a bird's memory for the songs it heard as a juvenile. Social interactions often alter individuals' hor-

mone levels, and the social experiences that an animal has might change the structure of its brain through hormone-related mechanisms, a profoundly significant insight.

Many aspects of social behavior are subject to modification by experience, such as by social learning (for example, mate choice copying) or Pavlovian conditioning (Heyes and Galef 1996; Hollis 1997; Domjan et al. 2000; Woodson 2002). Even hormone levels themselves are subject to Pavlovian conditioning. For example, when a male mouse smells a female, his levels of luteinizing hormone and testosterone rise within minutes. The same hormonal response can be elicited by previously neutral stimuli that have come to signal the imminent appearance of a female (Graham and Desjardins 1980). Pavlovian conditioning, a universal property of nervous systems, enables animals to anticipate what is to come and enhances reproductive success (Hollis et al. 1997; Domjan et al. 1998; Adkins-Regan and MacKillop 2003). Research also indicates that some hormones, including testosterone and estradiol, induce states that the animal can detect and discriminate between, and that have rewarding or aversive properties (Alexander et al. 1994; Frye et al. 2001; Wood 2004). Responses of the immune system are also subject to Pavlovian conditioning (Ader and Cohen 1992), which may be relevant to their contribution to potential costs of hormones (to be addressed later in this chapter) and their role in social behavior evolution (to be addressed in chapter 2).

The developing brain is the epitome of plasticity. Although adults learn a lot, the capacity for drastic change is reduced. This toning down of brain plasticity is a mechanistic basis for the phenomenon of critical periods in development, special times when experiences have long-term consequences of a sort that are not possible outside of an early "window of opportunity." Filial and sexual imprinting are well-known examples of early learning with this special property.

The critical period concept is central to the important organizational hormone theory first proposed by Phoenix, Goy, Gerall, and Young (1959, see also Young et al. 1964). The theory distinguishes between organizational and activational hormone effects and posits three important differences between them. First, organizational effects establish the substrate for the future behavioral sex of the animal, whereas activational effects merely stimulate (activate) the substrate that has already developed. Second, organizational effects are permanent, lasting for the life of the animal even though the hormonal condition that produced them may be long gone, whereas activational effects are reversible, disappearing if the hormonal condition subsides. Third, organizational effects are only possible early in development, during a relatively limited critical period, whereas activational effects normally occur in adulthood.

Nowadays "activate" sounds a bit strong given the permissive nature of hormone actions on social behavior. Also, subsequent research has shown that it is not always possible to make a clean distinction between organization

and activation (Arnold and Breedlove 1985), as, for example, when hormone treatment in adulthood turns out to have a long-term effect on behavior. The theory predates the discovery of structural plasticity in the adult brain, but that discovery itself does not undermine the original distinction between organization and activation. What is essential to those concepts is not whether structural change occurs, but whether such structural change is permanent and whether it can happen only during an early critical period.

The organizational hormone theory is still extremely useful and continues to be an important stone in the conceptual foundation of the field of hormones and behavior. The idea that hormones acting early in life can have different effects from those acting later has important implications not just for understanding sexual differentiation of social behavior (chapter 4), but also for thinking about hormones as mechanisms of condition-dependent signaling, as mediators of trade-offs, and as architects of life histories. Steroids with potential organizational effects include not only those produced by the young individual, but also those to which it is exposed through the mother (via internal gestation or egg yolks), siblings (intrauterine position effects), or both. Organizational effects are relevant to insects and possibly other invertebrates as well as vertebrates (Nijhout 1994; Elekonich and Robinson 2000). Because organization is permanent, such effects add to the increasing concern about exposure of wild animals to anthropogenic endocrine disrupters in the environment (Guillette et al. 1995; Palanza et al. 1999; Ottinger and vom Saal 2002).

The mechanisms underlying organizational effects of steroids are not yet well understood. What is clear is that steroid metabolites produced in the brain are key in understanding organizational as well as activational effects (Negri-Cesi et al. 2000). Studies of the regulation of the P450arom gene in young and adult brains may help explain why early hormone effects are so qualitatively different during organization compared with activation (Lephart 1997). It is also clear that binding of steroids to intracellular steroid receptors is involved, because steroid-receptor antagonists that work in adults to inhibit steroid-dependent behavior also work in young animals to prevent normal development of the behavior. But how do these steroid actions permanently sculpt the circuitry for future behavior and why is this sculpting only possible at young ages? These questions will be taken up in chapter 4.

How the Necessary Control of Steroids by the Environment Is Achieved: The HPG and HPA Axes

Animals do not achieve fitness in a vacuum but in a physical and social environment to which their hormones must respond appropriately. In many temperate zone birds, the increasing daylengths of spring stimulate massive growth of the gonads and a corresponding increase in sex steroid production (Wing-

field and Farner 1993). A stressful stimulus causes release of adrenal corticosterone, which interrupts mating in a male newt (Moore and Orchinik 1991). A dominant female coral reef fish turns into a male when the prior male of the group disappears (Shapiro and Boulon 1982).

How is it possible for the stimuli of the world outside an animal's body to have such a huge impact on what goes on inside it? Those external stimuli are detected by sensory organs that then convey signals to the brain or head ganglion. When the relevant hormones are neurohormones produced by brain neurosecretory cells, as in many invertebrates, the answer is straightforward: neurons that know about the stimulus tell those other neurons to produce or release the hormones. But in vertebrates, the gonadal and adrenocortical steroids of interest come from nonneural tissue located far from the brain. These hormones are regulated by yet other hormones from the anterior pituitary (fig. 1.4). Now that we're in the pituitary we're physically closer to the brain, but there's still a problem receiving information about the outside world, because the anterior pituitary is nonneural tissue. In tetrapod vertebrates, it is not even innervated. So how does sensory information get translated into the endocrinology of the anterior pituitary to then regulate the gonads and adrenals?

The solution lies in a set of discoveries that rank as great achievements in organismal biology (Harris 1955; Scharrer 1959; see also Raisman 1997). The story has been best told in mammals. A special little portal circulatory system (the hypothalamo-hypophysial portal system) connects the hypothalamus and the anterior pituitary. The hypothalamic neurosecretory cells produce a set of peptides, some excitatory and some inhibitory, that are released into this system to reach the cells of the anterior pituitary to regulate their hormone production. This allows communication between brain and anterior pituitary so that the external physical and social world can influence the animal's gonads and adrenals. Hypothalamic peptides also regulate the production of prolactin by other cells of the anterior pituitary. In mammals dopamine (which is not a peptide, and which acts as a neurotransmitter elsewhere) acts as a prolactin inhibitory factor.

GnRH is the hypothalamic releasing peptide that increases the levels of the polypeptide hormones FSH (follicle-stimulating hormone) and LH (luteinizing hormone), the gonadotrophic hormones that stimulate increases in gonadal sex steroids. LH is released in a pulsatile manner as a result of rhythmic activity in what is called the GnRH pulse generator (a set of hypothalamic neurons). Higher frequency pulses result in higher blood levels, as when LH is triggering ovulation. A set of largely negative feedback loops between the gonadal hormones (the sex steroids along with some nonsteroidal hormones such as inhibin and activin), the anterior pituitary, and the hypothalamus then maintains hormone levels within some reasonable range. If testosterone gets too high, less GnRH and LH is produced, which turns down the testosterone level. If it is too low, GnRH and LH go up, raising testosterone. Positive feedback occurs

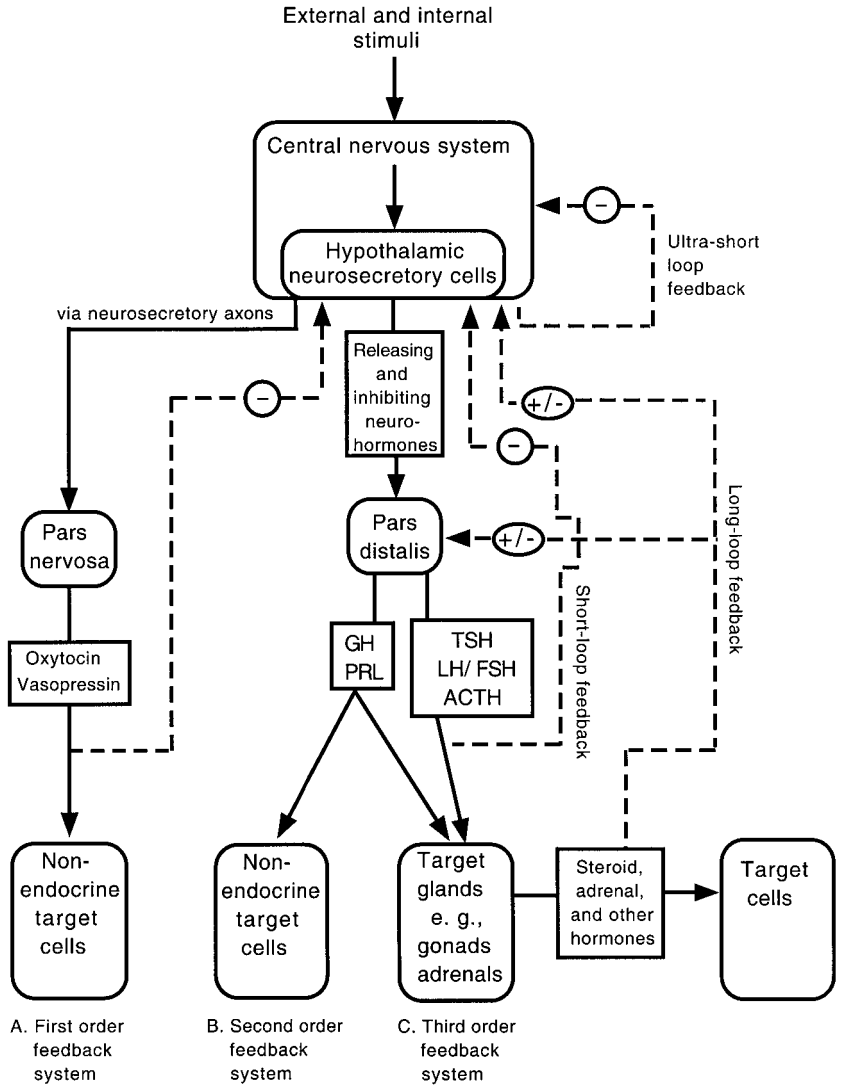

FIGURE 1.4. The hypothalamic–pituitary axes of mammals, including the hypothalamic–pituitary–gonadal (HPG) and hypothalamic–pituitary–adrenal (HPA) axes. The releasing and inhibiting hormones are transported via the hypothalamo-hypophysial portal system. At each level in the axis, hormones or neurohormones exert feedback on one or more other (usually higher) levels. Such feedback loops can be positive (+), negative (–), or both, depending on other factors. Many features of these axes are seen in other vertebrates. GH, growth hormone; PRL, prolactin; TSH, thyroid stimulating hormone; LH/FSH, luteinizing hormone/follicle-stimulating hormone; ACTH, adrenocorticotrophic hormone. The pars distalis is the anterior pituitary. The pars nervosa is the posterior pituitary (neurohypophysis). Redrawn from Norris (1996). © Academic Press (Elsevier).

when rising estrogen levels from developing ovarian follicles cause an increase in the GnRH pulse frequency, thus producing the spike of LH that triggers ovulation. This three-tiered system is called the hypothalamo–hypophysial–gonadal axis or hypothalamic–pituitary–gonadal axis (HPG axis). Levels of GnRH that are either too high or too low will cause suppression of the rest of the axis.

A three-tiered system analogous to the HPG axis, the HPA axis, connects the hypothalamus, anterior pituitary, and adrenal cortex, forming the pathway for the steroid hormone limb of the classic stress response (fig. 1.4). In response to an environmentally challenging (homeostasis perturbing or "stressful") situation, peptides in the paraventricular nuclei (PVN) of the hypothalamus such as CRF are released into the portal system. Upon reaching the anterior pituitary, these stimulate release of ACTH and other hormones into the circulation, which in turn stimulate glucocorticoids from the adrenal cortex. Negative feedback loops bring the hormones back to baseline levels after the challenge is over, but positive feedback can also occur, as when a very aversive event produces sustained glucocorticoid elevation. The sites where glucocorticoids exert feedback include the PVN but also regions outside of the hypothalamus, such as the hippocampus for negative feedback (via type II receptors) and portions of the amygdaloid complex for positive feedback (Lathe 2001). The adult "tone" of the HPA axis (its resting state and response threshold) is influenced by organizational actions of glucocorticoids resulting from experiences in early life (Levine and Mullins 1968; Carsia and Harvey 2000; Walker et al. 2002).

As the site of the releasing hormones, PVN is a control center of the top tier of the HPA axis. When HPA activation occurs in response to simple physical stressors such as cold, blood loss, or pain, the information that there is a problem comes up from the spinal cord or lower brainstem to the PVN in the upper brainstem, the categorization of the stimulus as meriting HPA activation is automatic and "hard-wired," and CRF is the principal releasing hormone for the response. Activation of the HPA in response to stimuli related to social behavior (sights and sounds of other individuals, social events, and psychological states) requires a more nuanced interpretation than the brainstem can provide. Is the stimulus a predator and therefore dangerous, or a conspecific presenting a golden reproductive opportunity? If it's a conspecific, is it a familiar individual or a stranger? Whether conspecifics are dangers or delights depends on who they are and what they have done to the observer in the past. As a receiver of processed sensory information from all modalities, the forebrain amygdaloid complex is well positioned to provide the necessary top-down interpretation, "deciding" how the animal should respond (flee? approach? court?). The mammalian "amygdala" is a complex of nuclei. Homologs of at least some of them are present in all jawed vertebrates (Butler and Hodos 1996). The discovery in mammals that some of these nuclei work together to

determine whether a stimulus is dangerous (fear inducing) enough to merit a stress response is an important chapter in contemporary brain science (LeDoux 1995). If the decision is that a stimulus is dangerous, the central nucleus of the amygdaloid complex then sends signals to the PVN and to other brainstem loci responsible for the physiological reactions to a fear inducing stimulus that enable the animal to deal with it.

Multiple hypothalamic peptides regulate the HPA axis, and there seem to be a greater number of them involved in regulating the axis in birds than in mammals (Romero and Sapolsky 1996). When the HPA is stimulated from the top down in mammals, the response at the hypothalalmic tier involves less CRF and more of the oxytocin family of peptides. The exact peptide signature (ratio of CRF to AVP/AVT to OT) depends on the psychological state resulting from the stimulus. Psychological state is widely recognized as a crucial intermediary in humans. Bungee jumping is a thrill for some but a trauma for others. The psychological component is almost certainly critical for nonhumans as well. In addition to individual differences, there are big seasonal and species differences in whether a stimulus is a stressor and activates the HPA axis (Orchinik 1998; Silverin 1998). Seasonal changes raise another important issue as well, whether "stress" is the right word to use for HPA activity. Characterizing adrenal activation as a "stress" response doesn't seem to capture what is happening when baseline glucocorticoid levels change markedly on a seasonal basis (Romero 2002).

As the final common signaling molecules from the brain that reflect integration of multiple inputs and orchestrate the responses of the lower tiers, peptides like GnRH and CRF have pivotal roles in reproductive success and survival (Ball 1993; Bonga 1997; Aguilera 1998). They regulate the HPG and HPA axes that ensure that steroid levels are appropriate for current and predictable future environmental and social conditions (Wingfield and Silverin 2002). These two axes can influence each other as well. Chronic stress, including social perturbation, nutritional stress, inflammation, parasites, and endotoxins, and glucocorticoid administration depress the HPG axis of many mammals and lizards, an effect that is caused by the increase in CRF and glucocorticoids (Sapolsky 1993; Dunlap and Schall 1995; Rivest and Rivier 1995; Kalra et al. 1998; Schneider and Wade 2000). The HPG axis of nonmammals seems to require more extreme HPA activation to be depressed, and moderate glucocorticoid elevation is often characteristic of the breeding period (Wingfield and Silverin 1986; Romero 2002; Moore and Jessop 2003). Sex steroid treatment tends to lower glucocorticoid levels in mammals but it elevates them in some birds (Hillgarth et al. 1997). Both GnRH and the HPA peptides (CRF, AVP/AVT, OT) are produced by other parts of the brain in addition to the hypothalamus and have other functions in addition to their roles in the top tiers of the HPG and HPA axes, including some direct behavioral functions such as aug-

mentation of anxiety by CRF (Habib et al. 2000) and facilitation of lordosis by GnRH (Dudley and Moss 1991).

The HPG and HPA scenarios spelled out earlier are based largely on research with mammals and to a lesser extent birds. Nonmammalian vertebrates also have hypothalamic regulation of the pituitary and have a similar array of hypothalamic peptides, but what these peptides do and whether they are involved in pituitary regulation is seldom understood (Norris 1996). In those birds studied so far, the peptide VIP seems to be the primary releasing factor for prolactin regulation (Sharp et al. 1989; El Halawani et al. 1990; Maney et al. 1999; Vleck and Patrick 1999). Fishes other than teleosts, lampreys, and hagfish have a vascular link between the hypothalamus and pituitary (Sower 1998). Teleosts, however, have direct innervation of the anterior pituitary by the preoptic area (the region anterior to the hypothalamus) (Peter et al. 1990). This is the mechanism for external regulation of the gonads in response to changes in the physical and social environment and of the glucocorticoid-producing interrenal tissue in response to stimuli that threaten survival (Bonga 1997; Norris 1996). Teleosts have GnRH neurons, but they have a wider distribution outside the hypothalamus compared to other vertebrates. In spite of these special derived anatomical features of the teleost HPG and HPA axes, GnRH and CRF are still the key molecules for the top tiers (Lovejoy and Balment 1999).

It is an oversimplification, of course, to imply that the problem of how steroid production is regulated adaptively is solved now that some links between the hypothalamus and the pituitary are known. Although a remarkable number of brain regions send some kind of neural projection to some part of the hypothalamus, nonetheless there are many gaps in understanding how sensory information about the social world reaches the hypothalamic cells producing the releasing peptides. Also, the hormone levels at the three tiers of the HPG and HPA axes aren't always well correlated. Behavioral effects of GnRH and CRF independent of their stimulating effect on peripheral steroids complicate the picture in potentially interesting ways.

Insect endocrinology shows some interesting parallels with the neuroendocrine axes of vertebrates (E. Scharrer 1959; Acher 1986). Insect hormones are regulated by external as well as internal cues via the brain by means of two- or three-tiered cascades with feedback loops that begin with brain neurohormonal peptides and end at peripheral target organs (Nijhout 1994). In one "axis," brain neurosecretory cells produce PTTH (prothoracicotrophic hormone, a neuropeptide), which is stored in and released from the corpora cardiaca; PTTH in turn stimulates the prothoracic gland, which makes the prohormone ecdysone, which is converted to ecdysteroids when it reaches its targets (e.g., epidermis). In another axis, brain neurosecretory cells produce the peptides allatotropin and allatostatin, which regulate the production by the corpora allata of juvenile hormones, their sole product, which in turn act on multiple targets including the nervous system. Communication between tiers occurs

mainly by direct innervation. There are no special portal systems to get the neurohormones out of the brain (there is no need given how insect circulation works), and are no gonadal steroids, but in other ways the organizational scheme is similar, reflecting a common need for hormonal systems to be adaptively regulated by the outside world. Some of the same peptides are used as well (Ottaviani and Franceschi 1996).

Diversity in Mechanisms

Many hormonal mechanisms are rather conserved in vertebrates but there is interesting diversity nonetheless. The special way that the hypothalamus communicates with the anterior pituitary in teleost fish is just one of several notable examples of derived features appearing only in one lineage, in this case a very large one. Diversity can arise through evolved adaptations to particular environmental circumstances (natural selection), through the behavior of conspecifics (sexual selection), or through the sheer passage of long periods of time with no selection against the occasional mutation (neutral evolution). The following are additional notable examples of diversity resulting from these processes:

1. Juvenile hormones (which are not steroids) appear to be a family unique to insects and crustaceans.
2. Insects cannot make steroids de novo because they don't make cholesterol. The ecdysteroids are made from sterols obtained from the diet (Rees 1985).
3. Vertebrate groups differ in whether the predominant glucocorticoid is cortisol (teleost fishes, primates) or corticosterone (rats and mice, birds, lizards).
4. 11-Ketotestosterone and other 11-oxygenated nonaromatizable androgens are characteristic of teleost fish, where they are the predominant circulating androgen and replace DHT as the other important androgen (Borg 1994). Testosterone is present as well but 11-ketotestosterone is often more potent than testosterone for male sexual characters and behavior (Brantley et al. 1993; Borg 1994). It is not clear how these steroids act or why teleosts have them and not other vertebrates. The recent discovery of two androgen receptor types in both teleosts examined may help answer the first question (Sperry and Thomas 1999a,b).
5. The presence in the brain of the steroid metabolizing enzyme 5β-reductase, which converts testosterone into 5β-dihydrotestosterone, is unique to birds. 5β-DHT has little or no biological activity and levels of it are highest in nonbreeding individuals and seasons. Thus, 5β-reductase seems to function as an inactivation shunt (Massa et al. 1979; Balthazart 1989). This ex-

ample illustrates the important principle that regulation involves inhibition or inactivation along with facilitation and upregulation.

6. The telencephalon of the speciose oscine passerine lineage of birds ("songbirds") has several striking features. In addition to the song system itself (a set of interconnected nuclei dedicated to song and song perception), there are intracellular androgen receptors in several of the telencephalic song system nuclei, there are estrogen receptors in one of the song system nuclei (HVC), and the caudal nidopallium has a large field of aromatase positive cells (Metzdorf et al. 1999; Schlinger and Brenowitz 2002). Intracellular androgen and estrogen receptors and aromatase are not seen in homologous parts of the telencephalon in other birds or in mammals and are unusual in other vertebrates as well.

How "Costly" Are These Hormonal Mechanisms?

The dominant theoretical framework in behavioral ecology emphasizes costs and benefits to individual animals of engaging in a behavior. Benefits must outweigh costs in the currency of fitness for a novel behavioral solution to a problem to be maintained and increase in frequency over evolutionary time. Also central is the concept of trade-offs that limit what is possible (Krebs and Davies 1997). These trade-offs occur at all levels including internal, behavioral, and life history (trade-offs between different fitness components), and are based not only on energetic costs but also on any other external or physiological resources that are in limited supply and on time limitations. If the brain uses more oxygen than usual, less is available for some other internal function. Two different behaviors cannot usually be done at the same time. Investment in the present could reduce investment in the future.

Behavior has direct energetic costs and some behavior that is good for social and reproductive goals increases predation risk. It is increasingly recognized that the costs of behavior also might include the costs of the mechanisms, including hormonal mechanisms, that support the behavior. Determining costs of mechanisms is difficult, and the concept of "costs" can be slippery. However, because it is an important part of some research on steroids and social behavior that will come up in several chapters, it is worth taking a look here at what the costs of steroids might be.

Some of the physiological costs of HPA activation and of chronic glucocorticoid elevation are well established, including atheroschlerosis, ulcers, immunosuppression resulting in increased disease risk, and, in some taxa, HPG inhibition (Cooper and Faisal 1990; von Holst 1998; Salzet et al. 2000; Jasnow et al. 2001). Such immunosuppression also occurs to natural stressors, including social conditions (Nelson et al. 2002). HPA activation is part of an emergency

response system whose costs can be borne because the alternative is likely to be death.

In a captive study simulating natural predation, estrous deermice (*Peromyscus maniculatus*) were more likely to suffer predation by a weasel than nonestrous deermice, and this was because of their odor, which is sex steroid dependent (Cushing 1985). Because this odor results in part from excreted steroid metabolites and serves to attract males, this is a particularly interesting illustration of the costs and benefits of steroids.

In categorizing potential costs of testosterone in male songbirds, Wingfield et al. (2001) consider both direct costs (those of the testosterone itself) and indirect costs (those resulting from the behavior that testosterone stimulates). Their list includes higher energetic costs, reduced fat stores, oncogenic effects of estrogenic metabolites of testosterone, increased mortality, interference with pair bonds, increased injury, interference with parental care, and immunosuppression. They refer to an idea first proposed by Naftolin (see Naftolin and MacLusky 1984) that steroidogenesis at the targets might be a mechanism to avoid costs of that steroid in the circulation. Because different behaviors involve somewhat different brain regions, limiting a potent steroid to the relevant target not only avoids systemic costs such as oncogenesis or immunosuppression, but also reduces costs resulting from inadvertent and unwanted stimulation of other behaviors. Another way to reduce the costs of a steroid might be to increase behavioral sensitivity to it (increase receptor numbers or sensitivity) (Hews and Moore 1997).

Steroid-stimulated behaviors, including general locomotor activity, can be energetically demanding and so steroids would be expected to increase metabolic rate (oxygen consumption) indirectly, because of these behaviors (Vehrencamp et al. 1989; Emerson and Hess 2001). Estradiol increases activity level and metabolic rate in female rats, whereas progesterone has the opposite effect (Wade and Schneider 1992). Brain tissue is energetically expensive with respect to oxygen consumption and the forebrain song system nuclei of songbirds are more metabolically active when they are testosterone stimulated (Wennstrom et al. 2001).

Do sex steroids also increase resting or basal metabolic rate (RMR or BMR), the "cost of living" (Hulbert and Else 2000)? In principle, steroids could have a direct effect on BMR if they change core body temperature or (because the brain is a big oxygen user) change neural activity during behaviorally inactive states such as sleep (Hänssler and Prinzinger 1979). In practice, measuring BMR so that it is unconfounded by locomotor activity (including minor locomotor activity like "fidgeting") and by the stress of confinement is challenging, especially for highly mobile animals with large home ranges. So is figuring out how to correct for the changes in body mass that usually occur when steroid levels are experimentally manipulated.

Any direct effects of sex steroids on BMR might be most likely to impact fitness in animals with small body size, rapid metabolism, and low fat stores, such as many small birds. There have been several attempts to see if testosterone affects BMR in birds (for example, Lynn et al. 2000); very few, however, have yielded positive results (Buchanan et al. 2001). The evidence from lizards is more positive. Female *Sceloporus virgatus* had lower BMRs than males when adjusted for body mass (Merker and Nagy 1984). Both testosterone and estradiol increased oxygen consumption in *Chalcides ocellatus* (al Sadoon et al. 1990).

In mammals the positive evidence comes mainly from humans, perhaps because it is easier to measure BMR in our species. Hamilton (1948) observed that castrated men have lower metabolic rates and live longer, and concluded that "a price is paid for a beard" (p. 315). Both testosterone and estradiol increase metabolic rate (Lyons 1969). Men have a higher BMR than women of the same height and weight, but this sex difference doesn't necessarily generalize to other species. Comparative analyses of BMR (for example, Bennett and Harvey 1987) do not mention the sex of the animals, as if there are no sex differences. Even glucocorticoids have not been shown to affect BMR in birds and mammals in spite of the fact that stress itself increases oxygen consumption. All told, the major physiological costs of sex steroids, if there are any, must lie elsewhere.

Folstad and Karter (1992) proposed that testosterone is costly in part because it suppresses the immune system, leaving animals more vulnerable to pathogens, and that this cost helps keep testosterone dependent male signals honest. Their proposal was based on rather little evidence, most of it from humans and a few laboratory mammals, although it is consistent with observed sex differences in immune function and parasitism rates (Nelson et al. 2002). It had the beneficial effect of inspiring a number of experimental attempts to test the hypothesis in birds and other nonmammals and a greater interest generally in the immune systems of nonmammalian vertebrates. Support for the hypothesis is mixed and seems to depend on the species, whether the animals are in captivity or free-living, and whether acquired or innate immunity is measured (Hillgarth et al. 1997; Hasselquist et al. 1999; Roberts et al. 2004). It is innate immunity that is seasonally regulated, has high nutritional costs, and is phylogenetically old (Lee and Klasing 2004). If immune function is redistributed (adaptive reallocation of energy) rather than suppressed overall, there would not necessarily be unavoidable costs (Raberg et al. 1998; Braude et al. 1999). In small rodents, immune system functioning is enhanced on short days (Nelson and Demas 1996), but this is due to melatonin, not steroids. The overarching conclusion that testosterone suppresses the immune system in animals appears to be premature. Furthermore, in those cases where positive evidence has been obtained, it is likely that other hormones, for example, glucocorticoids, and not the testosterone itself, are the culprits. Administering testoster-

one increases corticosterone in songbirds and corticosterone is a well-established immunosuppressant (Hillgarth et al. 1997; Evans et al. 2000; Casto et al. 2001). Regardless of what the relevant hormone is, immune status clearly has fitness consequences, not just for survival (disease and parasite resistance), but also for the likelihood of being chosen as a mate (Nelson and Klein 2000).

Little attention has been paid to potential costs of estrogens, even though they are oncogenic in mammals, males of some species have surprisingly high circulating levels, and some male courtship behavior is based on estrogens as well as androgens (see chapter 2). Experimentally administered estrogen has aversive properties (Ganesan 1994). Birds are sensitive to toxic effects of estrogens administered systemically, so much so that there is a fine line between a behaviorally effective dose of estradiol and a lethal dose (Warren and Hinde 1959). Small wonder then that estradiol usually circulates in amounts a hundred times lower than testosterone, with aromatization of androgens at the target serving to augment the local level. In mammals estradiol can either enhance or depress immune function, and again these effects could be mediated by changes in other hormones, including peptides (Grossman 1985; Whitacre et al. 1999; Geary 2001; Nelson et al. 2002). Both DHEA and prolactin are immune enhancing in those mammals studied (Nelson et al. 2002).

What about the steroids themselves and their mechanisms of action? Are these likely to be costly? Steroids are small molecules containing abundant elements, but both the enzymes required for their synthesis and conversion and their receptor proteins would have similar costs to other bodily proteins. Glucocorticoids are highly oxidized molecules of the sort that tend to be toxic and mutagenic. The GnRH and oxytocin families of peptides are relatively small as peptides go but prolactin is a much bigger molecule. In many species of animals and especially in females, gonadal steroids quickly drop when animals are food limited (e. g., Aubret et al. 2002), but this could be because food scarcity is a signal that it is a bad time to think of investing in reproduction rather than because the hormonal mechanisms themselves require more calories than are currently available. In birds and some other vertebrates the gonads (not just the gamete-producing tissue, but also the steroid-producing tissue) shrink to nearly nothing during the nonbreeding season, as if something about this tissue, its products, or their consequences is too costly to afford in the off-season. For birds, the cost of carrying gonadal weight while flying might be significant, but are there other costs as well?