Hormone Effects on Behavior

54

Yoav Litvin and Donald W. Pfaff

Abbreviations

a-MSH	A-melanocyte-stimulating hormone
ACTH	Adrenocorticotropin hormone
ADHD	Attention deficit hyperactivity disorder
AgRP	Agouti-related peptide
cAMP	Cyclic adenosine monophosphate
CA	*Cornu ammonis* region (hippocampus)
CCK	Cholecystokinin
CRH	Corticotropin-releasing hormone
DHT	Dihydrotestosterone
DNA	Deoxyribonucleic acid
E_2	Estradiol
EPM	Elevated-plus maze
ER	Estrogen receptor
GAD	Generalized anxiety disorder
HPA	Hypothalamic-pituitary-adrenal
HPG	Hypothalamic-pituitary-gonadal
HPT	Hypothalamic-pituitary-thyroid
mRNA	Messenger ribonucleic acid
NPY	Neuropeptide Y
P	Postnatal day
PTSD	Posttraumatic stress disorder
PVN	Paraventricular nucleus of the hypothalamus
SNP	Small nucleotide polymorphism

Y. Litvin (✉) • D.W. Pfaff
Laboratory of Neurobiology and Behavior, The Rockefeller University, New York, NY, USA
e-mail: ylitvin@rockefeller.edu, pfaff@rockefeller.edu

D.W. Pfaff (ed.), *Neuroscience in the 21st Century*,
DOI 10.1007/978-1-4614-1997-6_59,

Brief History

Brief Historical Introduction

During the last century, clues that hormones might affect behavior arose both from laboratory experiments and in the clinic. Frank Beach, working with animals at the University of California, Berkeley, demonstrated the activation of male sexual behavior by testosterone injections, as well as the facilitation of female sexual behavior by treatments with estrogens and progesterone. In the clinic, it was clear that hyperthyroid patients could be nervous and irritable, while hypothyroid patients would be sluggish and dull. As well, eunuchs (lacking testosterone from the testes) had no libido, and thus, clinical experience went hand in hand with Beach's experimental demonstration.

Introduction

Research on hormone/brain/behavior relations has led to phenomenal discoveries during the past 50 years. In addition to fulfilling our innate curiosity on the underpinnings of life in general and human nature in particular, basic and clinical research of neuroendocrine mechanisms has been successfully applied toward medical interventions aimed at prevention and treatment of a wide variety of human conditions. These include anxiety disorders, major depression, hypo-/hyperthyroidism, obesity, diabetes, and osteoporosis. In addition, knowledge of hormone/brain/behavior relations has led to drugs that have revolutionized human sexual behavior and reproductive habits, such as contraceptives and aphrodisiacs.

A *hormone* is broadly defined as a chemical messenger that is secreted by cells or glands, travels to other parts of the body, and affects their cellular activity. Hormones maintain homeostasis and facilitate survival by regulating behavioral neuroendocrine systems. To serve these functions, hormones bind to specialized receptors in the brain and periphery and promote behaviors that (1) preserve internal states such as nutrition, fluid balance, and body temperature; (2) steer away from threats involving pain and stress; and (3) support reproductive fitness through courtship, parental, and sexual behaviors.

Hormones travel through the circulation, enter the brain (some are already present in the brain), and act on neurons and glia. Hormones can act on cells via various mechanisms: (1) *Genomic actions* – as steroids, hormones easily cross the lipid membrane of target cells and bind to receptors present in the cytoplasm or nucleus. The hormone forms a complex (hormone-receptor complex), which binds to areas in the DNA termed hormone-response elements. This binding either potentiates or inhibits gene transcription, thus promoting or inhibiting protein synthesis and ultimately adjusting the function of the cell. (2) *Indirect genomic actions* – hormones bind to receptors located in the

membrane of cells and activate second messenger cascades that reach the nucleus and promote transcription of genes. (3) *Nongenomic actions* – hormones can also act directly at ion channels at the synapse, affecting cells' function and/or genesis.

Hormones are produced and secreted by numerous central and peripheral organs. By far, the brain is the largest gland in the body, referred to as the "master gland," with the hypothalamus and pituitary as its major production and secretion centers. The hypothalamus, which is located in the ventral part of the diencephalon in humans, serves as a nexus for numerous brain regions that are involved in a variety of behaviors. Hormones that are synthesized within the hypothalamus coordinate physiological adaptations by integrating and modulating the functions of the autonomic, endocrine, and immune systems. The hypothalamus is particularly interconnected with evolutionarily ancient parts of the brain that mediate functions related to emotion, behavior, and memory, commonly referred to as the limbic system. These include the hippocampus, amygdala, septal nuclei, thalamus, cingulated cortex, and orbitofrontal cortex. Excitatory and inhibitory signals reach the hypothalamus where they are translated into efferent neural and chemical signals that regulate hormone synthesis and release from the pituitary gland. Subsequently, pituitary hormones affect endocrine glands throughout the body (e.g., testes, ovaries, thyroid, adrenal). Hormones in the brain and periphery have a reciprocal relationship; i.e., pituitary-hormone secretagogues promote activation of endocrine glands, which release hormones that often travel back to the brain, providing negative and/or positive feedback.

The evolution of a hypothalamic-pituitary axis in vertebrates was a seminal event that enabled complex individual and social behavioral repertoires. These include sophisticated courtship, nurturing parenting, elaborate aggression and defensive patterns, and intricate social hierarchy formation. Examples of neuroendocrine systems include the hypothalamic-pituitary-adrenal (HPA) axis, hypothalamic-pituitary-thyroid (HPT) axis, and the hypothalamic-pituitary-gonadal (HPG) axis (Fig. 54.1).

The accumulation of data on hormone/brain/behavior relations has rendered it possible to assemble a number of underlying principles that define and elucidate mechanisms by which hormones affect a wide variety of behaviors, such as eating, drinking, fighting, and socializing. This chapter will include eight fundamental principles of hormone/brain/behavior relations, some distinct and some with overlaps. Each principle will include a brief and general description followed by numerous relevant examples from influential historic discoveries and/or from the recent scientific literature. We will begin by outlining six basic principles of hormone/brain/behavior relations and conclude by examining the effects of familial/genetic (Principle 7) and environmental/epigenetic (Principle 8) factors on behavioral neuroendocrine systems. For a detailed and exhaustive survey of the principles of neuroendocrinology, we refer the reader to the 5-volume second edition of *Hormones, Brain and Behavior* (see "Further Reading").

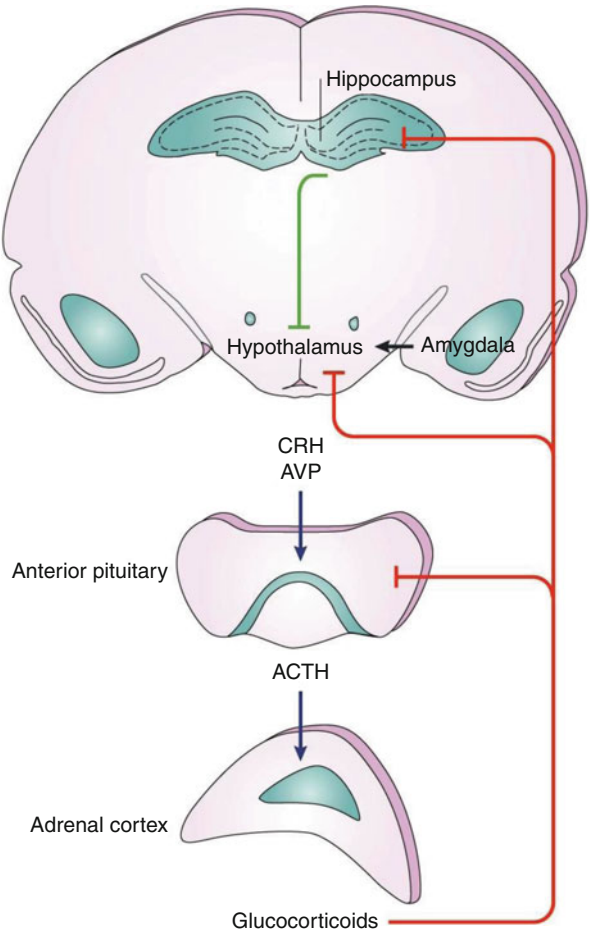

Fig. 54.1 The hypothalamic-pituitary-adrenal (HPA) axis, its inhibitory feedback mechanisms, and its neural control. Activation of the HPA axis results in the production of corticotropin-releasing hormone (*CRH*) and vasopressin (*AVP*) in the paraventricular nucleus of the hypothalamus (PVN). Both peptides are released into the pituitary portal system, which leads to the anterior pituitary where they stimulate the production and secretion of adrenocorticotropic hormone (*ACTH*). ACTH stimulates the release and synthesis of glucocorticoids from the adrenal cortex (cortisol in humans, corticosterone in some animals, such as rodents and chicks). Appropriate regulatory control of the HPA axis is crucial for health and survival, and it is accomplished through negative-feedback mechanisms that involve both rapid and genomic actions of glucocorticoids at the pituitary and at many sites in the brain, including the hippocampus. Conversely, inputs that arise from the amygdala elicit activation of the HPA axis (Taken, with permission, from Sandi 2004)

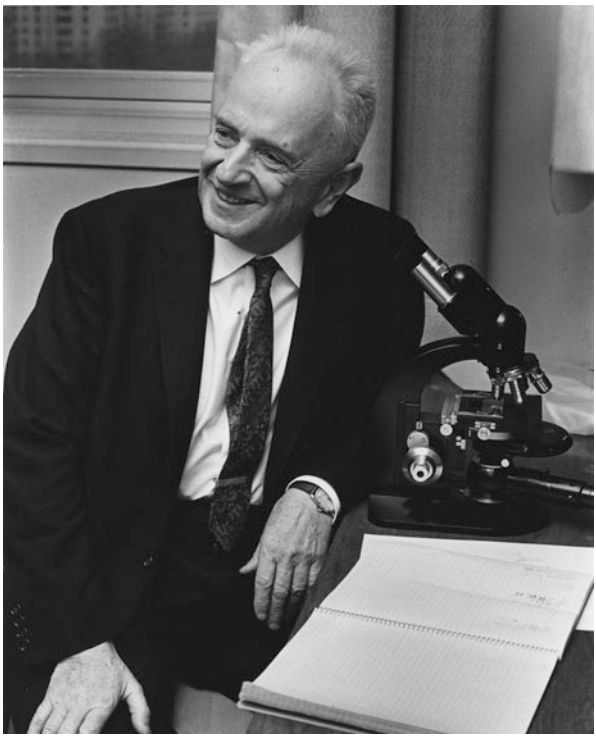

Fig. 54.2 Theodosius Grygorovych Dobzhansky (1900–1975)

Principle 1. Neuroendocrine Mechanisms Have Been Conserved to Provide Biologically Adaptive Body/Brain/Behavior Coordination

"Nothing in biology makes sense except in the light of evolution." This phrase, coined by prominent geneticist and evolutionary biologist Theodosius Grygorovych Dobzhansky in 1973 (see Fig. 54.2), has become an axiom for researchers in all branches of the life sciences as well as a political slogan used by anticreationists globally. In *Genetics and the Origin of Species*, Dobzhansky synthesized evolutionary biology and genetics by defining evolution as "a change in the frequency of an allele within a gene pool." Dobzhansky's work was instrumental in spreading the idea that it is through mutations in genes that natural selection takes place. The foundations of Dobzhansky's assertion were laid in Charles Darwin's *The Expression of Emotion in Man and Animals*, in which Darwin suggested continuity from lower mammals to humans in body, brain, and behavior. In fact, Dobzhansky's axiom is especially accurate when examining neuroendocrine systems across animal phyla; an abundance of evidence has accumulated in support of evolutionary continuity, attesting to both between- and within-species

Table 54.1 Examples of hormone molecules that have been highly conserved for hundreds of millions of years, as evidenced by their presence in a wide range of species

Mammalian Hormone	Found in other species	Function in mammals
Neuropeptide Y	*Caenorhabditis elegans*	Feeding
Angiotensin	Freshwater/saltwater fish	Fluid balance, vasoconstriction, apoptosis
Arginine-vasopressin	Invertebrates, vertebrates	Antidiuresis, vasoconstriction
Oxytocin	Invertebrates, vertebrates	Lactation, parturition, social behavior
Insulin	*Drosophila melanogaster*	Carbohydrate synthesis, growth, and development
Cortisol	Birds/rodents	Stress regulation, catabolism, memory
Prolactin	Fish	Sodium excretion
Aldosterone	Dipnoi (Lung fish)	Fluid balance, thirst
Gonadotropin-releasing hormone	Vertebrates	Reproduction
Estrogen	Insects	Sexual behavior and development, bone density
Somatostatins	Fish	Digestion, inhibits growth hormone
Leptin	*Xenopus*	Feeding, energy balance, reproduction, vascular permeability, bone mass
Thyroid hormone	Reptiles	Regulation of growth and metabolism

links, in hormone-controlled brain mechanisms that guide behaviors, which in turn facilitate survival and underlie the basis for natural selection.

Hormonal signaling systems have evolved to facilitate homeostasis and survival by promoting physiological and behavioral mechanisms, both in the brain and in the periphery. Hormone-controlled behaviors include but are not limited to feeding, drinking, stress, sexual, social, defensive, courtship, aggressive, and parental behaviors, systems that are evidently paramount for both survival and reproduction. Hence, it is not surprising that numerous classes of neuroendocrine signaling molecules have been conserved over millions of years of evolution, sometimes for overlapping functions in organisms ranging from fish to humans. In fact, the ancestral antecedents of some human hormones are still present in primitive vertebrates and even invertebrates such as insects, squid, and octopus. Thus, the study of homologous hormone/brain/behavior systems in laboratory animals sheds light on human mechanisms and on their perturbation in associated medical conditions.

Compelling examples of the evolutionary conservation of hormonal signaling molecules across taxa include neuropeptide Y (NPY), angiotensin II, oxytocin, arginine vasopressin, insulin, and more, all hormone molecules that have been highly conserved for hundreds of millions of years (for examples, see Table 54.1). How does this conservation take place on a molecular level? Simply stated,

hormones are products of the central dogma of biology. Hence, gene sequences encoding for hormones are transcribed from DNA into mRNA, which can be subsequently translated into peptide hormone molecules by dedicated cellular machinery. Over millions of years, these genes underwent random mutations in individual nucleotide bases, leading to the development of different variants of hormones. However, research clearly shows that the biologically active component of the gene sequence has remained tightly conserved. Moreover, in contrast to the short gene sequences that encode hormone ligands, gene sequences that encode hormone receptors are quite long and thus more susceptible to mutations. It is likely that for this reason individual ligands have numerous receptor subtypes.

Oxytocin and Arginine Vasopressin

Oxytocin and arginine vasopressin are structurally related neuropeptide molecules that are believed to have resulted from a gene duplication of an ancestral vasotocin gene (see Fig. 54.3). Their genes are found on chromosome 20 in humans and are oriented in opposing transcriptional directions. Both compounds are nonapeptides (peptides comprising nine amino acids) with a disulfide bridge that differ only in the third and eighth amino acid residues. Moreover, oxytocin and vasopressin have been shown to cross-react, further implicating the evolutionary and functional relatedness of these neuropeptide systems. Oxytocin and vasopressin are primarily synthesized in the paraventricular (PVN) and supraoptic nuclei of the hypothalamus from which they are transported either to the pituitary gland on to the periphery or directly to various targets within the brain. Oxytocin has been implicated in lactation, parturition, sexual orgasm, and a variety of prosocial behaviors such as maternal care and pair bonding via actions on the oxytocin receptor. Vasopressin regulates fluid balance by action on V2 receptors in the kidneys and has pressor effects via $V1_A$ receptors. $V1_A$ receptors are also present in the brain where they are involved in a variety of behaviors related to mood and sociality. A dense population of $V1_B$ receptors in the anterior pituitary has been implicated in HPA axis stress regulation, particularly during chronic stress, and $V1_B$ in the limbic system are believed to be involved in the coupling of sensory information with the appropriate behavioral response and mood regulation (e.g., aggression, anxiety, social behavior).

Homologs of oxytocin and vasopressin have been discovered in birds, reptiles, amphibians, fish, and various invertebrates as well.

Principle 2. Hormones Can Either Facilitate or Repress Behavioral Responses

A plethora of evidence implicates the power of hormones to both facilitate and repress behavioral responses. In fact, the same hormone can produce differential effects depending on the sex of the organism, developmental stage, time course, and site of action.

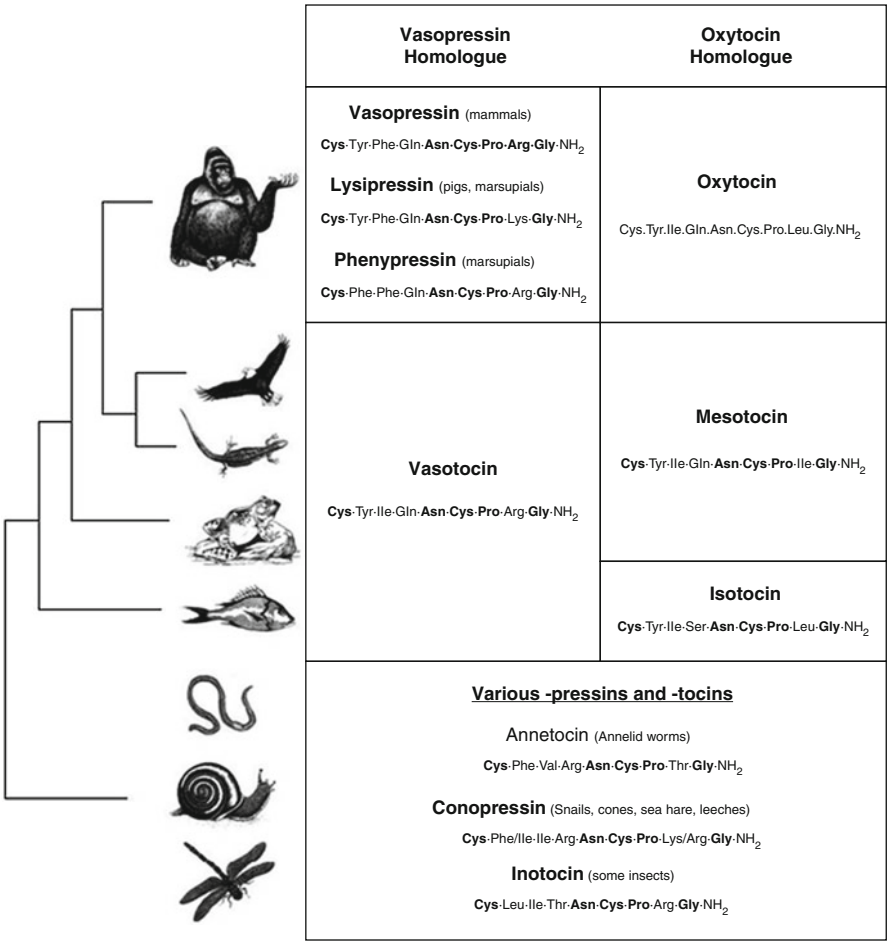

Fig. 54.3 Oxytocin and vasopressin homologues demonstrate the evolutionary conservation of neuropeptide systems (Taken, with permission, from Donaldson and Young 2008)

Play Fighting

Steroid hormones during perinatal development affect neural systems that are involved in male- and female-typical agonistic behaviors in juveniles/adults. For example, rats and hamsters engage in play fighting as early as motor activity has fully matured (around postnatal day (P) 15). Play fighting is extremely common in mammals, and some believe it may serve as a precursor for adult-typical aggressive interactions. Play involves mechanisms of reward; during bouts of play, rats emit 50 kHz ultrasonic vocalizations that have been typically associated with positive valence, such as copulation. In fact, rats emit these vocalizations when they are

gently tickled around the nape of the neck, a region typically nuzzled during play, with this behavior associated with laughing in humans. Throughout postnatal development, play fighting evolves and is typically divided into three stages: (1) *infant* (P15–P25), (2) *juvenile* (P25–P40), and (3) *adult* (P40–) on the basis of defensive and offensive behavioral patterns. For example, in hamsters, the frequencies and the target of the attacks gradually change from juvenile to adulthood: nape, cheeks, and flanks in play fighting and back and belly in adult aggression. Indeed, studies using sophisticated behavioral analyses show a shift from playful interactions to rougher agonistic interactions from the juvenile period (P30) to adulthood (P60). In hamsters, the transition from delicate nibbling to biting is accompanied by the development of agonistic territorial behaviors such as flank marking. The onset of play fighting coincides with the end of the "stress-hyporesponsive period," a period characterized by minimal activity of the HPA axis. Thus, some suggest that either corticosterone or androgens released by the adrenal activate play fighting.

There are clear quantitative and qualitative sex differences in play fighting in rats, suggesting a role for gonadal steroids. Males will typically instigate more attacks, pin their opponents, and generally engage in more physical contact, whereas females will more quickly evade an opponent. In addition, puberty in males signifies a transition from playing to rougher aggression, whereas females maintain elements of play throughout their lives. Castration does not cause a substantial decrease in play fighting, though males that are castrated at weaning will most likely develop into subordinate adult animals.

Aggression

One of the most oft-cited relationships in behavioral neuroendocrinology is that of androgens/anabolic steroids and the expression of aggressive behaviors. Aggressive behaviors are broadly defined as behaviors between same-sex individuals that affect dominance and access to resources, such as food or females.

Testosterone is a ubiquitous androgen, which can facilitate male-typical aggression via direct effects, effects of its metabolites dihydrotestosterone (DHT), estradiol (E_2), or synergistic/combinatory effects. Androgen receptor distribution is highly sexually dimorphic, perhaps accounting for the differential effects of testosterone on aggression in males and females.

The effects of testosterone on aggression have been demonstrated in studies of castration with or without androgen replacement. It typically takes 2–3 days for hormonal replacement to reactivate aggression in gonadectomized males. This time frame is consistent with the dependency of these effects on genomic activity. In fact, in line with the reductions in aggressive behaviors after castration, androgen receptors show a rapid decline postcastration, which is rescued by testosterone propionate administration. In contrast, the activation of male-typical aggression in gonadectomized females typically takes 16–21 days of androgen treatment, though androgen receptor levels increase within 24 h of androgen replacement. Researchers have suggested that the extended time required to produce aggression

in females may indicate an interaction between androgens and certain growth factors, i.e., testosterone-dependent remodeling. Corroborating evidence comes from studies of male canary birds that show a testosterone-dependent elevation in brain-derived neurotrophic factor in neurons of the vocal center.

Testosterone may mediate its effects on aggression via interactions with the vasopressinergic system. Vasopressin is prominent in brain areas shown to promote intermale aggression, such as the lateral septum and bed nucleus of the stria terminalis. Accordingly, when locally injected into these regions, vasopressin potentiates aggression. Vasopressinergic pathways that lead from the bed nucleus of the stria terminalis and medial amygdala to the lateral septum have been implicated in aggression. These pathways are testosterone dependent. In fact, castration diminishes these pathways in addition to curtailing aggression.

Additional evidence for the relationship between androgens/anabolic steroids and aggression comes from research on the abuse of these compounds by athletes. Anabolic steroids have been used and abused by athletes due to their ability to enhance muscle mass. Abuse of anabolic steroids can sometimes produce "steroid rage," which are bouts of aggression, mood disturbance, hypomania, irritability, and depressive episodes.

Estrogens facilitate a variety of behaviors in females but also in males. Estrogens differentially affect aggression depending on gender, age, and nature of the aggressive encounter. Estrogens bind to two receptor subtypes in the brain, ERa and ERb. Studies using ERa knockouts show that ERa plays a primary role in the facilitation of male aggression and in the ability to differentiate between "friend and foe." Specifically, ERa enables a male animal to initiate offensive attacks in response to instigation from a male competitor. Castration with or without testosterone replacement in ERa knockouts (animals in which a gene is genetically "deleted") or wild types (unaltered controls) indicates that the aromatization product of testosterone, namely E_2, is necessary for aggression, as it rescues aggression in castrated wild types, but not in ERa knockout males. Furthermore, the knockout of the *ERa* gene may also dramatically affect the development of brain pathways involved in the expression of aggression. ERa knockout males exhibit aggression toward sexually receptive females, indicating an inability to assess appropriate targets of intermale aggression. In contrast to the facilitatory role of ERa on male aggression, ERa knockout females show an opposite phenotype, i.e., reduced aggression. On the other hand, binding to ERb seems to inhibit aggression; ERb disruption results in heightened aggression in males, as indicated by shorter latencies to attack and higher frequencies. Interestingly, the magnitude of this phenotype attenuates with age, with the highest levels evident just after puberty.

Glucocorticoids have also been implicated in aggression. Interestingly, both acute high and chronically low levels of glucocorticoids have been linked to aggression. In particular, high glucocorticoid levels are associated with impulsive-reactive-hostile-affective aggression, whereas low glucocorticoid responses, with controlled proactive-instrumental-predatory aggression. Thus, it seems that glucocorticoids affect aggression (though different styles of it) in a "U"-shaped fashion, with high aggression at the extremes of glucocorticoid expression.

Principle 3. Hormone Metabolites, Combinations of Two or More Hormones, and the Sequence of Hormone Treatment Can Be Important for Influencing an Individual Behavior

Hormone Metabolites

Steroid hormones control cellular proliferation and death, neuronal migration and differentiation, and neurite extension and elaboration. Steroid hormones are synthesized from cholesterol by dedicated enzymes. For example, cholesterol is converted into pregnenolone in the mitochondria by cytochrome P450scc, which is then modified into progesterone by the actions of two enzymes, 3-beta-hydroxysteroid dehydrogenase and delta 4–5 isomerase. Progesterone can be further modified into cortisol by 11-deoxycortisol in the adrenal cortex.

The enzymatic metabolism of a subset of hormones termed prohormones produces steroid molecules that exert behavioral effects, a process termed steroidogenesis. Many hormones are synthesized in the adrenal gland. For example, the steroid glucocorticoid hormone cortisol is synthesized from cholesterol. This metabolic process involves several enzymatic steps that take place in the mitochondria and endoplasmic reticulum of cells in the *zona fasciculata* of the adrenal cortex. Cortisol and equivalent glucocorticoids in other mammals (e.g., corticosterone in rodents) elevate blood sugar via the process of gluconeogenesis, inhibit the immune system, and facilitate fat, protein, and carbohydrate metabolism. Cortisol is also centrally involved in stress reactivity, recovery, and memory (see Introduction and Principle 4 for details on cortisol and the HPA axis stress response). The adrenal cortex is also the central site of synthesis of aldosterone, a mineralocorticoid (in the *zona glomerulosa*) and some sex hormones (in the *zona reticularis*). The adrenal medulla synthesizes catecholamines such as epinephrine (adrenaline) and norepinephrine (noradrenaline).

Testosterone is an androgen primarily produced in the testes and adrenal gland and subsequently released into the circulation. All vertebrates express testosterone or a related form (e.g., in fish, 11-ketotestosterone), yet again demonstrating evolutionary continuity and preservation in hormone structure. Testosterone regulates virilization of internal structures such as the testes and prostate. The three-step conversion, termed aromatization, of testosterone to E_2 within the brain is especially important for the development of the masculine brain (see Fig. 54.4). Accordingly, the aromatase enzyme is abundant in brain regions implicated in sexual differentiation, such as the hypothalamus.

Testosterone and its hormone metabolites influence many tissue types throughout the body and brain where they bind to receptors and have widespread effects. In fact, the absence or presence of testosterone at a particular stage of ontogeny, late gestational or neonatal periods (also termed the critical period for brain sexual differentiation), produces significant sexual dimorphism in the rat, which underlies sex differences in neuroendocrine systems in adults. Indeed, several brain structures show marked sexual dimorphism. For example, the medial preoptic area of the hypothalamus is 2.5–5 times larger in males than in females. As this structural difference suggests, the medial preoptic area is centrally involved in male sexual

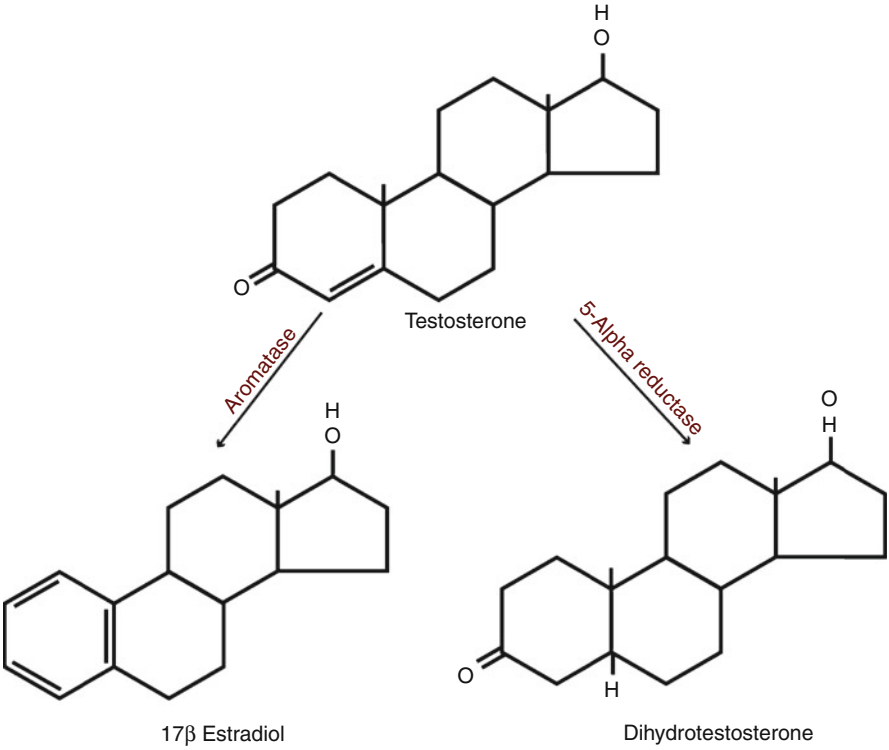

Fig. 54.4 Metabolism of testosterone into 17b estradiol (E_2) and dihydrotestosterone by aromatase and 5-alpha reductase, respectively

behavior. In addition, the bed nucleus of the stria terminalis and the ventromedial nucleus of the hypothalamus are both larger in males compared to females. Males have a larger posterodorsal nucleus of the medial amygdala, the volume of which is determined by circulating androgens. Indeed, castration of adult males reduces its volume to that of females, and administration of testosterone to adult females causes growth of the nucleus to a size comparable to males. In females the anteroventral periventricular nucleus is abundant with neurons expressing ERb, whereas in males, this structure is absent. The anteroventral periventricular nucleus plays a significant role in female cyclicity, a process that is initiated at puberty and involves a preoptic mechanism for estrogen-sensitive positive feedback on luteinizing hormone.

Testosterone is also metabolized by 5a-reductase into the male androgen DHT. By binding to dedicated receptors in the periphery, DHT is critical for the development of male secondary sex characteristics, such as body hair, bone density, and muscle mass. In females, E_2 exposure during puberty promotes feminine secondary sexual characteristics such as breast development and body composition.

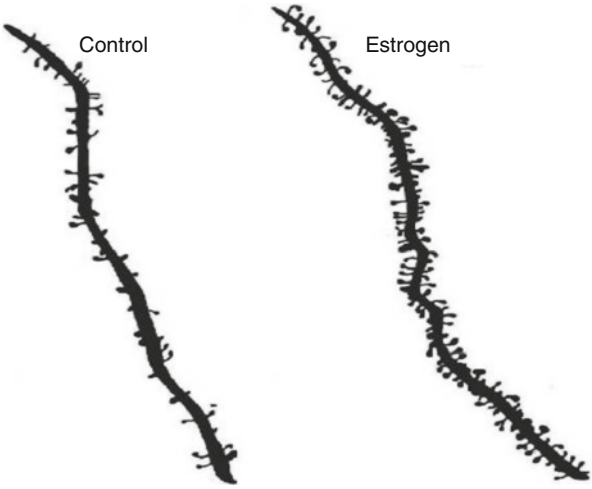

Fig. 54.5 Camera lucida drawings of a dendrite from a female not experiencing estrogenic stimulation (*left*, control) compared to a female exposed to estrogen (*right*, estrogen). Note the marked increase in spine density in the estrogen-treated female (Adapted from Woolley and McEwen (1992). Taken, with permission, from Romeo et al. 2004)

Yet another example of a sexually dimorphic brain structure is the hippocampus. The hippocampus is involved in emotion, learning, memory, and spatial navigation. Steroid hormones affect cellular morphology in several brain regions, which in turn may have profound effects on behavior. When comparing the arcuate and ventromedial nuclei of males and females in dendritic spine density, estrogen-treated females have significantly more spines than males. In addition, ovariectomized females have less dendritic spines on the apical dendrites in hippocampal *cornu ammonis* 1 (CA1) pyramidal cells when compared to ovariectomized females that received E_2 replacement (see Fig. 54.5).

Combination of Hormones

Different combinations of particular hormones have been shown to affect behaviors. For example, a combination of hormones regulates social recognition, particularly in females. Studies show that these social behaviors depend on estrogens affecting oxytocin and oxytocin receptor gene expression profiles.

Oxytocin is a highly conserved nonapeptide that functions as a neurotransmitter and neuromodulator with widespread effects (also see Principle 1). Research in animals and humans suggests that oxytocin is prosocial, facilitating trust and affiliation and inhibiting systems involved in fear and anxiety. Oxytocin synthesized in the PVN converges on oxytocin receptors in the medial amygdala with sensory information relayed by the accessory olfactory system to control trust and social behavior. Oxytocin acting on the central amygdala has been shown to regulate autonomic components of fear by integrating signals from the basolateral amygdala and cortex and projecting to the hypothalamus and brain stem. The medial amygdala is ideally

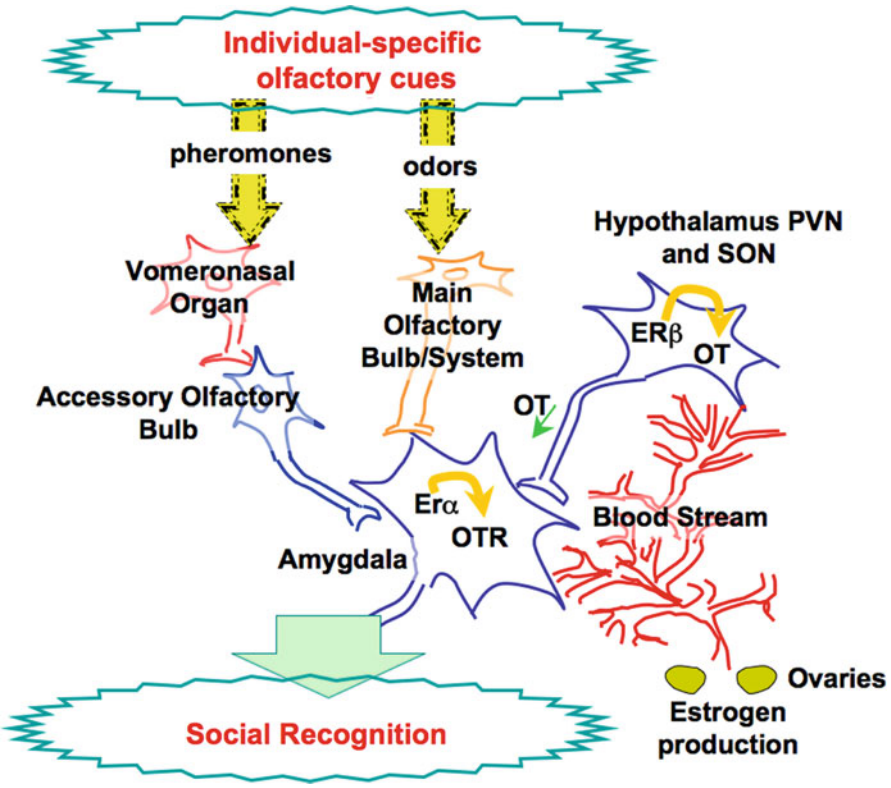

Fig. 54.6 Genomic and neuronal mechanisms for hormonal influences on social recognition. Estrogens produced in the ovaries circulate to the paraventricular nucleus of the hypothalamus (PVN) where, following binding to ERb, they stimulate oxytocin (OT) transcription. OT is carried to the amygdala. Estrogens circulate to the amygdala where, following binding to ERa, they stimulate OT-receptor transcription. OT, operating through OT-receptors in the amygdala, fosters social recognition. In mice, highly olfactory animals, olfactory signaling through both the main and accessory olfactory systems provides the stimuli to the amygdala to which the mice react using the estrogen influenced OT/OT-receptor system (From Pfaff et al. 2004, p. 53; Adapted from Choleris et al. (2003))

located to mediate social anxiety as it is involved in pheromonal processing through projections to both accessory and main olfactory bulbs and is at the center of a defensive behavioral circuit. Oxytocin in the medial amygdala facilitates social recognition and behaviors in the mouse; oxytocin knockout mice show marked deficits in social recognition, with intracerebroventricular and intramedial amygdala oxytocin replacement before the initial encounter rescuing the response.

Estrogens act in concert with oxytocin to promote social behaviors by attenuating social anxiety and facilitating social recognition and interaction. Estrogens produced in the ovaries travel to the supraoptic and paraventricular

nuclei of the hypothalamus where they bind to ERb, which promote the synthesis of oxytocin. Estrogens also travel to the medial amygdala where they bind to ERa and promote the synthesis of oxytocin receptors. Oxytocin travels from the hypothalamus to the amygdala, where it binds its receptor and facilitates social behaviors. In line with this model, oxytocin receptor mRNA levels have been shown to fluctuate in accordance with estrogen levels and throughout the estrus cycle (see Fig. 54.6).

Sequence of the Hormone Treatment

The same combination of hormones may have different effects on behavior depending on the timing of synthesis and release. In females, a priming treatment of estrogens (over 48 h) followed by an acute exposure to progesterone facilitates sexual behavior. A complete neurocircuit for the primary female sexual behavior in rodents, lordosis, has been delineated. In a remarkable example of evolutionary adaptive utility, estrogen priming followed by a brief progesterone exposure facilitates both lordosis and ovulation, thus synchronizing physiology and behavior necessary for reproduction. However, prolonged progesterone administration does not promote lordosis. The reverse sequence, i.e., administration of progesterone followed by estrogen, does not promote lordosis in the same manner as the original estrogen followed by progesterone sequence. Estrogen levels remain high throughout pregnancy and at parturition, whereas progesterone levels are high to begin with and decline approximately around birth. The combination of high levels of estrogens and reductions in progesterone are required for the display of maternal behaviors (see Fig. 54.7).

Principle 4. Hormones Can Facilitate Rapid or Prolonged Physiological and/or Behavioral Actions

The actions of hormones can be rapid, potentiating responsivity within seconds, or can be prolonged. These may correspond to the modes of action mentioned earlier, with a genomic cascade typically taking longer than a nongenomic one.

The Stress Response

The rapid activation of the stress response is evidently crucial for survival. Upon encountering a stressor, cascades of endocrine and autonomic processes facilitate appropriate defensive behaviors. The endocrine response entails induction of the HPA axis. This process commences when neuroendocrine cells in the medial parvocellular division of the PVN release a 41-amino-acid neuropeptide/hormone corticotropin-releasing hormone, as well as oxytocin and arginine vasopressin. Corticotropin-releasing hormone (CRH) axonally travels to the median eminence

	Ovulatory Release of Leuteinizing Hormone (LH)	Expression of Lordosis Behavior
No hormones	0	0
Estrogens (E) Only	↑	↑
Estrogens (E) followed by Progesterone (P) (Tested P + 2-5 hrs)	↑↑↑	↑↑↑
E followed by P administration longer (Tested P + 20-25 hrs.)	0	0
P (long administration) concomitant to E	↑	↑
P (long administration) concomitant to E, *followed* by high E & declining P	0	0 *

* This hormonal pattern is optimal for parental behavior

Fig. 54.7 There are many parallels between the hormonal conditions sufficient for ovulation in the female laboratory animal and those sufficient for the primary sex behavior lordosis (From Pfaff et al. 2004, p. 52)

and is subsequently released into the hypophysial portal system, which leads to the anterior pituitary. At this location, it binds to corticotrophs to stimulate the release of adrenocorticotropin hormone (ACTH). As a result, ACTH is released into the bloodstream where it causes the secretion of glucocorticoids from the adrenal cortex, cortisol in humans and corticosterone in rodents. The HPA axis is tightly controlled by a number of central negative-feedback sites; specifically, the hypothalamus, pituitary, and hippocampus have been identified as major brain targets of glucocorticoid-mediated regulation (see Fig. 54.1, Introduction). While both CRH and glucocorticoids aid in the *rapid* facilitation of an appropriate response to stressors that ultimately enhances chances of survival, *prolonged* release of these hormones has been shown to produce deleterious effects to a number of bodily processes, such as immunity, digestion, learning and memory, and reproduction.

The stress response also entails rapid autonomic nervous system activation. Autonomic activation supplies essential nutrients such as glucose and oxygen to muscles that are required for active defensive responses such as fighting or fleeing. Autonomic activation begins at the central nucleus of the amygdala, wherein CRH is synthesized and subsequently released onto the locus coeruleus, stimulating the release of norepinephrine and epinephrine into the general circulation, which in turn activates the peripheral autonomic response including cardiovascular responses (heart rate and blood pressure), body temperature regulation, perspiration, and bronchiole dilation. Abnormally high levels of CRH and norepinephrine have been implicated in various

psychopathologies related to anxiety and depression. In fact, antagonism of the beta-adrenergic receptor is effective against some forms of anxiety (e.g., stage fright), and CRH antagonists show promise as potent anxiolytics.

Sex Differences in Stress Reactivity

There are marked sex differences in the effects of stress on behavior and brain, suggesting a role for gonadal steroids in stress responsivity. In fact, the prevalence of stress-related psychopathologies is clearly sex dependent, with some studies reporting a double-fold preponderance of women to develop anxiety and depression. Gonadal steroids have been shown to profoundly affect mood. A change in E_2 levels in females, such as during periods of postpartum, perimenopause, and postmenopause, can lead to negative affect and HPA dysregulation, with E_2 replacement as an effective treatment. Testosterone also has effects on mood, especially in males (see Principle 2).

The hippocampus and amygdala are central to both stress responsivity and the learning and memory of aversive situations. Chronic foot shock, a highly stressful event, causes opposite effects on hippocampal cell proliferation (in dentate gyrus region) in males and females, suppressing it in the former and proliferating in the latter. Males and ovariectomized females show retraction of dendrites in the CA3 region after chronic restraint, with cycling females resilient to this effect. Chronic restraint has also been shown to produce deficits in males in a spatial learning task (hippocampal-dependent cognition), while having no effect or even enhancing learning in females. Interestingly, chronic restraint produces opposite effects in dendritic growth in neurons in the amygdala. The amygdala regulates both innate and learned components of fear. Thus, chronic restraint impairs hippocampal-dependent cognition, yet enhances amygdala-dependent fear, observations consistent with the opposite effects of stress on hippocampal and amygdalar structure.

Sexual Responsivity

Testosterone regulates male-typical sexual behaviors in rodents. However, testosterone promotes mounting behavior, pelvic thrusting, penile insertions, and ejaculation only after days or even weeks of prolonged administration.

In females, a prolonged priming period of estrogen is needed for high levels of lordosis behavior. In a cycling female, 48 h of estrogen priming is sufficient, but in gonadectomized females, the longer the animal has been devoid of circulating estrogens, the longer the priming period required to induce lordosis. In molecular terms, the prolonged absence of estrogens leads to a reduction in nuclear coactivator proteins, which are necessary to eventually translate the binding of estrogens to dedicated receptors into the transcription of behaviorally relevant genes. For example, transcription of the highly relevant gene for the progesterone receptor is induced as a result of estrogen priming. The lordosis response is optimally expressed if a short burst of

	Ability to generate ovulatory surge of LH release?	Sex Behaviors?	
		Male-like?	Female-like?
Normal male	NO	YES	NO
Neonatally castrated male	YES	NO	YES
Normal female	YES	Minimal	YES
Female given testosterone neonatally	NO	Increased	NO

Fig. 54.8 Removal of testicular androgens from the neonatal male rat permits his neuroendocrine system to generate a pulse of GnRH sufficient for ovulation (if he has been transplanted with ovaries) and to do female sex behaviors such as lordosis. Conversely, injection of testosterone neonatally into the female rat abolishes her ability to ovulate and to respond normally to estrogens with lordosis behavior. Male-like sex behaviors change, some less dramatically, in exactly the opposite direction from female behaviors (From Pfaff et al. 2004, p. 134)

progesterone follows estrogen priming. Interestingly, 2–5 h after an acute progesterone injection, luteinizing hormone is released from the pituitary and lordosis is expressed. However, if progesterone is continually applied, both luteinizing hormone and lordosis are inhibited (see Fig. 54.8).

Principle 5. Hormones Do Not Cause Behavior; They Alter Probabilities of Responses to Given Stimuli

As the title of this chapter indicates, a clear relationship exists between hormones in the brain and behavior. However, this leads to the misconception that hormones directly produce behavioral responses regardless of incoming stimuli.

In response to certain environmental stimuli, a behavioral response is evoked in quantifiable probabilities. Specifically, scientists examine the number of occurrences, their latency, and the amplitude of a behavior in a particular context in individuals without the hormone in question. Then, the hormone is administered and these parameters are reevaluated. A hormone is considered to facilitate a behavior when the frequencies increase, the latencies decrease, and/or the amplitude increases and to inhibit it when these parameters are reversed.

Predation and Defense

Predation is a form of aggression that is induced by specific stimuli. First, a prey must be present, and second, the prey must exhibit certain behavioral responses that elicit attack, such as flight. Gonadal steroids affect predatory behavior if and only if these stimuli are present. Defensive behaviors in response to predatory attack are also highly dependent on incoming stimuli. As mentioned in Principle 4, CRH and

norepinephrine are both instrumental for appropriate reactivity to an impending threat, such as a predator. Administration of these hormones in vacuo will produce autonomic activation associated with fear and anxiety and a generalized behavioral form of fear, such as hyperarousal. However, in response to a predator or predator-related cues (e.g., the odor of a predator), CRH and norepinephrine (among other hormones) will induce species-typical behaviors in rats and mice in the direction of the threatening cue, such as freezing, risk assessment, avoidance, and burying that will facilitate survival and an eventual return to homeostasis.

Intermale Aggression

Male rodents engage in a stereotypical form of territorial aggression. These behaviors are dependent on androgen levels (Principle 2). Male rats in a resident-intruder model will typically face off in a boxing stance that protects their dorsum, the primary target of intermale aggression, and will engage in a range of ritualized stances. Offensive dominant postures include being on top of your competitor, biting its back, and exhibiting lateral attacks toward its dorsum. Defensive submissive postures include boxing, lying on the back, and attempting to flee from your opponent (jumping, running).

In a seminatural visible burrow system aimed at mimicking a natural rodent mixed-sex social environment, subordinate male rats have significantly reduced testes weights, lower testosterone levels, higher basal corticosterone levels, and reduced corticosterone binding globulin (indicating more free-circulating corticosterone) than their dominant counterparts. These results suggest that in addition to the role of basal hormone levels in altering probabilities of responses to given stimuli, chronic subordination stress may exacerbate the neuroendocrine status of the organism, potentially leading to pathology.

Social Behavior

Oxytocin is centrally involved in social behaviors (see Principle 3). If rats or mice are given oxytocin in the absence of a social stimulus, affiliative behaviors are not evoked. In order for oxytocin to promote social behavior, olfactory signatures from conspecifics (pheromones indicating, e.g., friend/foe or ovulating female/nonovulating female) must be detected in the vomeronasal organ, travel via the accessory olfactory system, and finally converge in the medial amygdala, where oxytocin binds to its receptors.

Lordosis

An additional classic example is the elicitation of the lordotic response in female rats. Lordosis in response to pressure applied to the skin of ovariectomized

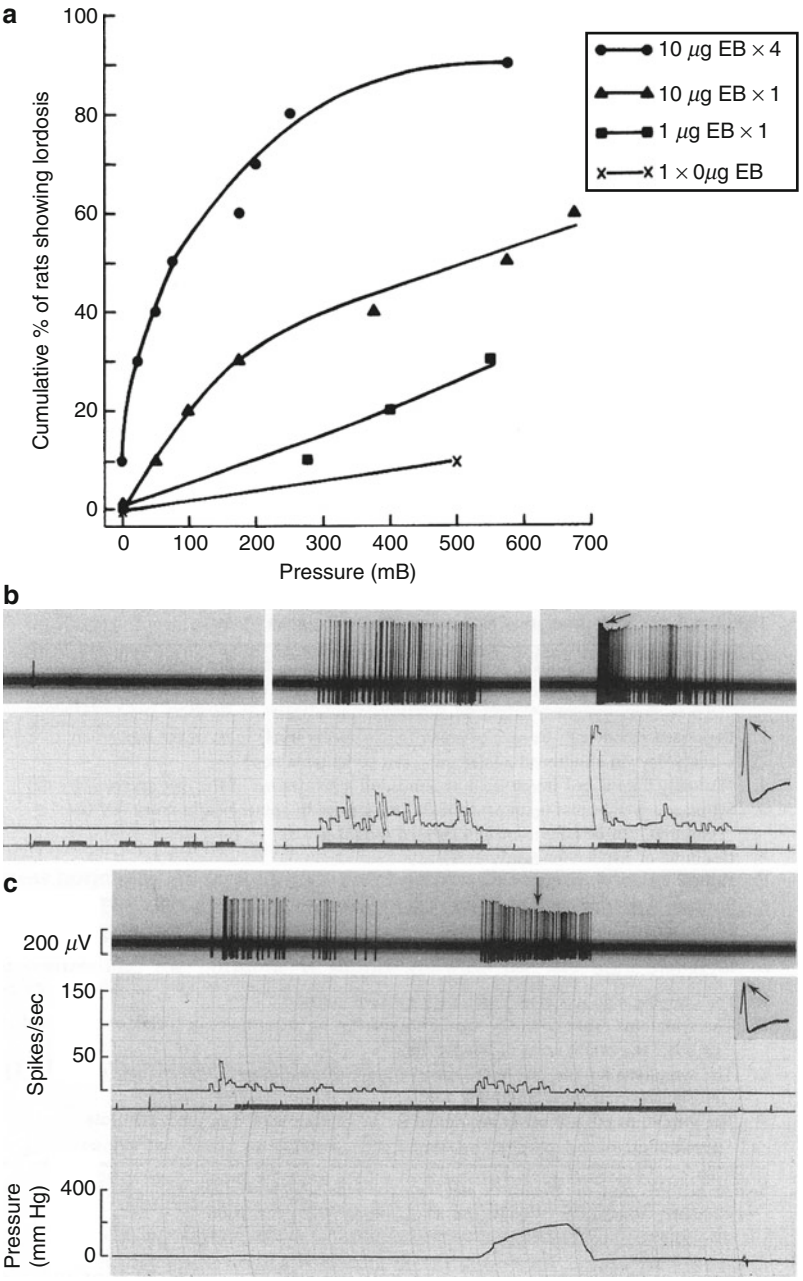

Fig. 54.9 (**a**) Estrogenic hormones act to increase behavioral responses (due to electrophysiological responses) to specific stimuli. Increasing doses of estradiol benzoate (EB) amplify the ramps of behavioral responses to pressure on the skin of ovariectomized female rats sufficient for eliciting lordosis. (**b**, **c**) Electrophysiological responses of single primary sensory neurons in the

females increases with doses of E_2. The increase in behavioral responsivity is only present if pressure to the skin is applied; without it, the animal will not display lordosis, irrespective of the dose of E_2. Electrophysiology of single primary sensory neurons in the dorsal root ganglion of an anesthetized female clearly demonstrates the effects of pressure and their potentiation in the presence of E_2 (see Fig. 54.9).

Principle 6. Effects of Hormones Can Be Widespread Across the Body; Effects in the Brain Consonant with Peripheral Effects Form Coordinate, United Mechanisms

As previous examples in this chapter have illustrated, hormones have effects on both central and peripheral systems. These hormonal routes facilitate two ways how an animal copes with challenges to homeostasis: (1) changes to the physiology of internal systems that work to maintain homeostasis and (2) behavioral modifications that utilize external resources to aid internal physiological adaptations.

Food Intake and Weight Regulation

The processes that control food intake and weight regulation involve hormones that affect hunger, satiety, metabolism, thermogenesis, and locomotor activity. Several pathways regulate weight and food intake (see Fig. 54.10):

1. Inputs from the stomach, gut, and liver converge in the vagus nerve, which feed into the brain stem. Cholecystokinin (CCK), an anorectic (i.e., inhibits feeding), is secreted from the small intestine into the circulation and stimulates contractions in the stomach, which are detected by stretch receptors of the vagus nerve that project to the nucleus of the solitary tract. Projections from the nucleus of the solitary tract inhibit feeding.
2. Leptin is a hormone that is manufactured in fat cells and is termed an adiposity signal. When fat cells become too large, they release more leptin. Leptin decreases food intake by inhibiting the release of the orexigenic (i.e., stimulates feeding) peptide NPY and by promoting the synthesis and release of the anorectic agent a-melanocyte-stimulating hormone (a-MSH). Leptin is released peripherally from fat cells, crosses the blood-brain barrier, and binds to leptin receptors in the arcuate nucleus of the hypothalamus, which lies next to the third ventricle. Leptin inhibits arcuate neurons containing NPY and agouti-related

Fig. 54.9 (continued) dorsal root ganglion of an anesthetized female rat to pressure stimuli on the skin (pressure intensity shown quantitatively on bottom trace of each figure). Sustained pressure led to action potentials in a neuron that had no spontaneous discharge. A sudden peak of pressure, as would be caused by stimulation from a male rat during mounting, causes a high rate of firing (From Pfaff et al. 2004, pp. 98–99; adapted from Kow et al. (1979))

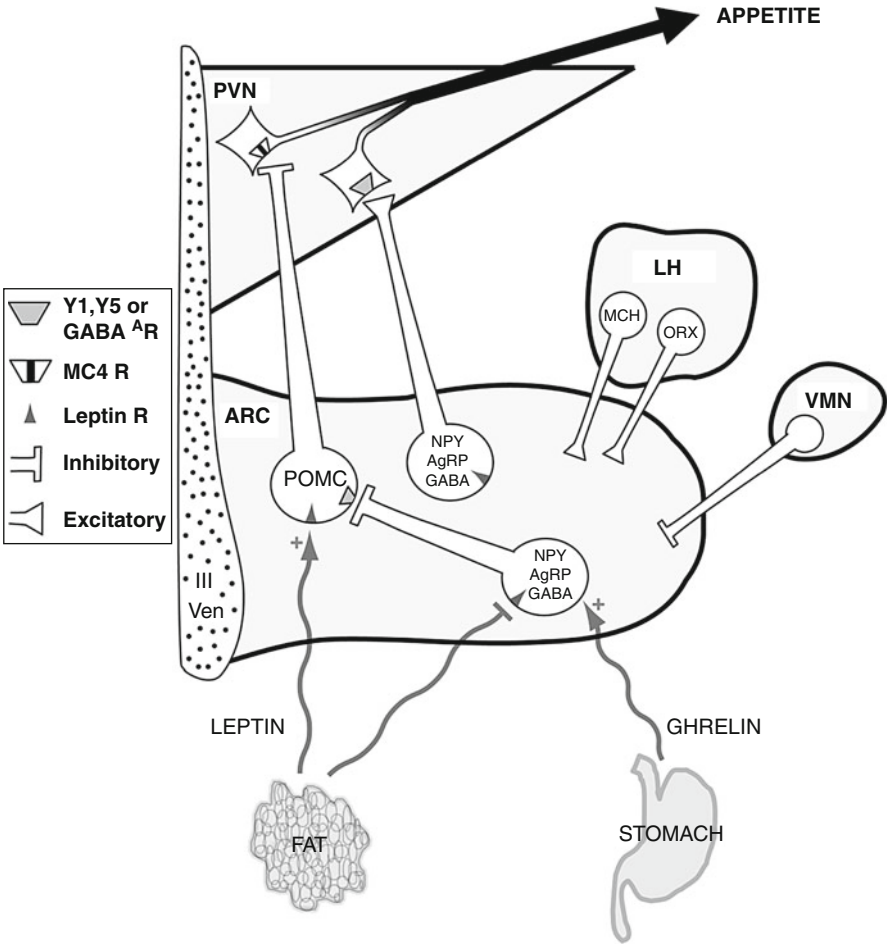

Fig. 54.10 Hormonal signals from stomach and fat impact neurons of specific chemical identities in the arcuate nucleus of the hypothalamus (*ARC*), lying next to the third ventricle (*III Ven*). These neurons, in turn, influence downstream neurons in the paraventricular nucleus of the hypothalamus (*PVN*) that facilitate food intake. A network of neuropeptide-expressing and neuropeptide-receiving neurons in the hypothalamus regulates appetitive behavior. The orexigenic peptides, neuropeptide Y (*NPY*) and agouti-related peptide (*AgRP*), along with g-aminobutyric acid (*GABA*) are coproduced by neurons in the arcuate nucleus (*ARC*) of the hypothalamus. Interspersed among these neurons are neurons that produce anorexigenic peptides, primarily a-melanocyte-stimulating hormone (a-MSH) derived from proopiomelanocortin (*POMC*). Both these neuronal populations project to the PVN, where a-MSH binds to MC-4 receptors to inhibit appetite. NPY and GABA released in the PVN activate the Y1/Y5 and GABA-A receptors, respectively, to stimulate appetite. Additionally, NPY released within the ARC nucleus binds to Y1 receptors on POMC neurons to reduce a-MSH synthesis. Thus, NPY stimulates appetite by direct action in the PVN and indirectly by suppressing POMC neuronal activity in the ARC. AgRP, coproduced with NPY,

peptide (AgRP) that normally inhibit cells containing melanocyte-stimulating hormone that project onto the PVN. In this manner, leptin disinhibits cells that produce a-MSH, thus inhibiting feeding. Leptin also directly activates cells that produce a-MSH to inhibit feeding. a-MSH activates melanocortin receptors, which induce intracellular cyclic adenosine monophosphate (cAMP). As a result, the organism will eat less, fat levels will decrease, and less leptin will be released.

3. Insulin, an additional adiposity signal, is released from the pancreas with the increase of glucose levels during feeding and reaches the brain where it facilitates several processes: (1) insulin facilitates glucose uptake from the circulation into target tissue, (2) insulin decreases endogenous glucose production by the liver, (3) insulin inhibits food intake, and (4) insulin promotes energy expenditure, i.e., anabolic processes. Insulin is believed to decrease food intake by reaching the arcuate nucleus and stimulating CRH and oxytocin release from the PVN.
4. Ghrelin from the stomach signals hunger in the arcuate nucleus. Ghrelin promotes the release of the orexigenic peptides NPY and AgRP.

Appetite for Salt; Natriorexegenia

Humans need salt (NaCl) for basic homeostatic physiological processes. Salt is essential for maintenance of body fluid balance, neuronal activity, and the function of both cardiac and skeletal muscle. Animals exhibit salt hunger that is motivated by hormones. Aldosterone is a mineralocorticoid vital for peripheral regulation of salt; aldosterone produced in the adrenal cortex controls sodium reabsorption in the kidneys, redistributes sodium in the salivary glands and the gut, and transports sodium from bone. High levels of aldosterone can also elicit a behavioral hunger for salt. As a lipid, aldosterone easily crosses the blood-brain barrier and binds to

Fig. 54.10 (continued) stimulates appetite by binding to MC-4 neurons in the PVN and preventing a-MSH action. Additional neuropeptides such as melanin-concentrating hormone (*MCH*) and orexins (*ORX*) from the lateral hypothalamus (LH) enhance appetite by upregulating the NPY network, and other unidentified signals from the ventromedial nucleus (*VMN*) inhibit this network. In sum, the appetite-regulating axis in the ARC and PVN is modulated by afferent hormonal signals that cross the blood-brain barrier and exert opposing effects. Food intake is suppressed by the adipocyte hormone leptin that conveys signals to the brain regarding the body's energy stores. Under conditions of positive energy balance, leptin binds to its receptors on NPY neurons to suppress NPY synthesis and release, and concomitantly, it activates receptors on POMC neurons to enhance a-MSH production. Conversely, hunger is signaled by ghrelin, a hormone secreted by oxyntic cells in the stomach. Blood ghrelin levels rise in conditions of negative energy balance and enhance appetite by activating NPY neuronal activity in the ARC (From Pfaff et al. 2004, p. 6–7; Courtesy of P. and S. Kalra, University of Florida. Also see Morton and Schwartz (2001) and Schwartz (2001))

mineralocorticoid receptors that are widely distributed throughout the brain. Notably, the joint release of glucocorticoids and aldosterone greatly enhances the effects of aldosterone alone, most likely due to activation of type-2 glucocorticoid receptors in addition to activation of type-1 receptors by aldosterone. The medial amygdala has been suggested to be a site involved in sodium hunger; direct injection of a selective mineralocorticoid antagonist into the medial amygdala inhibits sodium appetite induced by peripheral aldosterone administration.

Angiotensin II is associated with the appetite for salt and salt-seeking behaviors. Specifically, angiotensin II is involved in water and sodium consumption. Angiotensin II is a peptide and thus does not readily cross the blood-brain barrier. For this reason, much attention has been focused on the circumventricular organs, as these do not contain a blood-brain barrier. Indeed, a high concentration of angiotensin type-1 receptors are present in the circumventricular organs, specifically the subfornical organ and other areas in the wall of the anterior ventral third ventricle.

Aldosterone and angiotensin II work in concert to promote salt intake. The *zona glomerulosa* of the adrenal cortex, the primary site of release of mineralocorticoids, contains a high density of angiotensin type-1 receptor, which upon stimulation by angiotensin II releases aldosterone into the blood.

Principle 7. Genes Coding for Hormones and Associated Systems Affect Behavior

An individual's genetic background can influence his/her sensitivity to a given hormone in a particular situation. For example, as mentioned earlier, it is apparent that differences in oxytocin receptor in the medial amygdala could affect social behaviors, and changes in androgen and/or estrogen receptors could markedly change aggression profiles. Additionally, familial traits leading to abnormalities in the development of both body and brain can significantly affect behavior.

Anxiety

Anxiety is a subtype of motivation, also referred to as an emotion. Anxiety is defined as a motivation typically associated with behaviors that occur in response to ambiguous threatening stimuli. Anxiety-like behaviors are highly selected reactions to animate and inanimate threats in the environment, which increase the chances of survival/reproduction of those who perform them appropriately. Natural threats may include predators and associated cues, conspecific attack, and dangerous features of the environment. When confronted with these threats, animals display a behavioral repertoire that is composed of innate and learned elements. Researchers investigating the innate aspect of anxiety have typically focused on exposing animals to predators and associated cues, novel environments, and elevated locations, while researchers investigating the learned component have predominantly utilized Pavlovian fear conditioning techniques. Although appropriate

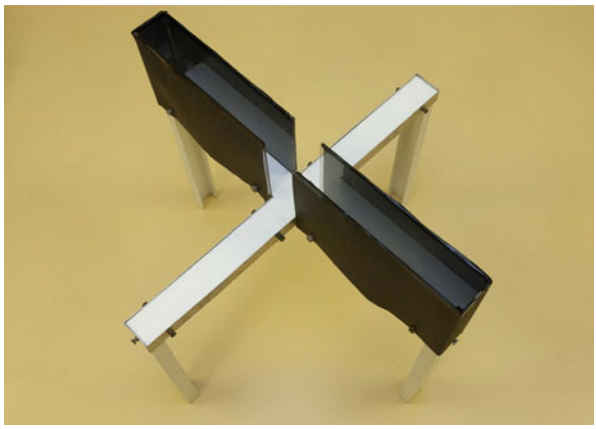

Fig. 54.11 The elevated-plus maze (EPM)

anxiety and subsequent behavioral reactions are vital for an individual, exaggerated or prolonged forms of anxiety are maladaptive in both animals and humans.

A wide variety of genes encoding for hormones and associated systems affect innate anxiety-like behaviors (trait anxiety). These include gonadal and adrenal steroid hormones (e.g., testosterone, estradiol, corticosterone) and various neuropeptide hormones (e.g., CRH, AVP, and oxytocin). In addition, hormones such as AVP and glucocorticoids are involved in components of fear conditioning to cues and/or context (e.g., acquisition, consolidation).

"Knocking out" a specific gene is a useful method to study its effects on behavior. For example, CRH is a neuropeptide hormone that functions as a major secretagogue of the HPA stress axis (see Principles 1 and 4) and directly binds to central sites to regulate anxiety-like defensive behaviors. Accordingly, CRH type-1 receptor knockout mice show extensive dysregulation of the HPA axis, including irregular secretion of ACTH and corticosterone from the pituitary and adrenal cortex, respectively, and display a marked attenuation in anxiety-related behaviors.

Scientists have been selectively breeding animals for traits that make them easier to work with in a laboratory environment, a process called laboratorization. Many generations of human handling have created a rift between laboratory and wild strains of rats and mice, with respect to both physiology and behavior. For example, defensive aggression has been bred out of most rat strains simply due to scientists not wanting to get bitten. Thus, when comparing laboratory with wild strains, it is important to consider the effects of laboratorization on exhibited phenotypes.

Selectively breeding mice or rats for high or low levels of a hormone-related trait is a useful technique to study the role of particular hormone-related genes (genes for hormones, receptors, binding proteins, etc.). Rats have been selectively bred for high- and low-anxiety phenotypes based on performance in the most widely used model of innate anxiety, the elevated-plus maze (see Fig. 54.11). The origin of the elevated-plus maze derived from the observation that when faced with a choice between alleys enclosed within walls versus unprotected open ones, rodents prefer

to stay in the protected areas. In short, the elevated-plus maze is based on a conflict between the propensity of rodents to explore a novel setting and the aversive properties of the open arms.

Study of rats selectively bred for high- and low-anxiety phenotypes on the elevated-plus maze has enabled researchers to identify both central nervous system pathways involved in trait anxiety and candidate genes and gene polymorphisms underlying trait anxiety. Rats that exhibit high levels of anxiety show marked similarities to psychiatric patients; they display a hyperactive HPA axis response, favor passive coping strategies, and have a pathological-like response to the dexamethasone/CRH challenge test, all depressive-like markers. Dysregulation of the HPA axis has been attributed to a number of anxiety-related psychopathologies, such as generalized anxiety disorder (GAD), major depression, and posttraumatic stress disorder (PTSD). Indeed, rats bred for high-anxiety-related behaviors show a more pronounced activation of the HPA axis when exposed to a nonsocial stressor (e.g., elevated-plus maze) and neuronal activation in the PVN, the medial preoptic area, and the locus coeruleus, the major production center of norepinephrine. Interestingly, rats bred for low anxiety show enhanced HPA axis activation in response to social stressors, accompanied by increased activation of the PVN and central amygdala. Plasma ACTH in response to a social stressor (the resident-intruder paradigm) was significantly elevated in rats selected for low-anxiety behavior compared to high-anxiety behavior and nonselected rats. In addition, higher levels of aggression were linked to elevations in the HPA axis stress response.

Vasopressin is centrally involved in anxiety behavior. Indeed, affective disorders such as major depression, anxious-retarded depression, and obsessive-compulsive disorder are characterized by atypical vasopressin levels or receptor activity. High cerebrospinal fluid concentration of vasopressin and CRH was found in depressed patients, with fluoxetine treatment attenuating levels of both neuropeptides. High plasma vasopressin values have also been associated with a family history of depression and mixed anxiety and retardation. Small nucleotide polymorphism (SNP) haplotypes in the vasopressinergic $V1_B$ receptor gene have been shown to protect against recurrent major depression, demonstrating increased susceptibility to affective disorders in patients with alternative polymorphisms.

Accordingly, male and female high-anxiety behavior rats show elevation in the vasopressin neuropeptide gene, though no changes were found in $V1_A$ receptor levels or binding. Intra-PVN infusion of a vasopressin V1-receptor antagonist d (CH(2))(5) Tyr (Me) AVP to high-anxiety rats decreases anxiety-/depression-related behavior. The vasopressin promoter of the high-anxiety rats shows ten polymorphisms that may lead to a disinhibition of the vasopressin gene. High levels of vasopressin in the PVN and other central sites may contribute to HPA axis dysregulation and anxiety- and depressive-like symptoms.

Oxytocin promotes social behaviors, such as pair bonding and maternal behaviors by attenuating anxiety. Recently, researchers have investigated a naturally occurring genetic polymorphism of the oxytocin receptor (rs53576) and its relationship to empathy and stress reactivity. Compared to individuals homozygous for

the G allele, polymorphic individuals with one or two copies of the A allele exhibited less empathy and displayed higher stress reactivity than GG individuals, as determined by heart rate response during a startle anticipation task and an affective reactivity scale.

Thyroid Hormone Resistance

The thyroid gland regulates general metabolism, i.e., growth, development, body temperature, and heart rate via synthesis of the thyroid hormones thyroxine (T4) and triiodothyronine (T3) and their actions on cells throughout the body and brain. Thyroid hormones affect development, metabolic rate (regulate protein, fat, and carbohydrate metabolism), protein synthesis, and neuronal maturation. In addition, thyroid hormones increase the sensitivity of cells to catecholamines (e.g., norepinephrine) by *permissiveness*.

A genetic trait leading to a resistance in thyroid hormone has vast ramifications on mood, energy, and intellectual and social capabilities. In fact, patients with thyroid hormone resistance often display symptoms of attention-deficit hyperactivity disorder (ADHD). Thyroid hormone resistance is characterized by a significant deficit in responsiveness to thyroxine. Scientists have implicated several mutations in the ligand binding domain and "hinge" region of the thyroid hormone receptor-beta in thyroid hormone resistance. These mutations lead to reduced thyroid hormone binding and an impairment of corepressor release.

Precocious Puberty

Abnormal early sexual development, often referred to as precocious puberty, has profound effects on social behavior and psychological development. Many cases of precocious puberty are idiopathic, i.e., for no apparent reason. Precocious puberty may occur as a result of central or peripheral causes. Central causes can involve trauma or infection of the hypothalamus and/or pituitary causing abnormal hormonal synthesis and release, whereas peripheral causes may involve disease or tumors of the adrenal or gonads. Such individuals are physically mature enough for sexual activity, though their emotional development is on par with their peers. In fact, individuals with precocious puberty can conceive at a very young age, with obvious behavioral effects. Susceptibility to tumors or disease is inherited. Some cases of precocious puberty are inherited, especially in boys.

Pseudohermaphroditism

Pseudohermaphroditism is a condition in which an individual has secondary sex characteristics that are different or opposite of those usually dictated by gonadal tissue. In males, if the enzyme that converts testosterone into DHT (5a-reductase) is

mutated rendering it dysfunctional, the external genitalia will appear ambiguous. These individuals have female external sex characteristics, in the presence of XY chromosomes, undescended testes, and a masculinized brain. The complexity of this disorder is especially pronounced when these individuals reach puberty; puberty initiates secretion of testosterone from the testes leading to a phenotypic reversal. Individuals grow a male phallus, exhibit erections, develop a masculine voice, grow body hair, and exhibit male psychological characteristics.

Thus, male pseudohermaphroditism resparked the debate of the dichotomous relationship between nature and nurture. Historically, individuals suffering from a mutation in the 5a-reductase gene were raised as females, with puberty signifying a dramatic crisis in their sexual identity and the primacy of nature over nurture. Nowadays, it is possible to identify this condition at an early stage and determine the most suitable rearing conditions, either as boys or as ambiguous girls.

Principle 8. Genes Interact with Experience to Influence Hormone Responsiveness in the CNS During Adulthood

Inherited factors and environmental experience interact to shape the phenotype of an animal. Genetic attributes predispose individuals toward enhanced or reduced sensitivity to a particular hormone (Principle 7). In addition to inherited factors, i.e., genomic factors, experience, otherwise referred to as epigenetic factors, has a significant influence on the adult phenotype.

Early-Life Experience Affects Adult Stress Reactivity

Prepubertal social experiences have a significant impact on the adult phenotype. In humans, early-life adverse events often precipitate the development of stress-related psychiatric disorders such as PTSD and major depression. Studies using primate and rodent models show that nonhuman mammals are adequate models for the assessment of early-life trauma or enrichment on subsequent emotional reactivity.

Monkeys separated from their mothers show considerable social deficits, withdrawal, and despair. In rats during the first stages of development, it is believed that the interactions between the newborn and the dam program future defensive reactivity by affecting the HPA axis and the sympathetic branch of the autonomic system. Numerous studies on rodents focus on the effects of early-life manipulations during the stress-hyporesponsive period, a period between P4–14 in which the dam suppresses basal and stress-induced circulating corticosterone, on neuroanatomical, endocrine, and behavioral indices of the adult stress response. Corticosterone actions are predominantly catabolic, and thus, its relative inhibition during the stress-hyporesponsive period is adaptive during development, a period characterized by anabolic processes. In accord, adrenalectomized animals show an increase in glucose utilization and in cerebral blood flow to the hippocampus, both processes necessary for neurogeneration and development.

Much attention has been focused on corticosterone and CRH as mediators of early-life adverse effects. Both hormones have been shown to affect hippocampal neurons when excessively released, and their respective receptors have been directly implicated in the effects of early-life manipulations. Long maternal separation has been shown to produce HPA axis hyperactivity characterized by enhanced synthesis and expression of CRH in the PVN, reduced binding to and downregulation of hippocampal type-2 glucocorticoid receptors, elevated CRH levels in the hippocampus, reductions in CRH type-1 receptors in the hippocampus (specifically in regions CA1 and dentate gyrus), deficits in learning and memory, and enhanced anxiety in adults. It has also been suggested that early-life events influence lifelong patterns of emotionality and stress responsiveness and alter the rate of brain and body aging by altering hippocampal structure and function, subsequently affecting HPA axis and limbic activity. Evidence supports this notion: the hippocampus is modulated by long maternal separation; showing reduced mossy fiber density and abnormally abundant mossy fiber terminals within region CA3. These effects are most likely due to elevations in CRH, as CRH injections during the neonatal period, but not corticosterone, can mimic these structural changes.

In contrast to the damaging effects of maternal separation, neonatal handling (typically 15 minutes) seems to have an ameliorating effect on the HPA axis. Brief handling of rat pups has been shown to cause hyporesponsiveness of the HPA axis characterized by reduced synthesis and expression of CRH in the PVN, enhanced binding to and upregulation of hippocampal glucocorticoid receptor, improved learning and memory, and attenuated anxiety in adult rats. Importantly, adult males and females vary in a number of behavioral and neuroendocrine measures with respect to vulnerability to the effects of early-life stress, suggesting the involvement of gonadal steroids.

Rat pups that received abundant attention from their mothers, in the form of licking and grooming, grow up to be less anxious and more maternal than pups that received less maternal care. The neuropeptides oxytocin and vasopressin (see Principle 1) have been implicated in this effect; males that receive more attention have higher levels of $V1_A$ receptors in the amygdala than ones that were less cared for. Females that received more maternal care showed elevated levels of oxytocin receptors in the amygdala and bed nucleus of the stria terminalis, regions involved in fear, anxiety, and affiliative behaviors.

Sexual Differentiation

In mammals, there are critical periods for sexual differentiation. Rodents are altricial, i.e., their central nervous system still develops after birth, whereas humans are precocial, i.e., critical periods for the development of the central nervous system including neuroendocrine function and sexual differentiation occur in utero. Gonadal steroid hormones are crucial for sexual differentiation. In fact, neuroendocrinological and behavioral responsivity to sex hormones in adulthood is

determined by neonatal androgen exposure. In mammalian fetuses, both Müllerian and Wolffian ducts are present. In the presence of the appropriate hormones in an XX animal, Müllerian ducts will develop into fallopian tubes, uterus, and ovaries in the female, and Wolffian ducts into the epididymis, the vas deferens, and the seminal vesicle in males. During the first 2–3 days of the (altricial) life of a rat, the presence or absence of circulating androgens will determine whether the pup will develop neuroendocrine and behavioral trademarks of a female or a male. If testosterone is injected to a newborn genetic female, she will not ovulate as an adult and will not display adultlike female sexual behaviors. If a newborn genetic male is castrated, as an adult, he will be capable of responding to estrogens and progesterone by organizing an ovulatory luteinizing hormone surge and consequently exhibiting lordosis, the typical female quadruped sexual behavior. It is possible to determine the exact timing of these changes. In females, administration of testosterone on P4 (during the critical period of sexual differentiation), but not on P16, results in reduced spine synapses in the preoptic area, acyclic luteinizing hormone secretion, and defeminization (reduced lordosis) and masculinization (increased mounting) of sexual behavior. In males, castration on P1, but not P7, results in increased spine synapses in the preoptic area, cyclic luteinizing hormone secretion, and a demasculinization of sexual behavior (reduced mounting, territoriality, and male-typical aggression) as well as a feminization (exhibit lordosis).

Outlook

The burgeoning field of behavioral neuroendocrinology has grown to occupy a major place in preclinical neuroscience research. Hormone/brain/behavior relations can be simply evoked and can be studied in the laboratory by integrating knowledge of behavioral mechanisms with the ever-rapid advancement in sophisticated cell biological, molecular, and electrophysiological methodologies.

The effects of hormones on behavior are dependent on the genetic makeup of the organism, its development in a dynamic environment, and the stimuli it encounters in the here and now. As such, research in behavioral neuroendocrinology will focus on (1) common and uncommon variations in genes that encode for hormonally responsive systems, (2) early-life epigenetic factors that regulate the expression of particular hormone-related genes, and (3) the effects of external stimuli on genetically and epigenetically controlled hormonally responsive systems that ultimately drive behavior. This breadth of knowledge will mature into novel prevention and/or treatment methods for hormone-related conditions and pathologies in human patients.

Further Reading

Blanchard DC, McKittrick CR, Hardy MP, Blanchard RJ (2002) Effects of social stress on hormones, brain, and behavior. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, Rubin RT (eds) Hormones, brain and behavior, vol 1. Academic, Amsterdam, pp 735–772

Choleris E, Gustafsson JA, Korach KS, Muglia LJ, Pfaff DW, Ogawa S (2003) An estrogen-dependent four-gene micronet regulating social recognition: a study with oxytocin and estrogen receptor-alpha and -beta knockout mice. Proc Natl Acad Sci USA 100(10):6192–6197

De Vries GJ, Simerly RB (2002) Anatomy, development and functions of sexually dimorphic neural circuits in the mammalian brain. In: Pfaff DW, Arnold AP, Etgen AM, Rubin RT, Fahrbach SE (eds) Hormones, brain and behavior, vol IV. Academic, San Diego, pp 137–192

Delville Y, Newman ML, Wommack JC, Taravosh-Lahn K, Cervantes MC (2006) Development of aggression. In: Nelson RJ (ed) Biology of aggression. Oxford University Press, New York, pp 327–350

Donaldson ZR, Young LJ (2008) Oxytocin, vasopressin, and the neurogenetics of sociality. Science 322(5903):900–904

Insel TR, Young LJ (2000) Neuropeptides and the evolution of social behavior. Curr Opin Neurobiol 10(6):784–789

Kow LM, Pfaff DW (1979) Responses of single units in sixth lumbar dorsal root ganglion of female rats to mechanostimulation relevant for lordosis reflex. J Neurophysiol 42(1 Pt 1):203–213

Kow LM, Montgomery MO, Pfaff DW (1979) Triggering of lordosis reflex in female rats with somatosensory stimulation: quantitative determination of stimulus parameters. J Neurophysiol 42(1 Pt 1):195–202

Landgraf R, Neumann ID (2004) Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication. Front Neuroendocrinol 25(3–4):150–176

McEwen BS (2007) Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev 87(3):873–904

Meaney MJ (2001) Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu Rev Neurosci 24:1161–1192

Morton GJ, Schwartz MW (2001) The NPY/AgRP neuron and energy homeostasis. Int J Obes Relat Metab Disord 25(Suppl 5):S56–S62

Neumann ID, Veenema AH, Beiderbeck DI (2010) Aggression and anxiety: social context and neurobiological links. Front Behav Neurosci 4:12

Ogawa S, Nomura M, Choleris E, Pfaff D (2006) The role of estrogen receptors in the regulation of aggressive behaviors. In: Nelson RJ (ed) Biology of aggression. Oxford University Press, New York, pp 231–249

Pfaff DW (1999) Drive: neurobiological and molecular mechanisms of sexual motivation. The MIT Press, Cambridge, MA

Pfaff DW, Phillips MI, Rubin RT (2004) Principles of hormone/behavior relations. Elsevier/Academic, Amsterdam

Romeo RD, Waters EM, McEwen BS (2004) Steroid-induced hippocampal synaptic plasticity: sex differences and similarities. Neuron Glia Biol 1(3):219–229

Sakuma Y (2009) Gonadal steroid action and brain sex differentiation in the rat. J Neuroendocrinol 21(4):410–414

Sandi C (2004) Stress, cognitive impairment and cell adhesion molecules. Nat Rev Neurosci 5(12):917–930

Schwartz MW (2001) Brain pathways controlling food intake and body weight. Exp Biol Med (Maywood) 226(11):978–981

Schwartz MW, Porte D Jr (2005) Diabetes, obesity, and the brain. Science 307(5708):375–379

Woolley CS, McEwen BS (1992) Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. J Neurosci 12(7):2549-2554; Erratum in: J Neurosci 12 (10):followi