CHAPTER

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Female Sexual Behavior

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INTRODUCTION

Interest in female sexual behavior and reproduction is likely as old as our species. Indeed, one of the oldest known medical texts, the Kahun Papyrus, found near the pyramid in El-Lahoun, Egypt, in 1889 and dated to at least 1800 BCE, is a gynecological text that outlines causes and treatments for women's reproductive health problems, including problems of fertility, contraception, and pregnancy.[1] Ancient erotic texts, such as Vatsayana's *Kama Sutra* (compiled from Hindu writings in the second century BCE), Ovid's *Ars Amatoria* (c.1 BCE), and the Taoist *Art of the Bedchamber* books (compiled between 206 BCE and 24 CE), devoted large portions to understanding how to court women properly; how to stimulate arousal, desire, and pleasure; and how women's receptivity to such courting and stimulation shifted across the menstrual cycle.[2] The striking similarity in descriptions, despite vast differences in culture, suggests some degree of commonality in the way women's sexual physiology and psychology are constructed and altered, respectively, by ovarian hormones.

The *Hippocratic Corpus*, dating from the fifth century BCE, contains seven early volumes written by Hippocrates himself that were concerned with women's fertility and diseases specific to women.[1] The first of these contains an oddly modern account of midcycle rises in women's sexual lust and receptivity, driven of course by the lunear impact on women's "psychic pneuma" (the four endogenous fluids—blood, yellow bile, black bile, and phlegm—that were thought to mediate the four basic emotions of passion, anger, sadness, and calm). Hippocrates also notes that "hysteria" should be treated by gentle stimulation of the clitoris and/or vagina to orgasm (or sexual intercourse, if the woman is married or has a knowledgeable sex partner). It was believed that orgasm would center the uterus back into its normal position. The second contains a then-comprehensive list of contraception methods, including the use of small scrubbed stones as intrauterine devices. This practice was based on the experiences of Egyptian and Bedouin camel drivers, who routinely placed stones in the uteri of camels to prevent pregnancy. It is likely that animal sexual behavior and reproduction have been studied since humans started domesticating animals, but largely from a veterinary and/or animal husbandry perspective, and only in those species used as pack animals (camels, sheep, donkeys), for agricultural work (e.g., oxen, horses), or for food (e.g., sheep, cattle). In his work *Historia Animalium*, Aristotle (384–322 BCE) described the routine spaying (ovariectomy, OVX) of adult sows and camels as common agricultural practice, done specifically to eliminate sexual behavior and control reproduction.[3]

Rudimentary psychological understanding of sexual arousal, desire, pleasure, and inhibition in women can be found in art and prose, from ancient religious stories (e.g., "Song of Songs"), plays (e.g., Shakespeare's "The Taming of the Shrew"), to myriad love poems written throughout the ages, mostly by men about women, but also written by women (e.g., the "nine earthly muses"

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of Ancient Greece, notably Sappho and Praxilla who wrote erotic, comic, and tragic poetry about the sexual desires of women and men). Most ancient polytheistic cultures had women depicted as goddesses of sex and fertility. The Aztecs had eight deities—seven of which were women—who controlled the intricacies of sex and reproduction. However, goddesses of love and carnality were associated with freedom, war, or mental illness. An example of the latter was the Moroccan lust goddess, Qandisa, who seduced men and then drove them insane.

Erotic art has existed for a very long time, starting with cave drawings of copulation (often between animals, but also heterosexual and homosexual copulation between adult humans). Fertility statues also exist, dating back well over 10,000 years, and almost always depicting a full-bodied, reproductively capable woman (e.g., the Venus of Willendorf). Erotic art from India, Japan, Greece, and Rome during ancient times depicts a variety of imaginable sex acts between two or more humans, humans and animals, and even humans and inanimate objects. Women were depicted masturbating and initiating sex. This did not change in Europe during the Middle Ages, Reformation, or Enlightenment periods, as Néret's[4] *Erotica* series depicts. Women are drawn by many artists with smiles and in full control of sexual interaction, again often initiating sex and in positions (e.g., female supine) that would maximize clitoral and inner vaginal stimulation. Women's orgasm was thought to be positively related to fertility; thus, hints of masturbation were found in artistic works throughout these periods. For example, Titian's "Venus of Urbino" of 1538 depicts her reclining sensually with her hand draped over her mons, her fingers easily in position to stimulate her clitoris. Ēdouard Manet's "Olympia" of 1863 has a similar depiction, this time with her hand outstretched over her pubis, but her forefinger hidden seductively between her legs. There is also a large portrayal of women in fetish circumstances during the eighteenth century, often controlling the man in the action. This is in stark contrast to the portrayal of women in a large part of nineteenth and twentieth century pre-Internet erotica as being either passive exposers of their genitalia or passive and emotionless recipients of male penetration.[5]

As science and rationalism took hold in Europe and the Americas, so did attempts at understanding behavior based on deductions about underlying physical causes. For female sexual behavior, this began with the nineteenth century phrenologists who attempted to understand the nature of female sex drive as a function of the size of bumps on the skull that they believed reflected the size of the underlying brain structure. Among these was the so-called amativeness center at the back base of the skull, just over the cerebellum, that allegedly controlled sexual and parental instincts in women.[6]

Early ethologists began to categorize reproductive behavior in the late 1800s. Notable among these descriptions was that of Darwin,[7] who discussed sexual selection in terms of flexible *female* choice for male epigamic and/or behavioral traits of strength. At the same time, physicians in France and the US began to define "hysteria" in women as a long-term complication of sexual frustration that should be treated with manual clitoral and/or vaginal stimulation. In the 1800s, "muscle beaters" used for massage were applied to the clitoris in the treatment of "hysteria". Some of these had long handles that women would use on their own to provide clitoral and vaginal stimulation. In the late 1800s, vibratory stimulation of the clitoris and/or vagina was seen as particularly effective in inducing orgasm, and was easily produced electrically using saline electrodes with faradaic current applied.[8] Thus the "vibrator" was used in clinical medicine explicitly as a means of "electrotherapy" for the treatment of "hysteria". Vibrators were sold by mail order (e.g., from successive catalogs of Sears, Roebuck and Co.) up to the start of World War II as "Aides that Every Woman Appreciates". Although these seemed to disappear in the 1940s and 1950s, they returned in the 1960s specifically as "sex toys" for women. A fascinating account of the development of the vibrator can be found in Maines.[8]

The ovaries had been known since the time of Aristotle to be involved in both the generation of offspring and in female sexual behavior (for a historical overview, see Ref. 9). Despite Aristotle's writings, it was not until 1672 that de Graaf explicitly described the ovarian follicle as an "egg" which turned into a corpora lutea when the female was impregnated. Van Leeuwenhoek in 1683 suggested that it was the egg itself that was impregnated with sperm. In the nineteenth Century, ovarian function was again the focus of scrutiny. The French physician Roberts reported that Indian women who underwent forcible OVX as prepubertal girls had no sex drive, no menstruation, and had retained a boyish appearance (meaning no breasts). Although Berthold in 1849 had suggested the existence of a floating substance secreted by the testes that masculinized body and behavior in roosters, nothing was mentioned about the ovaries until Brown-Séquard's claim in 1890 that multiple injections of guinea pig and rabbit ovarian extracts could refeminize and excite the passions of OVX, hysterectomized women.[1] Berthold's experiment was repeated in 1896 by Knauer, but this time grafting ovaries into the abdominal cavity of OVX dogs, rabbits, and guinea pigs, and restoring estrous cyclicity and sexual behavior.

The early twentieth century saw experiments aimed at discerning the function of the corpora lutea in the timing of ovulation and the maintenance of pregnancy. Heape in 1900 coined the terms estrus, proestrus, diestrus, metestrus, and anestrus to describe cytological changes in vaginal epithelium, which were used subsequently in 1922 by

Long and Evans to link the stage of the vaginal epithelial cycle with sexual ("estrous") behavior. In 1923, Allen and Doisey determined that vaginal cell cornification could be used as a bioassay to determine which of the ovarian secretions induced it. Parkes and Bellerby in 1926 referred to the active secretion, "oestrin", as the cause of cornification, and in 1930 abundant sources of oestrin had been found in the late pregnancy urine of Canadian women and sold by Ayerst Labs as an orally active source of the hormone. In 1929, Butenandt and Doisey determined the crystalline structure of estrogens, and in particular estradiol-17β, which was the same in the late-pregnancy urine of cows, sows, horses, and humans. Progesterone was isolated in 1934 and was found to inhibit pregnancy when injected alone to a variety of gonadally intact, ovulating animals. Work by Zuckerman in 1937 showed that menstruation was the result of atrophy of the corpora lutea. These findings together led to the creation of steroid biochemistry, and ultimately the isolation of different estrogens and progestins, and the formation of oral contraceptives in 1953 by Pincus and Chang.

The other important endocrine question was behavioral. In 1939, Boling and Blandau found that sequential injections of estradiol followed 48 h later by progesterone induced sexual "heat" (lordosis) in OVX female rats. This pivotal paper coincided with experiments throughout the 1930s–1950s by Stone, Ball, Beach, Larsson, Yerkes, and Young, among others, examining the sexual behavior of female rats, cats, and nonhuman primates, such as macaques and chimpanzees, and in particular its expression around the time of ovulation and how it declined after OVX, and was stimulated. An important comparative approach was taken to this, and careful analyses were made comparing the expression of hormone-driven sexual behavior in animals to humans (e.g., Ref. 10). Since the 1930s, oral estrogens derived first from urine, and then made synthetically, were used to treat menopausal symptoms such as hot flashes. With the advent of the birth control pill and its subsequent reformulations, hormone replacement therapy was born. In the late 1960s, binding sites for estradiol were found in the brain independently by Pfaff, Sar, and Stumpf, and localized largely in the mediobasal forebrain, notably in regions of the hypothalamus and limbic system. The molecular actions of those receptors were characterized in the 1980s and 1990s by McEwen, Pfaff, and others, and their molecular role in the generation of neurotransmitter actions was elucidated.

Drugs of abuse, such as heroin and cocaine, had been known since the 1920s to alter reproductive function in both women and men and to inhibit sexual arousal and desire, along with anorgasmia, in female addicts.[11] The effects of alcohol on sexual arousal, desire, and reproductive function were legendary.[12,13] The study of drug effects on sexual behavior began in the 1950s with the work of the Soulairacs in France. The role of the newly discovered monoamines, dopamine (DA) and serotonin, was examined on lordosis in female rats using systemic pharmacological treatments by Swedish pharmacologists Meyersson, Ahlenius, Södersten, and Malmnäs starting in the mid-1960s through the 1970s. The discovery of brain-born neuropeptides and their receptors in the 1970s and 1980s increased the complexity of the pharmacological targets, and individual brain regions, especially in the hypothalamus, began to be examined. Large brain lesion studies in the 1940s and 1950s showed that decorticate male rats could not copulate, but that OVX females receiving estradiol and progesterone could still display lordosis.[14] More specific brain lesion studies conducted largely in rats during the 1960s showed that ablation of the medial preoptic area (mPOA) reduced male mounting behavior[15] and lesions that included the ventromedial hypothalamus (VMH) reduced lordosis in female rats.[16,17]

The analysis of female sexual behavior in different species became more sophisticated during the 1980s and 1990s (see below), as did the pharmacological and molecular tools applied to its study. Transgenic mouse models were made with specific genes deleted (knockouts (KOs)) or overexpressed (knock-ins); antisense technology allowed researchers to KO specific gene products in different brain regions. Microdialysis and voltammetry allowed certain monoamines, such as DA and serotonin (5-HT), and small molecule neurotransmitters such as acetylcholine (Ach), glutamate, and GABA, to be analyzed directly in brain regions. Cellular techniques allowed cytoplasmic proteins or their mRNA to be labeled in brain slices, and the use of immediate-early gene products such as Fos allowed cell-level localization of activated neurons following copulatory stimulation.[18] Similar advances were made in human brain imaging using functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) scanning. These techniques allowed clinical researchers to examine brain activation by erotic stimuli and orgasm in women (see below).

Sophistication also grew in behavioral analyses of sexual function and dysfunction in women, although not without heated controversy. In 1948, Kinsey and colleagues published *Sexual Behavior in the Human Male*,[19] which was greeted with a sense of accomplishment and intrigue in the postwar West. Five years later, their publication of *Sexual Behavior in the Human Female*[20] was greeted with scandal and denouncement. Nevertheless, Kinsey's work, along with the advent of *Playboy* as a mainstream publication, and the reaction a decade later to *Human Sexual Response* by Masters and Johnson,[21] gave rise to a "sexual revolution" during the 1960s and 1970s that had women question traditional sex roles and traditional sexual behavior, especially the role of the clitoris as *the* gland of women's orgasm and sexual pleasure, rather than the "mature" vaginal orgasm that Freud[22] had thrust

into psychoanalytical therapy and psychiatric medicine. Hite[23,24] wrote two reports on male and female sexuality in the sexually liberated 1970s, which saw changing female sexual attitudes. However, she still noted sex differences in which women were viewed as more "sensate focused" and needing more than visual erotic stimuli to arouse their sexual interest. Such sex differences in response to erotic visual stimuli, however, appear to have waned with the advent of the Internet, and with a new generation of women who have had easy access to such stimuli.

Sexual disorders, which had been cataloged by psychiatry in various editions of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) in North America and the *International Classification of Diseases* (ICD) in Europe, eliminated homosexuality as a disorder in the mid-1970s and reworked sexual behavior disorders in women as those of sexual arousal, desire, and orgasm, with sexual pain disorders, such as dyspareunia and vaginismus, and gender identity disorders as separate disorders. Work around the turn of the millienium sought to characterize those disorders as sets of symptoms and apply both physiological and subjective assessment techniques that could predict symptom severity. As with the Kinsey experience a generation before, the advent of oral treatments for erectile dysfunction in men was hailed in light of a new sophisticated neuropharmacology of sex, whereas the search for a "female Viagra" has been vilified by special-interest groups who are against the so-called "medicalization of women's bodies" by the pharmaceutical industry.[25,26] Although androgens were reported to increase sexual arousal and desire in both pre- and postmenopausal women with those disorders, a transdermal patch was rejected by the U.S. Food and Drug Administration. Despite this, more compounds entered clinical trials for the treatment of sexual arousal and desire disorders in the early twenty-first century. Part of that effort was aided by preclinical research in rodents and nonhuman primates, showing conclusively that drugs that elevated female solicitations in rats also increased measures of subjective sexual desire in women. The study of the neuroanatomy and neurochemistry of sexual behavior has become another "sexual revolution" of neurobiologically-relevant information about sex—one that moves almost seamlessly between species. This begins to realize the challenge put forth by Beach[27] to examine the physiology of behavior across different species and the dream of a truly translational clinical science.

COMMONALITIES, HOMOLOGIES, ANALOGIES, AND MODELS

Sexual function is required for the propagation of all mammalian species. It not only allows for reproduction, but it also provides a natural reward that is easily accessible to any sexually mature individual in good health. Its benefits can be short-term by instilling feelings of satiety, intimacy, and well-being, while long-term benefits may include the memory of the sexually satisfying event, bonding with the partner, in addition to the pregnancy and the delivery of offspring. Sexual dysfunction, in contrast, results in feelings of inadequacy and a loss of feelings of intimacy and well-being, which induces significant distress on individuals and their relationships. The etiology of the dysfunction can be due to physiological, psychological, or a mixture of factors. As such, sexual function in humans involves a complex integration of the individual's biology and physiology (including genetics, hormonal states, and neurochemical regulation), personal history, expectations, context, and culture—all of which impinge on the brain to create or inhibit a sexual response.

In all species, sexual behavior is directed by a complex interplay between steroid hormone actions in the brain that give rise to sexual arousability, and experience with sexual reward or pleasure that gives rise to expectations of competent sexual activity, including sexual arousal, desire, and elements of copulatory performance.[28] Sexual experience allows animals to form instrumental and Pavlovian associations that predict sexual outcomes and thereby direct the strength of sexual responding. Although the study of animal sexual behavior by neuroendocrinologists has traditionally been concerned with mechanisms of copulatory responding related to reproduction (e.g., lordosis in females and erections, mounts, intromissions, and ejaculations in males), more recent use of conditioning and preference paradigms, and a focus on environmental circumstances and experience, has revealed sexual behaviors in a variety of species that are driven by reward-related mechanisms in the brain and that are analogous or homologous to human sexual desire.[28–31] From both a biological and psychological perspective, this makes logical sense: animals must be able to respond to hormonal and neurochemical changes that signal their own sexual arousal and desire, and be able to interact with external sexual incentives. Animals must be able to identify external stimuli that predict where potential sex partners can be found and subsequently seek them out, solicit, court, or otherwise work to obtain them; distinguish sensory cues and behavioral patterns of potential partners from those that are not interested or receptive; and pursue desired sex partners once sexual contact has been made.

The Sexual Brain

The brain organizes sexual stimulation into an evolutionarily conserved set of pathways or "modules" (e.g., Ref. 32; see below for lordosis) that reflect different levels of processing and interpretation[33] (Figure 50.1).

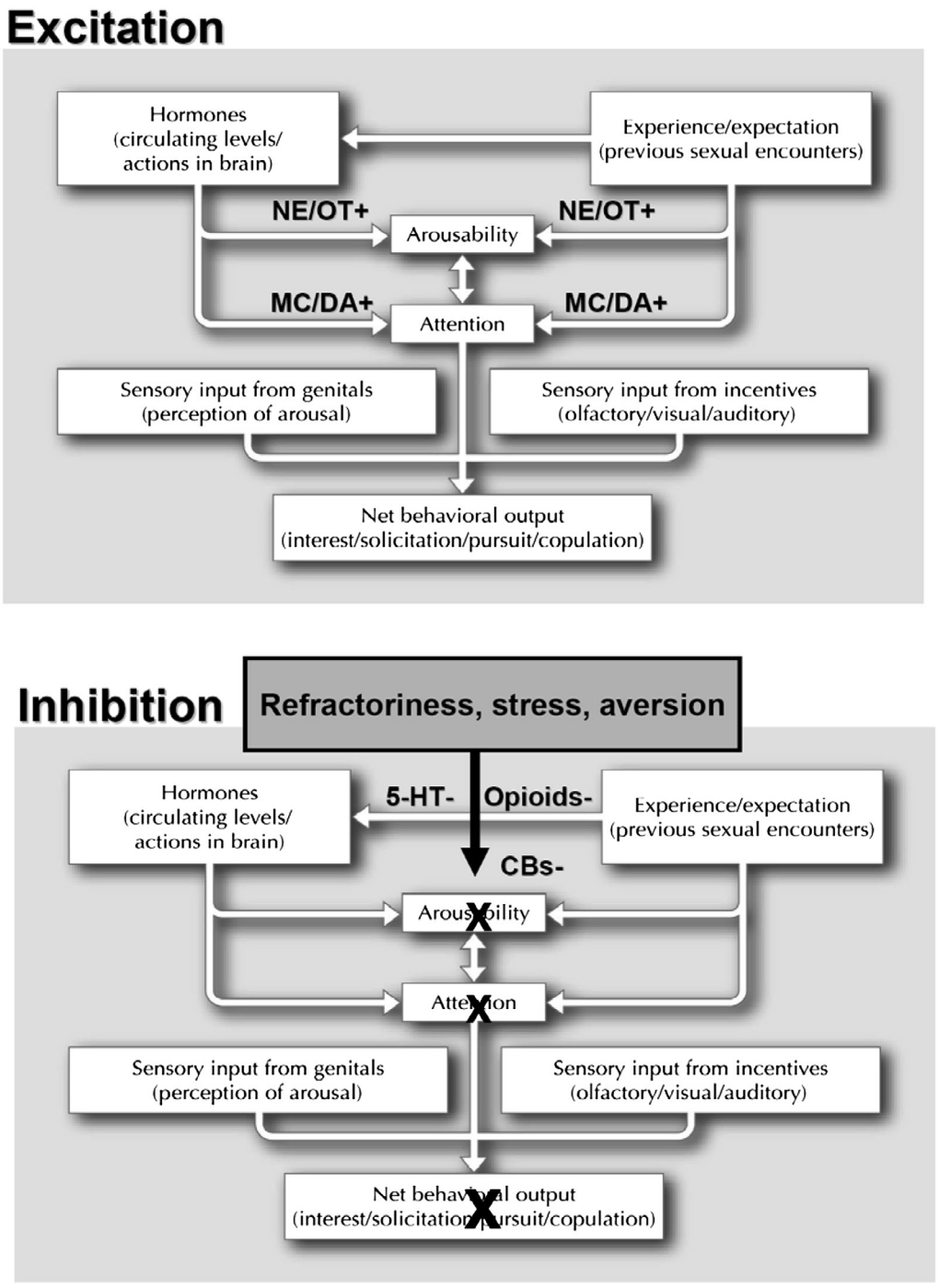

FIGURE 50.1 Interactive model depicting the regulation of sexual behavior by hormonal and associative learning (experiential) systems that subserve sexual arousal, desire, and behavior. Top: Excitation produced by hormone action and/or experientially derived activation of arousability (via activation of norepinephrine (NE) and oxytocin (OT)) and attention (via the activation of melanocortin (MC) and dopamine (DA)) that mixes with peripheral arousal and sexual stimuli to drive net behavioral output. Bottom: Inhibition from refractory states, stress, or aversion. Inhibitory systems activate serotonin (5-HT), opioids, and endocannabinoids (CBs) to induce satiety, pleasure, and sedation, respectively, although such systems are activated in stressful or aversive circumstances. *Source: Adapted from Pfaus and Scepkowski.*[33]

These pathways integrate endogenous sex "drive" (e.g., gonadal hormone status and energy metabolism) with autonomic arousal in the hypothalamus, the intensity of incentive sexual stimuli (unconditioned and conditioned stimuli that activate or "prime" attention and movement from distal to proximal to interactive) in the hypothalamus and limbic system, and the evaluation of sexual context and executive function as it relates to sexual excitation or inhibition overall in the cortex. In particular, cortical activation controls the coding of information

into "gestalts" (e.g., sets of physical or interpersonal characteristics that individuals find conditionally attractive or unattractive, contexts that are suitable or unsuitable for sexual activity, etc., following from Pavlov[34]) and involves the activation of medial prefrontal cortex (mPFC) and the descending inhibition of motor acts as part of executive function. Within each are excitatory and inhibitory neurochemical systems that control sexual responding at any given time. These systems are activated or suppressed by steroid hormones, as well as by experience-driven changes in gene expression and neurochemical function.[35] It is through these systems that priming stimuli or drugs alter sexual responding by changing the interpretation of stimuli and context.

Attentional and emotional components are encoded largely in limbic structures, notably in the nucleus accumbens (NAc), septum, and amygdala, which allow the animal to focus on pleasure- (or punishment-) related stimuli in the environment. The hippocampus provides spatial maps of the external world and episodic memory for important sexual encounters, and the paleocortex (e.g., anterior cingulate gyrus) regulates autonomic function along with anticipation of reward, decision-making, and empathy.[36,37] Along with limbic activation, hypothalamic structures, notably the mPOA and VMH, activate sexual responding in relation to hormonal status and metabolism, and in concert with regions, such as the paraventricular nucleus (PVN) and supraoptic nucleus (SON), coordinate autonomic activation with elements of sexual desire (e.g., solicitations, pursuit). Those structures also participate in the generation of partner and mate preferences. The mPOA is well suited as a central processor in the linking of metabolic need, hormonal status, and autonomic outflow, with the stimulation of mesolimbic DA neurons in the ventral tegmental area (VTA). The mesolimbic DA system projects to several important limbic and cortical structures, notably the NAc, corticomedial amygdala, lateral and medial septum, and mPFC, and is critical for all animals' attention to incentive stimuli.[38] Thus, regulatory, attentional, and emotional systems are engaged at the same time following the hormonal stimulation that occurs around ovulation, linking reward-related incentive motivation to reproduction.

Finally, although sexual responses can include thoughts and fantasies (at least in humans), they are reflected in all animals as behavior. Coordinated purposeful behavior comes from the activity of both fine and gross motor acts that are derived from the coordinated activation of motor cortex and the basal ganglia, along with other motor structures in the midbrain and the cerebellum. In addition to coordinating body movements in space and time, these structures crystallize motor memory, a function that is critical for motor habit formation (the phenomenon whereby motor acts at the beginning of behavioral learning are choppy and uncoordinated, but become virtually automated with practice). Although the formation of motor habits in males with extensive sexual experience protects sexual behavior against treatments or situations that might disrupt it, including novel environments, stress, genital anesthesia, brain lesions, and even castration or hypogonadism (reviewed in Pfaus et al.[28]), it is not yet known whether sexual experience provides females with similar protection.

Structure of Female Sexual Behavior

For all animals, sexual behavior occurs as a sequence or "cascade" of behavioral events. Beach[39] recognized the heuristic value of separating sexual behavior into *appetitive* and *consummatory* phases. Essentially, this scheme followed from the work of early twentieth century ethologists and experimental psychologists,[40,41] who defined appetitive (or "preparatory") behaviors as those which bring an animal from distal to proximal and into contact with goal objects or incentives, such as potential sex partners. In contrast, consummatory behaviors are performed once an animal is in direct contact with the incentive (i.e., to "consummate" the goal). Consummatory sexual behaviors tend to be species-specific, sexually differentiated, and stereotyped, whereas appetitive behaviors are more flexible. This also makes sense as survival often depends on behavioral flexibility—on an animal's ability to learn a variety of strategies to obtain goals in different environments or appetitive circumstances.[28,42] As in animals, human sexual desire and subjective sexual arousal fit into an appetitive framework,[28,31,43] whereas the more stereotyped patterns of copulatory behavior fit into a consummatory framework. Perhaps the most well-known description of human sexual response is that of Masters and Johnson's "EPOR" (Excitement-Plateau-Orgasm-Resolution) model[21] (Figure 50.2). This model flows in time as a cascade of behavioral and neurophysiological events, starting with sexual excitement (blood flow to the genitals and other erogenous erectile tissues), then plateau (parasympathetic maintenance of genital blood flow during sexual intercourse), culminating in orgasm (a defining moment of euphoria, ecstasy, and pleasure in which sympathetic systems move blood out of the genitals), followed by resolution (also called a refractory period during which inhibitory systems of the brain are activated to reduce the salience of external and somatosensory sexual stimuli). The EPOR model describes at least three distinct patterns for women that vary in the structure of the plateau, the intensity and number of orgasms, and the temporal offset of arousal during the resolution phase, although it does not differentiate the particular characteristics of the sexual stimuli used to achieve orgasm (e.g., external clitoral

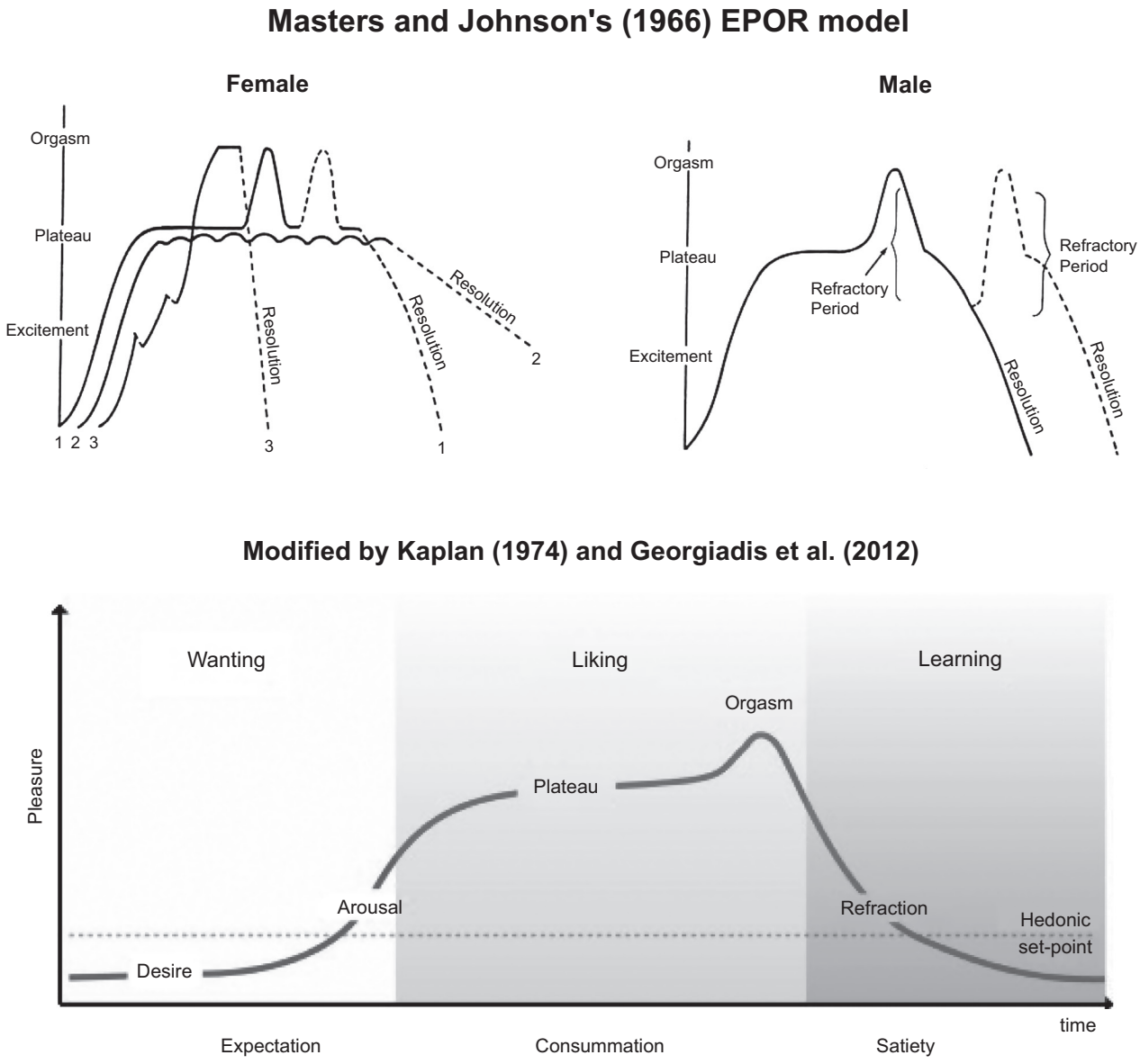

FIGURE 50.2 Top: The EPOR model of human sexual response by Masters and Johnson.[21] Bottom: Modifications of the model by Kaplan[44] to include sexual desire before arousal, and further modifications by Georgiadis et al.[45] to include theoretical phases of expectation, consummation, and satiety, along with the conceptual framework of Berridge et al.[46] of wanting, liking, and learning.

only, external and internal clitoral, cervical, blended clitoral and cervical, extragenital, etc.), nor was it based on an analysis of actual genital blood flow. Subsequently, Kaplan[44] added a phase of sexual desire, consisting of fantasies and thoughts about sexual activity, along with behavior aimed at obtaining sexual partners and/or sexual gratification.

Despite overarching theoretical models of human and animal sexual response that did not posit sex differences in the basic response structure, female sexual behavior has, until fairly recently, been considered "passive". This is due in part to a general social construction in Western society of female sexuality as something that is "done to", relative to more active male sexuality that "performs", and to the labeling of female sexual behavior in both animals and humans as "receptive", consisting largely in animals of estrogen- and progestin-dependent behaviors that allow females to accept male initiation (e.g., mounts) and be open to vaginal penetration by engaging in postural changes such as lordosis, the characteristic arching of the back that raises the rump to allow penile intromission. Similarly, in humans, hormone- and context-dependent "responsive desire" has been viewed as allowing females to be responsive to a partner's active pursuits.[47] However, it is clear that women and some other primate females can have sexual intercourse anytime during the ovulatory cycle. This can even occur without hormone priming in hypogonadal individuals and, indeed, without

prior desire or consent.[48] Although sexually receptive behaviors clearly exist in females of all species, they are far from passive when it comes to sex. Based on observations of a variety of species, Beach[39] proposed that female-initiated sexual behaviors can be partitioned into a cascade of essentially three temporal phases: *attractivity* (behaviors such as approach or scent marking that lure males to the females), *proceptivity* (behaviors that precede receptive behaviors and focus the male on pursuing the female), and *receptivity* (behaviors such as lordosis and lateral tail deflection in rats and hamsters, respectively, or leg spreading in the human) that allows the male to gain vaginal penetration. More recently, Basson[47] (Figure 50.3) described how innate sexual desire (potentially induced at ovulation, for example by the combined action of estrogens and androgens in the hypothalamus and limbic system) activates attention to incentive sexual stimuli, sexual arousal, and sexual receptive behaviors, that, if positively reinforced, lead to a sensitization of attention and sexual arousal in the presence of salient and competent incentive sexual cues. Her model is easily applicable to all species and is similar to incentive models for sexual motivation produced by others.[2,43] Inherent in all models of sexual behavior is the notion that the components are separable. This would require different brain regions or networks to control the components, feedback systems that link them together, and molecular mechanisms that allow their activation to be altered by steroid hormones and experience.

Clearly, females and males engage in mutual and complementary patterns of sexual activity; however, it is the females that initiate and control successful sexual interaction, including the initiation and temporal patterning of copulation. This occurs by a complex interaction of *appetitive precopulatory behaviors* that attract and solicit sex from males. These behaviors are taken to reflect sexual desire and may well be informed by or sum with sexual arousal. Once copulation begins, females engage in *receptive behaviors* such as lordosis, *pacing behaviors* that control the rate of sexual stimulation received during sexual interaction and copulation, and *defensive behaviors* used either to pace the copulatory contact if females cannot otherwise do so or to terminate the sexual interaction.[2,49,50] These behaviors serve to optimize the rate and strength of sexual stimulation received by females, which in turn initiates neuroendocrine reflexes associated with fertility and pregnancy.[51–53]

Ovarian Hormones Set the Stage

The cyclic actions of estradiol, testosterone, and progesterone in females leads to changes in sexual responding and increases in sexual arousal and desire around the time of ovulation in all vertebrate species, including humans,[54,55] although a smaller increase in arousal and desire has been reported around the time of menstruation.[56] Of the major steroids released from the ovaries of mammalian females, estradiol and testosterone are at their highest level in the circulation around the time of ovulation (Figure 50.4). Progesterone levels rise before, during, and after ovulation, depending on the species. This hormonal milieu during the periovulatory follicular phase alters the way in which visual sexual stimuli are processed in women,[58–61] which presumably leads to a shift in the incentive value of the stimuli. Analogous findings have been reported in other primates, for example, in approaches and solicitations made around the time of the mid-cycle estradiol peak in rhesus macaques,[57] and in the appetitive and consummatory sexual behaviors that characterize the periovulatory period of female rats.[31,32,39,49,50,62] Steroid hormones drive sexual arousal

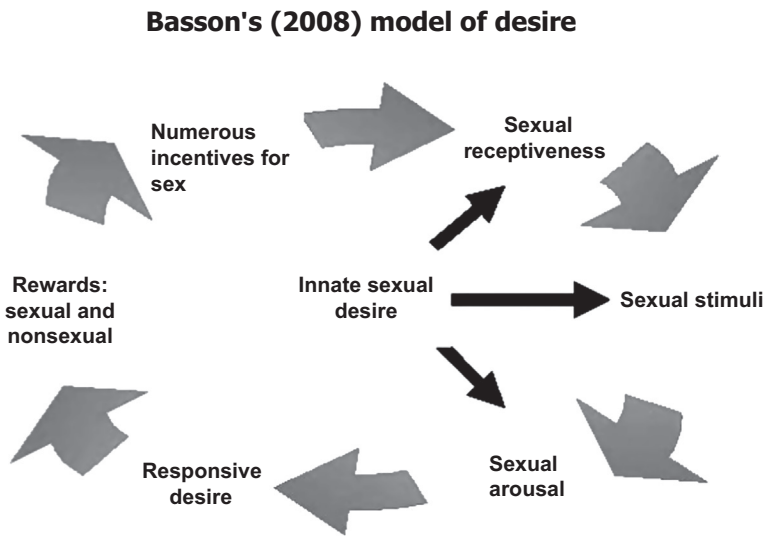

FIGURE 50.3 Circular model of female sexual response by Basson.[47]

and desire in response to competent incentive stimuli. In turn, experience with sexual reward (and inhibition) modulates the strength and trajectory of responses to incentive sexual cues. This is timed in most female mammals to the period around ovulation, thus stimulating females to engage in the most rewarding behaviors under the most reproductively relevant circumstances (see below). This contrasts with the relatively stable and continuous testicular androgen secretion in mammalian males (and its aromatization to neural estradiol in different regions of the brain) that maintains sexual arousability and responsiveness in a relatively continuous manner[63] (see Chapter 49).

Animal Models of Female Sexual Behavior

Although human sexual behavior is best studied in humans, it is often impossible to do so with experimental precision or at a level that allows any degree of finely-grained neural or molecular analysis. Recent advances in brain imaging and eye-tracking technology have allowed cortical and subcortical activation and visual gaze to be assessed in women viewing erotic visual stimuli, and some important paradigms have emerged to correlate aspects of subjective sexual arousal, desire, and orgasm to overall brain activation patterns.[64] Such data reveal a great deal about the cognitive and limbic control of different aspects of female sexual behavior under different hormonally-modulated, pharmacological, or experiential conditions, and in ways that confirm data from females of other species. Nevertheless, these paradigms lag behind the scope of neuroanatomical, neuropharmacological, histochemical, and molecular methods that can be utilized in situ with animal models.

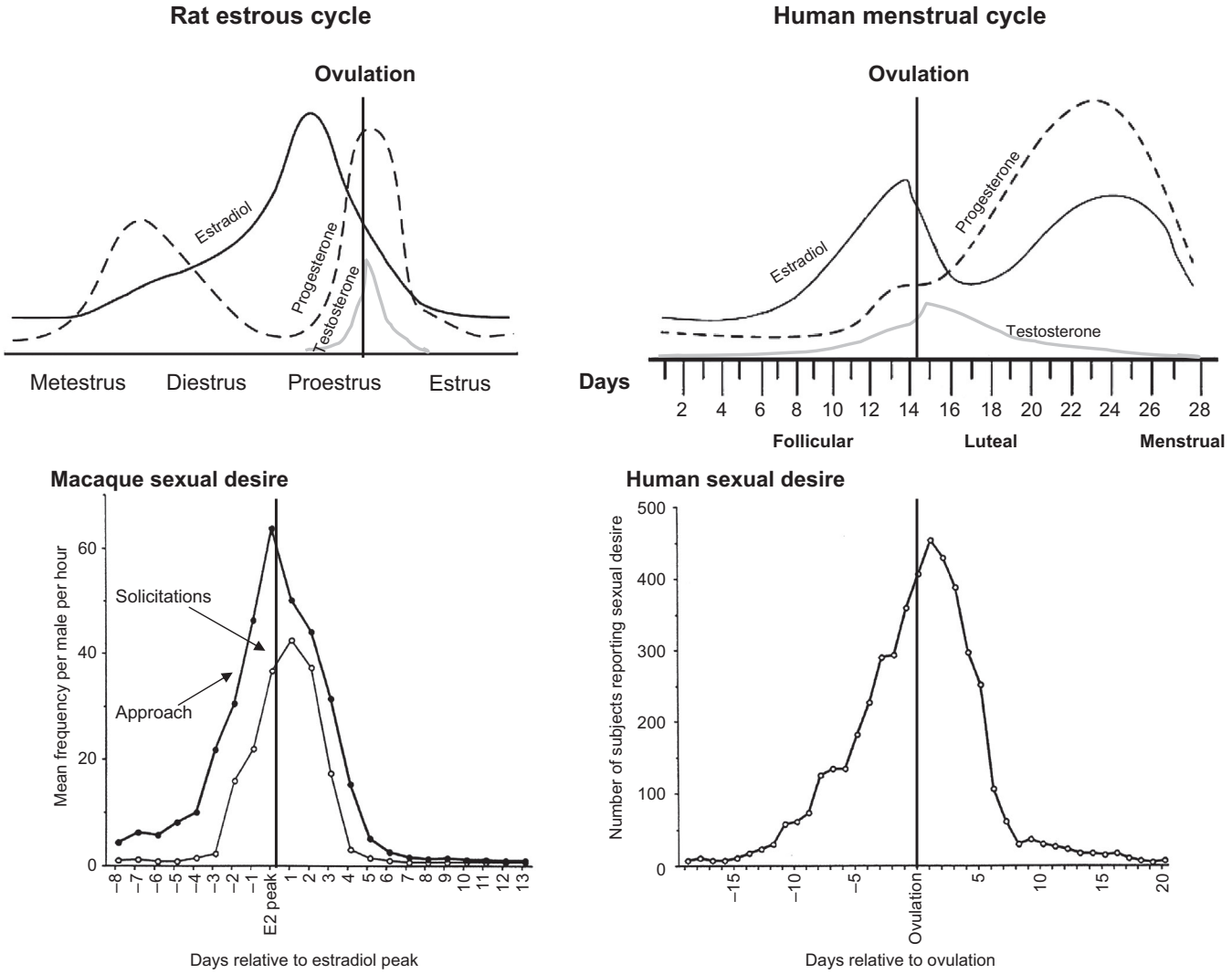

FIGURE 50.4 Top: Schematic changes in estradiol, progesterone, and testosterone in rats and humans across their respective estrous and menstrual cycles. Bottom: Sexual approaches and solicitations by female rhesus macaques, and expressed sexual desire of women, relative to ovulation. *Source: Adapted from Refs 54,57.*

Most of the research done on the neurobiology of female sexual behavior comes from rodent subfamilies, such as rats, mice, hamsters, gerbils, voles, and musk shrews, lagomorphs like rabbits and myomorphs like guinea pigs, less so from primates like rhesus and Japanese macaques, and even less so from humans. Understanding the behavioral structure of each species' appetitive and consummatory phases is vitally important for a sophisticated understanding of the neurobiological mechanisms that control it. In many studies the female's receptive lordosis posture is taken as an index of her copulatory or "mating" behavior, rather than the full repertoire of appetitive and consummatory sexual responses. It is often the case in laboratory settings that females are tested in small chambers that do not allow them to approach or escape the male or, in the case of females that pace the copulatory contact, allow them to regulate the timing and intensity of that contact. When appetitive responses are taken together with lordosis, it becomes immediately apparent that there is extensive conservation of the neurochemical mechanisms that control sexual behavior which generates homologies in the way that sexual stimulation is perceived by the brain and induces competent responses. Behavior is the ultimate arbiter, and can never be supplanted by the processes that underlie it. For example, release of the neurotransmitter DA in mesolimbic terminals like the NAc may be involved in all forms of appetitive motivation toward rewarding incentives like sex partners, but just observing DA released there does not allow the viewer to conclude that whatever the animal was doing is positively hedonic. Understanding the neurobiology of animal sexual behavior also allows the consideration of models of sexual function and dysfunction that are directly applicable in the preclinical testing of drugs or other treatments.

Rats

Female rats are cyclic ovulators whose sexual behavior is timed to the periovulatory period. The ovulatory cycle last approximately 4–5 days, and as mentioned above, is split into phases that reflect the actions of ovarian hormones on vaginal cell morphology and sexual behavior, including diestrus, metestrus, proestrus, and estrus. A rise in estradiol, during metestrus, followed by a peak in progesterone during the afternoon of proestrus, just prior to ovulation, primes the neural circuits necessary for sexual responding. As such, sexual behavior is expressed only during an approximate 12–20 h window, when she is fertile. As mentioned above, sexual behavior can be completely eliminated by bilateral OVX, but reinstated by priming with estradiol and progesterone (P) administered by subcutaneous injections, 48 and 4 h prior to testing, respectively,[65] to mimic ovarian steroid secretion.

A typical copulatory bout in rats begins by anogenital investigation by either the female or the male, followed shortly thereafter by a solicitation made by the female. Solicitations begin with a head-wise orientation toward the male followed by a runaway of varying lengths, some of which are short and in the male's vicinity and are typically referred to as "hops and darts".[30,31,49,50,66–71] This behavior entices the male to chase the female. As the female comes to a stop, she initiates a present posture, facilitating the ability of the male to mount the female when he arrives. Flank stimulation during a mount elicits lordosis, the concave arching of the back that raises the rump and anogenital region, and is absolutely critical for vaginal penetration, or intromission, to occur. Male mounts are almost always accompanied by pelvic thrusts which make direct contact with the female's clitoris. If the male has an erection, then the pelvic thrusts will push his penis into her vagina. Such vaginal intromissions provide the female with stimulation of the vagina, including the internal clitoris and possibly also the cervix. Immediately after a vaginal intromission the male dismounts and grooms his penis into detumescence. The female may run away again or stay in the male's vicinity and hop over him to induce another mount with intromission. In this way, the female regulates or "paces" the rate of vaginal penetration, which controls the rate of vaginal, clitoral, and possibly cervical stimulation. After several mounts with intromission regulated by the female, the male ejaculates. Ejaculation consists of a deep penetration that is held in the vagina as the ejaculate congeals into a "vaginal plug" that surrounds the cervix and protects sperm transport. This also produces a large and sustained amount of cervical stimulation and is responded to by the female with a bursting dismount and runaway. After a few minutes of absolute then relative refractoriness and behavioral quiescence, the female shows increasing interest in the male and stays in his vicinity. This is responded to by the male with the resumption of mounts with intromission and a second ejaculatory series. Males typically have 7–10 ejaculatory series before becoming "sexually exhausted".[72,73] However, long before male exhaustion, females display a progressive reduction in solicitations and enforce increased time between intromissions either by running away or fighting. In the wild, more dominant females tend to reenter the burrow after only a few ejaculations, whereas more subordinate females take more ejaculations before they stop copulating. The progressive decline in solicitations and increase in agonistic behavior is characteristic of "estrus termination", an inhibitory state that signals a period of female refractoriness and the endocrine transition to pseudopregnancy or pregnancy.[70,74,75]

Female and male rats have also been used in studies of sexual reward, and to understand the plasticity of appetitive responses and their neurochemical control under different sexual circumstances, including different contexts, experiences, level of sexual reward, and partner

density.[28,30,76–79] Indeed, rats will copulate in a variety of circumstances and testing chambers, including small cylindrical or square chambers,[80] unilevel pacing chambers,[49,77,78,81–84] bilevel pacing chambers,[70,75,85,86] and open fields[31,50,87] (Figure 50.5).

Mice

There are well over 1100 naturally-occurring species of mice, with a far larger number of transgenic lines made with specific gene deletions or insertions. Those lines have been useful in identifying the role of estrogen, androgen, and progestin receptors using lines with specific KOs of those receptors or their subtypes, e.g., estrogen receptor alpha (ERα) and ER beta (ERβ), or steroid synthesizing enzymes, e.g., aromatase or 5α-reductase. The sexual behavior of wild house mice (*Mus musculus*) was documented by Estep, Lanier, and Dewsbury,[87] and consisted of a period of appetitive courtship that included mutual grooming of the body and genitals, sniffing of the genitals, and rooting (in which one partner lifts up the body of the other with its head). Females were observed to orient away from the male prior to the males initiating mounts. Like hamster females, these wild mice hold lordosis for a long duration of male thrusts with intromission. Upon ejaculation, males typically fall over, pulling the females with them. Females then hold lordosis on their sides with the male's penis still inside the vagina for approximately 20–30 s, after which the male dismounts, and the two groom their own genitals. Unlike the female rat, the female mouse stays in close proximity to the male during his postejaculatory interval. Despite obvious differences in copulatory behavior

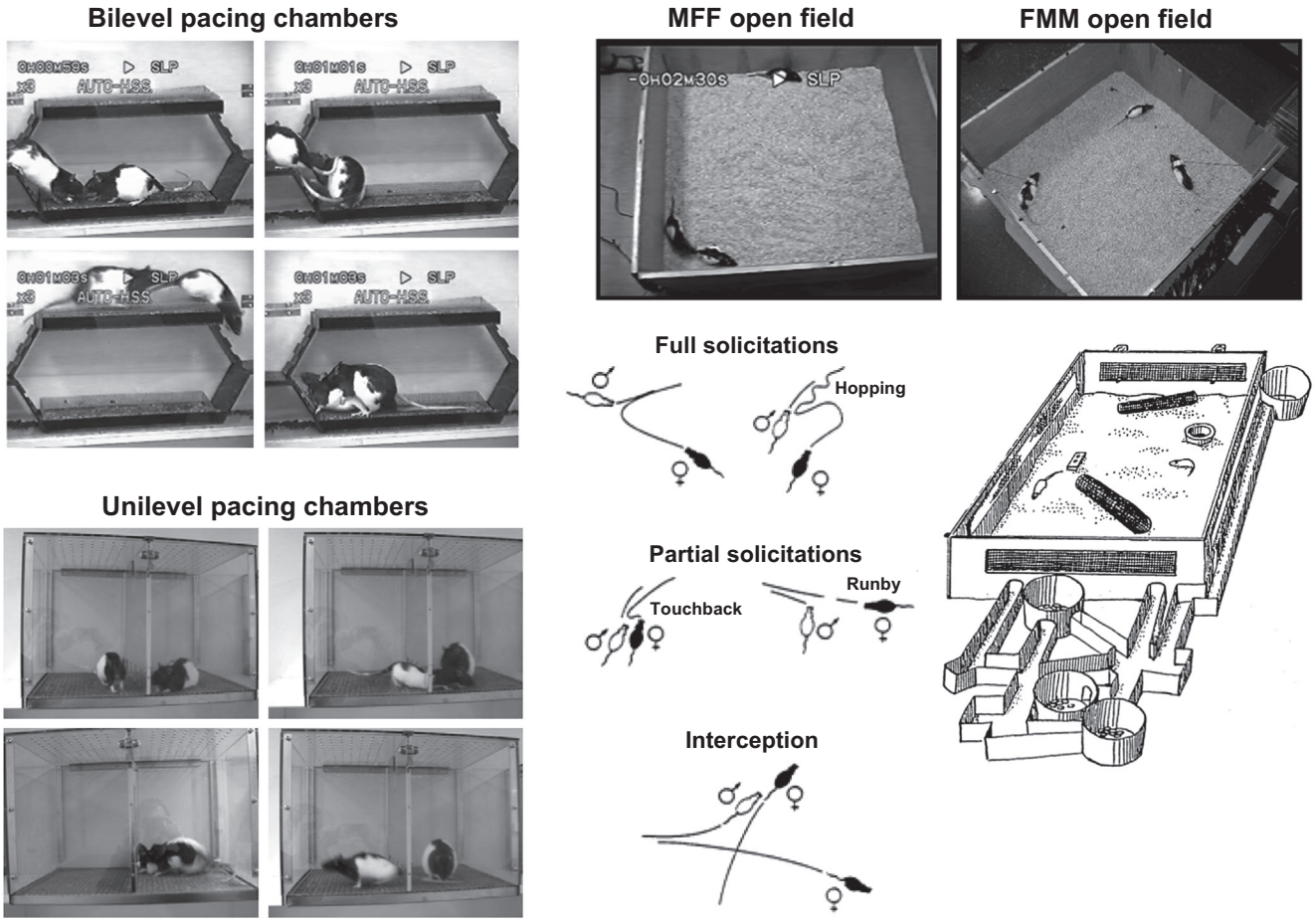

FIGURE 50.5 Rat copulation in several chambers. Top left: Bilevel pacing chambers. Bottom left: Unilevel pacing chambers. Bouts of copulation are typically initiated by the female through solicitations. In bilevel chambers the female solicits as she would in the wild by making a headwise orientation to the male and then either darting or hopping away on the same or other level. This forces the male to chase her and females regulate the rate of copulation by running from level to level. The endpoint of each solicitation and runaway is lordosis, allowing the male to mount and gain vaginal intromission, after which the male dismounts and grooms his penis. This is repeated several times until the male ejaculates. Unilevel pacing chambers are bisected by a Plexiglas or mesh partition with one or more holes cut out of the bottom that are big enough to allow the female to pass through but too small for the male. This restricts the male to one side. Females initiate copulatory contact by moving to the male's side, and regulate the rate of contact by running from side to side. Top right: Open fields used for tests of conditioned sexual partner preference for one male choosing among two free-ranging females (MFF) or for one female choosing between two tethered males (FMM). Bottom right: Large open field used by McClintock[50] to examine group mating patterns and preferences in female rats.

between mice and rats, nearly identical pacing behavior was observed recently in female mice.[88] Females placed into a smaller version of a unilevel pacing chamber bisected by Plexiglas dividers with four holes that only the females can pass through displayed exits and returns from the male's side after bouts of intromissions. This indicates that mice pace the copulatory contact like rats do when given the opportunity. It is not yet known whether female mice find sexual stimulation in paced conditions rewarding.

Guinea Pigs

Guinea pigs were used extensively to understand the role of ovarian hormones in the elicitation of lordosis, and also to understand the functions of progesterone. Both females and males engage in a courtship dance prior to copulation in which the female approaches the male swinging her hips back and forth and making a vibrating sound often referred to as "purring". Males respond in kind, and will often sniff and lick the anogenital region of the female, who does the same. Males have been observed to purr and sway their hips at nonreceptive females, but if the females do not respond sexually, then copulation is not attempted by the male.[89] This is similar to sexually experienced male rats that investigate sexually nonreceptive females but do not attempt copulation.[90] Females in heat express lordosis in response to male flank palpation during mounting. Like rats, males have a multiple intromission pattern prior to ejaculation and, as in rat pacing behavior, females run away from the males between intromissions causing the males to chase them. As the period of estrus terminates, females display more and more aggressive rejection responses to male pursuits and mounts.

Hamsters and Gerbils

The copulatory behavior of these subfamilies have been studied in less detail than that of rats, and mostly for effects of hormones, drugs, or brain region lesions on lordosis,[91] but also to examine social conditions like group size on sexual receptivity,[92] and sexual recognition.[93] Like rats, hamsters become sexually receptive every 4 days during their ovulatory phase, but are much more consistent than rats in their estrous cycles. Gerbils are sexually receptive approximately every 6 days. Female hamsters and gerbils both display appetitive responses that attract and solicit males to chase them. Female gerbils, for example, display a rhythmic "thumping" of the hindlegs that attracts males to them (males display similar behaviors with other males, although typically in response to a threat). Females of both species also display lordosis upon somatosensory stimulation of the flanks and perineum by males. Although both species are seasonal breeders in the wild, sexual behavior can be induced at regular intervals by injections of estradiol and progesterone, as in the rat. True receptivity to intromissions in the hamster, however, is not assessed by lordosis alone. Female hamsters display the lordosis posture and remain in it while the male mounts, intromits, and dismounts several times. To allow vaginal penetration, the females' tails must deflect laterally or dorsally, making "tail displacements" the true measure of receptivity to vaginal penetration.[94] In addition, female hamsters do not run away from males, or if they do it is for a very short distance and still while in a semi-lordosis posture, so there is little chasing behavior. Males simply mount and intromit repeatedly until ejaculation. Like rats, female hamsters will attack males that attempt to mount them when they are not sexually receptive, when the period of sexual receptivity is terminating, and when they are OVX and primed with low doses of estradiol, with or without progesterone. Female gerbils engage in appetitive thumping, causing males to thump back, as the rhythmic leg thumps are a form of social communication. This typically occurs prior to the female presenting to the male, which is followed by the male mounting. Estrus termination in both species is induced by vaginocervical stimulation (VCS) and accompanied by an increase in female fighting if males persist in attempting to mount. It is more typical, however, for females to take themselves out of the situation by moving to a different space or into a burrow system.

Voles

Voles have been studied largely to understand the neurochemical and genetic basis of "monogamous" vs "promiscuous" sexual partner and mate preferences. Both female and male prairie voles show social and sexual partner preferences for their first sexual partner, relative to an unfamiliar partner, and they form relatively stable pair bonds for the nurturing of their pups. In contrast, closely related meadow voles do not display such preferences. However, prairie voles are not sexually exclusive and will copulate with other partners under certain circumstances. Differences in mating strategy have been linked to the differential actions of oxytocin (OT) in females and vasopressin in males,[95] in particular in males to the greater expression of the vasopressin 1a (V1a) receptor in the ventral pallidum, a motor structure that receives input from the NAc. DA is also important in driving the partner preference in the presence of familiar—and presumably olfactory—incentive cues.[96]

Female voles make precopulatory scent marks when they are sexually receptive, and they will spend more time near male scent marks. Females also approach males and both engage in side-by-side contact, sometimes referred to as cuddling. Males mount females who display lordosis, and although males appear to set the pace, females copulate with tethered males, suggesting that females can initiate copulation. Vole mating in the wild takes place over

several days, during which males ejaculate several times with either one (monogamous) or several (polygamous) females. Like rats, male voles display a multiple intromission pattern prior to ejaculation, suggesting that females receive clitoral stimulation (CLS) and VCS at a particular rate. It is not known which stimuli are responsible for terminating sexual receptivity in the vole.

Musk Shrews

Musk shrews are a primitive eutherian mammal. Female shrews are induced or reflex ovulators that require behavioral stimulation to ovulate and induce increases in circulating estradiol and progesterone. The torturous process of a male "taming" a female shrew into sexual receptivity has been known for a very long time. Indeed, Shakespeare's 1592 play of the same name is a comic analogy about human heterosexual marriage. Rissman and colleagues studied the copulatory behavior of musk shrews extensively in order to make comparative analyses of hormone-brain interactions. Their work established clearly that female musk shrews display a period of intense appetitive aggression toward males which includes biting and scratching, and if the females live in large natal groups, the aggression against potential suitor males sometimes results in death.[97] Virgin females attack males more frequently than sexually experienced females, and exposure to male olfactory cues reduce aggression in virgin females.[98] The most persistent male will eventually gain access to her flanks and mount, which induces tail-wagging, a first sign of sexual interest, followed by a lordosis posture thus rendering her sexually receptive to his intromissions. Testosterone produced by the ovaries and adrenal glands is the most abundant circulating steroid at the time the males begin their approach,[99] and may explain the increased female aggression during this appetitive period. Indeed, crystalline implants of testosterone in the mPOA or VMH of OVX shrews were more likely to induce a full complement of sexual behaviors compared to implants to the bed nucleus of the stria terminalis (BNST), whereas implants of estradiol in the mPOA or VMH induced lordosis and immobility, and reduced the time taken for females to become receptive. This suggests that aromatization of circulating testosterone into estradiol is necessary for the switching from aggression to sexual receptivity. High levels of cortisol are also observed during the induction of sexual receptivity, and blocking cortisol production reduces sexual receptivity.[100] As with rats, extended periods of sexual receptivity also facilitate the induction of pregnancy.[98] It is not known what type of sexual stimulation may contribute to this.

Rabbits

As with rats and guinea pigs, rabbits have been used to examine a host of female reproductive functions, from hormone and brain lesion effects on sexual behavior and scent marking to sexually dimorphic development of the olfactory system (e.g., Refs 101,102). Female rabbits typically approach males and stay motionless beside them. The two gradually rub noses and nibble at each other's fur. If receptive females are eating when a male approaches, he may rush past them and then gradually get closer, often circling stiff-legged with his rump and tail raised high in the air. If the female ignores the male's advances, he often scent marks her with urine. Once the male mounts and intromits, he typically bites the back of the female's neck. After a few thrusts he ejaculates, and like the rat often falls off the side of the female and remains motionless for a while. As the male approaches exhaustion and the female approaches estrus termination, the two start nibbling more and more at a food source.

Macaques and Other Nonhuman Primates

Rhesus macaques have been used in a variety of ways in sex and neuroendocrine research, notably like rats in the study of hormonal and neurochemical systems underlying appetitive and consummatory sexual behavior, but also to study the social conditions under which very different types of sexual responding are induced. Female rhesus macaques living in large natal groups approach males early in their appetitive phase, several days before their estradiol peak, followed by solicitations of males more closely linked to the estradiol peak.[57,103] Solicitations or "invitations" include characteristic "hand reach", "head-duck", and "head-bob" behaviors in the vicinity of the male.[104] Females also assume a lordosis posture when mounted, and males typically mount with a number of intromissions and pelvic thrusts, sometimes occurring in bouts prior to ejaculation. Females display a characteristic clutching reaction after ejaculation in which the female reaches back and clutches the face of the male.[105] These behaviors cease soon after copulation terminates, as plasma estradiol levels decrease. Like rats and dogs, female rhesus macaques also mount sexually inactive or naïve males as a supersolicitational behavior to induce the males to mount back.[106] In contrast to females living in large groups, single female rhesus macaques living in dyadic conditions with one male show a far greater propensity to submit to mounts on the part of the male throughout the ovulatory cycle, although there is an increase in successful mounts by the male around the time of the estradiol peak.[103] Thus, a dyadic context in which the female is always in the vicinity of the male and cannot escape stimulates more mounting behavior on the part of the male, which is reacted to by consummatory sexual behavior on the part of the female throughout her ovulatory cycle, although the number of male ejaculations still increases during ovulation. This suggests that contextual

cues and sexual stimulation interact with neuroendocrine systems to stimulate sexual responding in female rhesus macaques that appears to be less tightly-linked to ovulation, a situation generally not found in rats and mice. In laboratory settings, female rhesus macaques can also be trained to bar-press to obtain a male, behavior that increases during their ovulatory phase.[107]

Female Japanese macaques, and to a lesser extent Stump-tail macaques, engage in homosexual mounting of other receptive females, often to the point of displaying ejaculation—like facial grimaces prior to dismounting and a period of rest,[108] suggesting that female–female mounting leads to genital stimulation that induces an ejaculation-like state in the female. Female Japanese macaques form stable homosexual consortships during the breeding season[109–112] that is not due to, nor affected by, the availability of males. Females mount one another repeatedly, and males entering into this consortship engage in intrasexual competition, often trying to fight the dominant (mounting) female to gain access to the other female, who typically rejects him over 90% of the time.[109] Female bonobos also engage in female–female mounting, usually as a method of conflict resolution when males are fighting.[113,114] Notably this behavior can occur at anytime during the ovulatory cycle. Although the patterns of female approach and solicitation are similar in many primate species, the copulatory stance of the Bonobos includes face-to-face, male-on-top "missionary" positions not observed in other primates except humans.

Humans

Like other primates, the sexual behavior, and especially sexual arousal, desire, orgasm, and sexual inhibition of the human female are exquisitely sensitive to context and social learning.[47] However, despite the general view that sexual behavior in women is "freed" from the dependence on steroid hormones, women display a characteristic increase in self-reported sexual desire and arousal during ovulation.[55] Across the ovarian cycle of women, steroid hormone levels fluctuate in a cyclical manner. Circulating levels of estradiol, progesterone, and testosterone rise around the time of ovulation, correlating with an increase in sexual interest, activity and fantasies.[55,115–118] Removal of cyclic steroid hormone release by long-term administration of estrogen-containing oral contraceptives often results in a decline in sexual desire, activity, and genital blood flow.[115,119,120] It is unclear whether the blunting of the cyclical induction of sexual activity and fantasies is directly due to the removal of the cyclicity of the hormones acting on relevant tissues, or whether it is secondary to downstream effects of chronic administration of estrogens. Long-term exposure to estrogens has a number of physiological effects that might disrupt sexual behavior. For example, estrogens upregulate steroid hormone binding globulin (SHBG) production by the liver, which are transport proteins that bind androgens with higher affinity than estrogens.[121,122] A recent study on premenopausal women's steroid hormone levels using liquid chromatography-tandem mass spectrometry revealed that serum testosterone, free testosterone, estradiol, estrone, and SHBG levels peaked at midcycle and remained higher in the mid-luteal phase, whereas the 5-α reduced androgen metabolite dihydrotestosterone (DHT) did not change across the cycle.[123] Chronic use of oral contraceptives containing combined estrogens and progestins lowers free testosterone levels and upregulates SHBGs.[124] Given the importance of free testosterone for sexual desire in women,[125] its reduction by chronic oral contraceptive use may be one reason why some women experience a decrease in sexual desire on the pill.

A decline in sexual desire and activity also occurs following surgical and natural menopause. Surgically menopausal women, induced either by bilateral oophorectomy and hysterectomy, experience a sudden and drastic decline in sexual arousal and desire.[126–129] These symptoms can be restored following adequate hormone replacement regimens, particularly with replacement of estrogens in combination with testosterone.[126,127,130–139] As such, ample evidence suggests that fluctuating ovarian steroid hormone levels are important in normal sexual function in women, as they are in other species.

Sexual desire in women is a matter of great controversy. Although sexual arousal and desire can be defined by subjective reports, only arousal has been defined objectively (as increased genital blood flow). There is not yet an objective measure of desire, thereby forcing it to be inferred from subjective self-report or intuitively observed behavior (e.g., flirtations). Desire appears to occur spontaneously in some women whereas in others it occurs in response to the right male(s) or females(s) making the right verbal and nonverbal gestures in the right contexts. As mentioned above, self-reported desire peaks during ovulation. This makes antecedent hormonal conditions—effects of estradiol, testosterone, and perhaps also progesterone, in the brain—likely motivational variables in its stimulation. Responsive desire, or the ability of the "right" stimuli to activate incentive motivational pathways in the brain and excite attention and behaviors that are indicative of desire, is also activated by steroid hormones. Desire is then expressed both as a spontaneous motivation and an attention toward competent sexual stimuli.[2,43] In both cases, the emergent conscious awareness of sexual desire activates movement from distal to proximal to interactive, like the approach and solicitations of rats and macaques. Humans thus learn a baffling array of appetitive responses that work differently in different cultures and contexts, or differently within a single culture at different epochs, and indeed

differently with different people. And cultures constrain women's responses, and indeed their own knowledge of their own sexuality, to appropriate times and places. The brain must balance these excitatory and inhibitory influences to achieve some kind of optimal level for pleasure. And it must do this with hormonal influences weighing it toward excitation, especially during ovulation, and experience directing attention and behavior toward individuals and stimuli previously associated with sexual reward.

The copulatory patterns of women are also fraught with problems of interpretation. Although more behaviorally stereotyped than appetitive responses, consummatory patterns of copulation in humans are nonetheless extremely variable, even in cultures where certain positions (e.g., missionary) are proscribed.[10,20,140] Some heterosexual positions, e.g., woman on top, can maximize her ability to get optimal external and internal CLS, possibly along with direct stimulation of the cervix, from the male.[141] Other positions may embellish other stimulus zones, and thus engage different motor patterns to maximize the stimulation achieved. And of course, some women are extremely sensitive to external CLS, and can only achieve orgasm in that way, whereas others achieve orgasms with blended internal and external CLS.[142–146] Interestingly, there is no human analogue to the lordosis reflex, although lordosis-like positions can be observed in women being mounted from behind. The arching of the back, however, is not a hormone-induced and/or facilitated spinal reflex; those do not exist in humans, a fact that continues to limit the human clinical application of the neuroendocrine work done on lordosis in animals. Being "receptive" to vaginal penetration in women involves a conscious decision to expose the vulva and open it to penetration. Experience with orgasm or other types of sexual pleasure and intimacy leads to expectancies which also constrain the sexual positions and patterns of both men and women.[145]

ANATOMY AND PHYSIOLOGY OF SEXUAL SENSORY SYSTEMS

The autonomic and peripheral nervous systems work together to send sensory information about genital and erogenous sexual arousal and stimulation to the spinal cord and brain, from which conscious feelings of desire and pleasure are derived (Figure 50.6). Genital (clitoral and cervical, but also involving sensitive regions of the labia and anus), and erogenous (nipples, lips) stimulation typically requires the engorgement of erectile tissues with blood (a parasympathetic activation). This engorgement increases the somatosensory surface area upon which stimulation can induce a response, essentially making the female more sensitive to tactile stimulation. Copulation typically involves more focused and localized genital stimulation that culminates in the buildup of a threshold amount of sympathetic arousal that brings about orgasm (or homologous responses in animals), and immediate sexual pleasure that activates inhibitory mechanisms related to euphoria (e.g., orgasm/reward), satiety and refractoriness.

Autonomic Control

The autonomic nervous system[147,148] consists of three divisions, sympathetic, parasympathetic, and enteric (the latter of which controls the gut). The role of the sympathetic system is to up- or down-regulate homeostatic and cardiovascular mechanisms to prepare the body for action (sometimes referred to as "the stress response" or the "fight or flight response"). Such action can be defined in terms of good stress ("eustress") or bad stress ("distress") depending on the nature of the event.[149] In either case, the sympathetic system initiates immediate pupil dilation (allowing for greater processing of the visual field), increased heart rate and blood pressure, dilation of the bronchioles of the lungs (to increase oxygenation of the blood), constriction of blood vessels, and inhibited digestion. It also is responsible for orgasm once sexual stimulation is underway. The sympathetic system activates the adrenal gland (located just above the kidney) to induce a massive release of adrenaline from the adrenal medulla (which activates and potentiates sympathetic outflow in most organs all at once, except the gonads). Adrenocorticotropic hormone (ACTH) released from the anterior pituitary stimulates the secretion of glucocorticoids such as corticosterone or cortisol from the adrenal cortex (which increases glucose concentrations in the blood, inhibits inflammation that might occur in response to injury, and potentiates arousal in the central nervous system and phenomena like place learning in the hippocampus of the brain's limbic system). The sympathetic nervous system extends from the spinal cord between the thoracic and lumbar divisions, and consists of short preganglionic nerves that contain the neurotransmitter Ach which excites postganglionic neurons, and long postganglionic nerves that contain norepinephrine (which inhibits prolonged muscle contractions). The ganglia collect incoming preganglionic fibers and distribute the postganglionic fibers to the organs in the abdominal and pelvic regions.

In contrast, the parasympathetic nervous system opposes the actions of the sympathetic system at each organ within each division. Its role is to calm the system down after stress, although it can be activated within each division on its own (e.g., as bright sunlight induces constriction of the pupil). The parasympathetic division is literally around ("para") the sympathetic division, and extends long fibers of the cranial nerves III, VII, IX, and

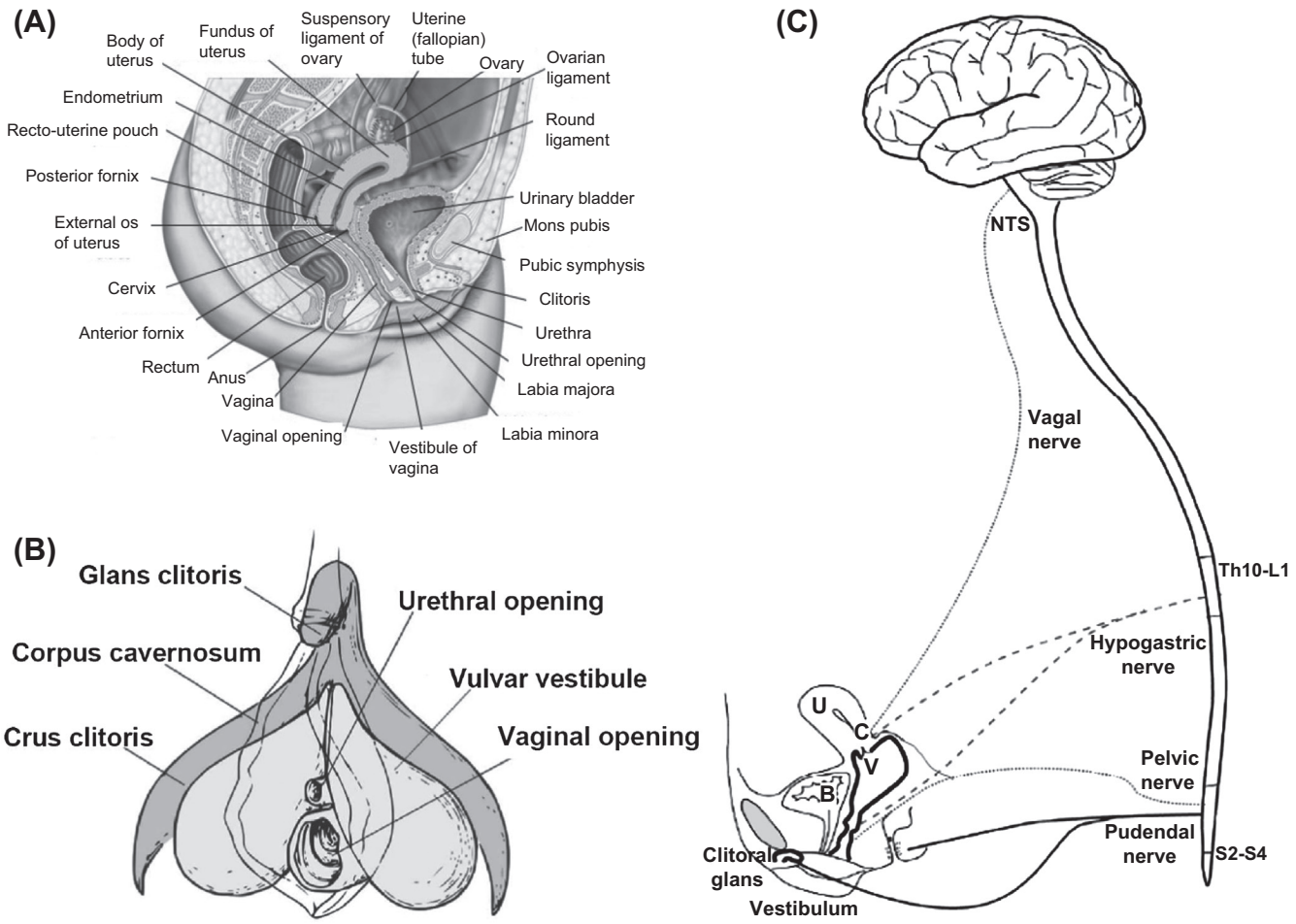

FIGURE 50.6 **Human female genital anatomy and neurophysiology.** (A) Cross-section of the human female genital and pelvic region. (B) The clitoral complex in relation to the urethra, vulva, and vagina. (C) Sensory nerve input to the spinal cord and brain from the genital and pelvic region, including pudendal, pelvic, hypogastric, and vagal nerve innervation. C, cervix; V, vagina; B, bladder; U, uterus.

X (occulomotor, facial, glossopharyngeal, and vagus) to innervate ganglia close to the organs. The postganglionic fibers that innervate the organs are short. Both pre- and postganglionic fibers contain Ach, which excites neurons and contracts smooth muscle. The pre-to-post relationship is more specific (e.g., 1:3) compared to the sympathetic division (which is greater than 1:10). Because the parasympathetic division causes dilation of blood vessels, it is critical for the stimulation of erection in labia, clitoris, and the vaginal epithelium, along with other erogenous erectile tissues (e.g., nipples and lips), and for draining the blood out of erectile tissues after orgasm.

Physiological sexual arousal can be defined as increased autonomic activation that prepares the body for sexual activity. In females this includes activation of the parasympathetic system that keeps blood in genital and erectile tissues, in particular the clitoris, labia, vaginal epithelium, nipples, and lips, and sympathetic blood flow from the heart to striated and smooth muscle that participate in sexual responses (Figure 50.7). Sexual arousal also includes a central component that increases neural "tone" or preparedness to respond to sexual incentives, and forms around an intricate interaction of hormone priming and noradrenergic activity in different regions of the brain. Both peripheral and central arousal may be detected as part of the perception of subjective sexual arousal, and both clearly lead to changes in responsiveness in genital tissues and control certain copulatory responses, such as the latency to orgasm (with shorter latencies indicating an increase in arousal). Both aspects are sensitized by estradiol and testosterone,[150] and are thus more likely to be experienced during the periovulatory period.

Peripheral autonomic blood flow is typically experienced far more readily than internal flow. Thus, women are less likely to be consciously aware of blood flow to the labia and clitoris relative to blood flow to the nipples. The reliance on vaginal arousal as a measure of sexual arousal may well account for the relative lack of concordance between physiological and subjective measures of sexual arousal in women relative to the concordance observed between physiological and subjective measures of sexual arousal in men.[151]

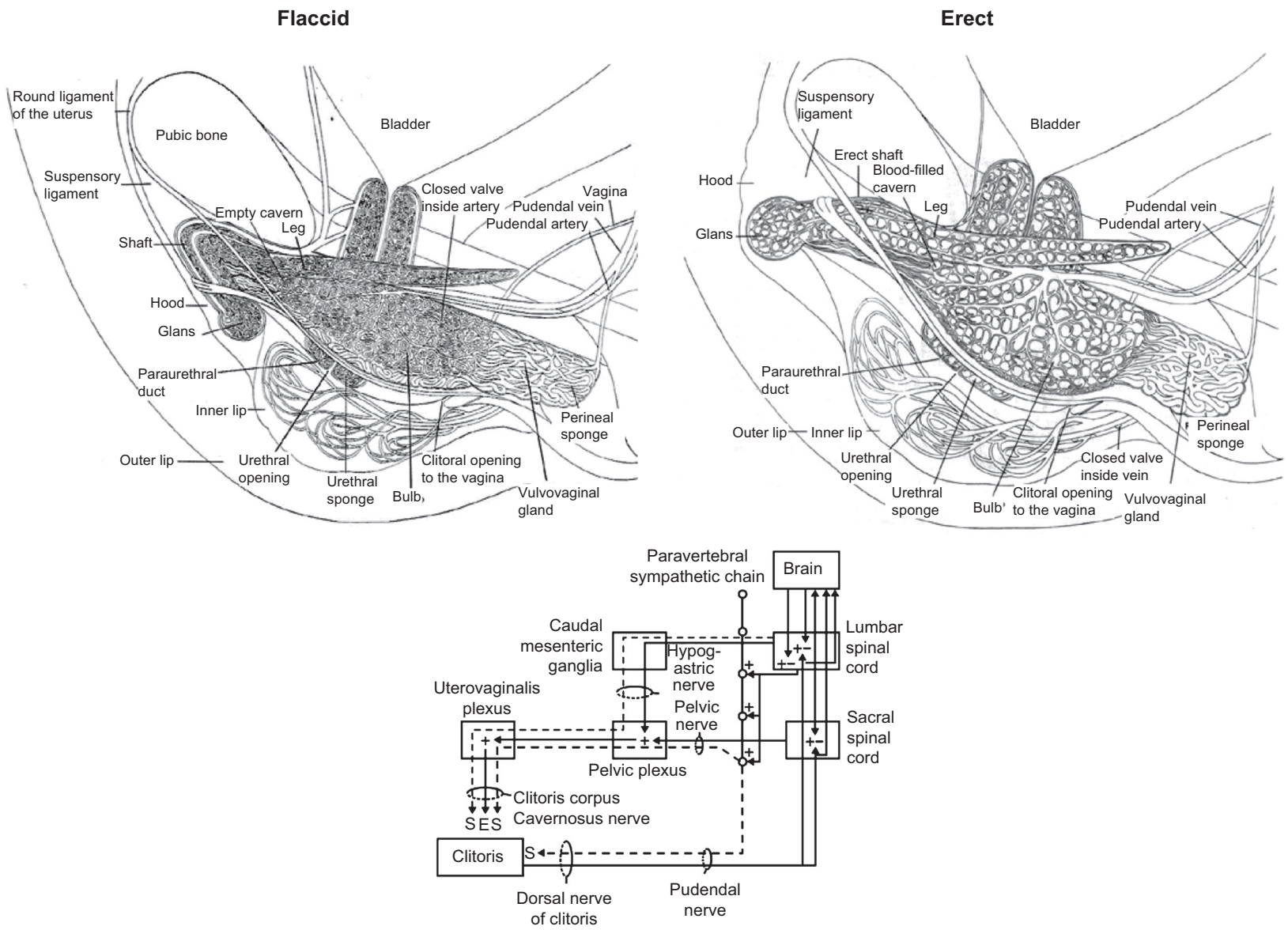

FIGURE 50.7 Top: The clitoral complex in its flaccid and erect states. Bottom: Wiring diagram of the sensory and autonomic pathways of the clitoral complex.

Genitosensory Stimulation

Both psychogenic and physical stimulation induce sexual arousal and desire that can lead to sexual behavior. Under normal conditions peripheral genital stimulation enhances excitability within sensory afferents that innervate spinal cord neural circuits. These spinal pathways contain the neural components for reflexive vasodilatation and orgasm and receive and send information to brain regions that perceive and modulate the signals that drive or inhibit sexual behavior. Although there are important differences in the brain and spinal pathways that mediate sexual function in males and females, largely related to sexual differentiation due to gonadal steroid hormone actions during fetal development (See Chapter 47), there is also substantial similarity between the CNS mechanisms mediating sexual desire, arousal and orgasm in males and females. In females, these include mechanisms involved in genital and psychological arousal, vasodilatation in the clitoris and vagina, smooth and skeletal muscle contractions, and rewarding sensations including orgasm.

Clitoral Stimulation

The clitoris is the main genitosensory organ of sexual pleasure in females,[130,132,134] and CLS influences vaginal muscle function.[152] It has been studied in rats with regard to its innervation and vasculature during sexual arousal[153–156] and its morphology in response to hormones.[157–159] However, its role in carrying sensory information during sexual activity in female rats has only recently been eludicated.[160–163] By engaging in lordosis, female rats not only receive vaginal intromission, but also CLS during mounts with pelvic thrusting. This interaction stimulates the female's flanks, rump, tailbase, perineum, and perivaginal surfaces which include the clitoris.[164] Intromission also stimulates the internal end of the clitoris in a region that overlaps in humans with the so-called "G-spot".[165–167]

In addition to CLS induced by male pelvic thrusting, direct CLS can be applied by an experimenter that mimics the distributed CLS a female rat might receive during copulation[160–163] (Figure 50.8, bottom left). Such stimulation on its own is rewarding in sexually naïve rats and stimulates preference for places and scents on partners associated with the reward state (see Section Consequences of Sexual Stimulation below). In contrast, CLS does not induce a preference in sexually experienced rats,[162] although it stimulates approach and solicitations of males behind a wire mesh screen.[161] Interestingly, the reward value of CLS is independent of steroid hormone priming, working as well in OVX rats as it does in OVX rats primed with estradiol alone or estradiol and progesterone.[162]

Clitoral afferents travel largely via the pudendal nerve (which branches into the dorsal penile nerve in males and dorsal clitoral nerve in females). This nerve provides the major sensory input from the external and internal clitoris and vagina, and provides the efferent (motor) innervations of the striated muscles of the pelvic floor and perineum.[170–172] Stimulation of the sensory branch of the pudendal nerve elicits vasodilation of the clitoris and vagina.[158,172–175] The increase in vaginal blood flow in response to sensory pudendal nerve stimulation was reduced by bilateral pelvic nerve cuts, suggesting that somatic afferents activate automatic spinal pathways to mediate the changes in vaginal blood flow that lead to vasocongestion.[174] The sensory field of the pudendal nerve is augmented by treatment with estradiol in OVX rats.[176]

Cervical Stimulation

The cervix is also stimulated during sexual interaction. Female rats, for example may receive VCS directly with each intromissive pelvic thrust from the male, whereas in humans the penis is generally not long enough to do this in most male-above positions, although it can occur in female-on-top positions.[177] Notably, however, orgasm in women also induces contractions of the cervix as part of what has been referred to as the "up-suck" reflex that aids sperm transport into the uterus.[178]

In rats small amounts of VCS potentiate lordosis whereas large amounts stimulate the termination of estrus. This stimulation-dependent excitation or inhibition depends on the hormonal status of the female and whether she can pace the copulatory contact with the male, as pacing increases the force with which the male intromits.[179–181] The augmentation of lordosis by small amounts of VCS is thought to be mediated at least in part, by the release of norepinephrine and possibly DA in the hypothalamus.[182,183] It may also be mediated by the release of OT in the spinal cord,[184] an effect that could also induce cervical dilation in preparation for sperm transport.

VCS can be applied by the experimenter using a smooth glass rod (Figure 50.8 bottom center) or plastic 1 cc syringe that approximates the width of the erect male rat penis. This stimulation can partially mimic the effects of intromissions by a male rat on reproductive physiology and behavior. This is somewhat surprising, however, because the probes provide pressure directly to the uterine cervix with mild distension pressure on the vaginal wall, whereas the erect rat penis is covered with keratinous spines,[185,186] which may more potently stimulate the vaginal wall even if the glans of the penis does not actually make contact with the cervix during intromission. Nevertheless, experimenter-administered VCS has many of the same effects on behavior and physiology as intromissions and is often used as a tool to simulate in a controlled manner the intromissions by the male. For example, experimenter-applied VCS increases heart rate[187] and elevates pain thresholds in both rats[188] and women.[189] VCS also

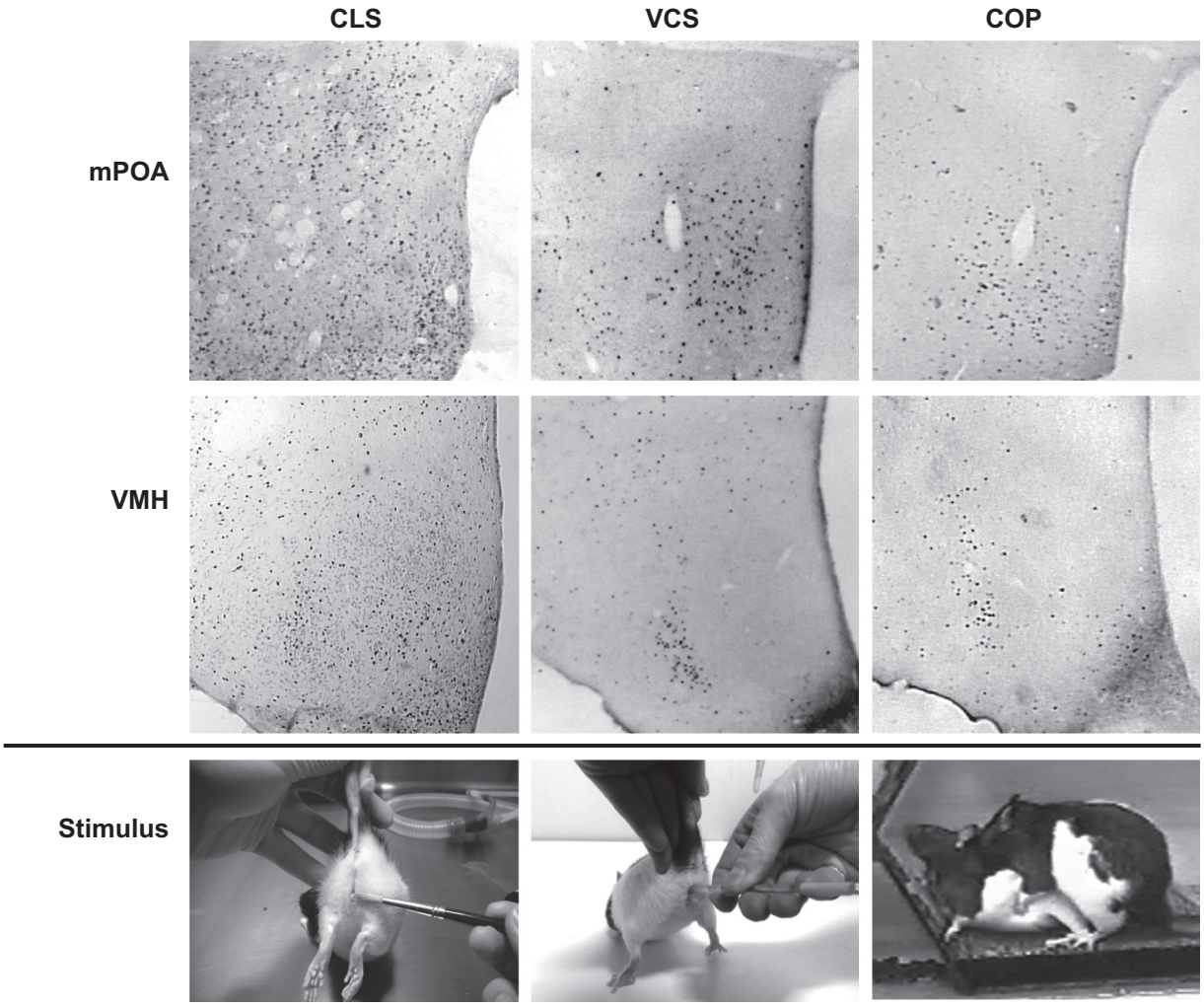

FIGURE 50.8 Fos induction in the mPOA (top) and VMH (middle) of OVX hormone-primed rats that received 50 distributed CLSs applied with a paintbrush,[160–163] 50 distributed VCSs applied with a lubricated glass rod,[75,168,169] or an hour's worth of paced copulation in bilevel chambers (bottom).

potentiates the ability of flank stimulation to induce lordosis in OVX rats not given hormone replacement,[190] and represents the only known stimulus, other than estradiol, to permit flank stimulation to induce lordosis.

Study of the relative contribution of intromissive, as compared to flank and perineal, stimulation provided by males suggested that nonintromissive stimulation is sufficient for mating-enhancement of lordosis, ear-wiggling and darting-and hopping in OVX/adrenalectomized (ADX) rats in a repetitive mating situation.[179] Therefore, VCS is not the proximate cause of mating enhancement in the repetitive mating situation, because, mounts without intromission were sufficient to enhance lordosis in OVX/ADX, estradiol-treated rats in the absence of progesterone. This makes logical sense; enhancement precedes the receipt of intromissions, because intromissions require that females display lordosis in response to mounts. Of course, the female does not show this posture until she is sexually receptive. In some experiments, rats have been OVX, and in others, they have also been ADX. Therefore, the interactions between adrenal ovarian hormones in the regulation of sexual responsiveness[182] and in the neuronal response to mating stimulation[191] must be considered. In some neuroanatomical areas, for example, OVX/ADX rats express the immediate early protein, Fos, in fewer cells in response to mounts without intromission than do OVX rats, but they express Fos in more cells in response to intromissions. This finding suggests that adrenal secretions may decrease sensitivity to low levels of mating stimulation.[191] Besides its influences on behavior, VCS influences luteinizing hormone (LH) release[192] and the twice daily surges of prolactin that then result in an extended period of diestrus[193] called pseudopregnancy or the progestational state.[194] More will be explained about the influences of VCS on estrus termination below. Cervical afferents travel largely via the pelvic nerve, and ablation of the pelvic nerve abolishes Fos induction in the brain by both VCS and copulation.[195]

Clitoral and Cervical Overlap

Both pelvic and hypogastric nerves convey sensory and noxious information from the internal reproductive organs, vagina and skin.[196] The cavernous nerve regulates both penile and clitoral erections. Stimulation of pelvic nerve afferents evokes an increase in vaginal blood flow, modeling genital arousal.[158] Vagal afferents from the uterus and cervix provide direct connections to the brainstem, and may sense orgasmic responses after spinal cord injury.[197] The distribution of the sensory afferents in the pelvis allows them to transmit considerable information relevant to mating, such as initiation and strength of sexual arousal, the location and movement of the penis inside the vagina, and orgasm or other pleasurable sensations derived from CLS. The clitoral glans contains specialized nerve endings that become very sensitive during erection and likely enhance sensation during intercourse, as is the case of the glans penis.[198,199]

Spinal Pathways

The sympathetic, parasympathetic, and somatic branches of the nervous system are coordinated and interconnected through important spinal pathways. This coordination allows sensory inputs to produce the appropriate sexual response, e.g., vasodilatation of erectile tissue, lubrication, increased sensitivity of erogenous zones, and muscle contractions during intromission and orgasm. In females the sympathetic spinal regions in lower thoracic-lumbar segments play a role in psychogenic arousal that occurs when distal cues (e.g., erotic visual cues in women; olfactory cues in rats) induce vaginal congestion.[200,201] However, genital responses occur only if the lumbosacral spinal cord that contains the pelvic and pudendal afferents and efferents is intact. Brainstem nuclei in the nucleus paragigantocellularis exert a serotonin-mediated tonic inhibition over the spinal pathways, and different regions of the brain (cortex, limbic system, mPOA) can be activated (directly or through visual or olfactory sexual stimulation) to initiate genital responses (reviewed in Ref. 196).

The pudendal nerve and pelvic nerve afferents course along the medial and lateral dorsal horn, respectively, of L5-S1 of the rat, with some afferent fibers penetrating into the dorsal gray commissure, and toward the parasympathetic preganglionic nucleus. In contrast, the hypogastric nerve afferents of the rat are relatively sparse and terminate in the superficial dorsal horn and medial gray of T13-L3.[171,195]

Fos Induction

Copulation, artificial VCS or CLS, urethral stimulation, or electrical stimulation of pudendal or pelvic nerves, all activate the immediate-early gene product Fos in neurons in similar spinal segments and subregions.[175,202] Activated spinal neurons are located in the superficial dorsal horn, the dorsal gray commissure of L5-S1 segments, overlapping with pelvic and pudendal sensory nerve innervations. Additional spinal neurons are activated in the intermediate gray and lateral gray dorsal to the parasympathetic preganglionic neurons. The distribution of activated spinal interneurons is similar in males and females. In parallel, electrophysiological studies identified similar spinal interneurons in the medial lumbosacral spinal gray matter after stimulation of the pudendal nerve and pelvic viscera.[203,204] However, VCS and CLS produce a different pattern of Fos activation in the rat brain (Figure 50.8, top). For example, distributed CLS that females find rewarding activates the medial nucleus of the mPOA,[160] whereas VCS activates a more medial region near the ventricles of the mPOA.[168,169] Paced copulation with a male (Figure 50.8, bottom right) activates both. In the VMH, CLS activates Fos throughout the dorsal and medial region, whereas VCS activates Fos exclusively in the ventrolateral region. Similarly, CLS activates Fos in limbic structures such as the posteroventral region of the medial amygdala (MEApv), whereas VCS activates Fos in the posteriordorsal region of the MEA.[18,160] In women, fMRI studies show that clitoral, vaginal, or cervical self-stimulation activates different regions of the sensory cortex.[205] Each of these are clustered in the medial paracentral lobe, a region that registers stimulation of the penis in the classic sensory homunculus of men.[206]

Tract Tracing

Neuroanatomical tract-tracing studies using neurotrophic viruses that are transported transneuronally through several synapses (for example, pseudorabies virus) have been used to map spinal and brain neurons that innervate the perineal muscles, clitoris, vagina, and uterus.[171,207–210] The majority of labeled neurons in the spinal cord are located in the dorsal gray commissure and in the vicinity sympathetic and parasympathetic preganglionic neurons, in the same regions as the Fos activated neurons. These neuroanatomical and electrophysiological studies reveal that genital afferents synapse on multiple interneurons in the spinal cord which then relay through a spinal pattern generator the preganglionic and postganglionic neurons and motoneurons to mediate, enhance, trigger, and maintain genital sexual responses. This complex spinal system allows coordination of sexual reflexes and sensorimotor modulation of these responses by different hypothalamic, midbrain, and brainstem regions (Figure 50.9).

Ultrastructural Changes in the Brain by Copulatory Stimulation

Sexual behavior itself influences neuronal morphology in rats[211] and hamsters.[94] After 1 h of mating in rats, there is a dramatic increase in expression of the cytoskeletal

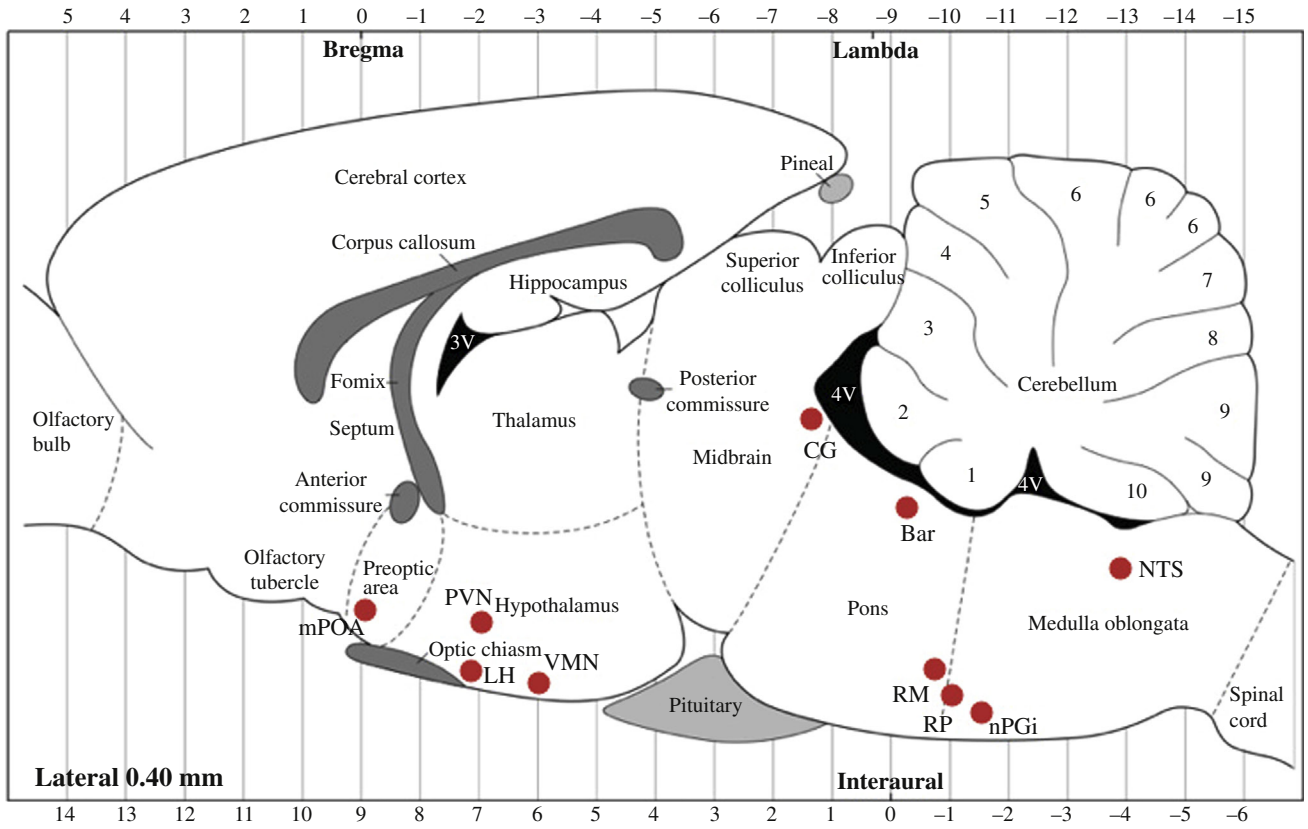

FIGURE 50.9 Dots depict areas of transneuronal staining in the brain 4 days after an injection of pseudorabies virus (a retrograde tracer) to the glans clitoris. *(Source: Adapted from Marson.[171,209])* mPOA, medial preoptic area; PVN, paraventricular nucleus; LH, lateral hypothalamus; VMN, ventromedial hypothalamus; CG, central gray; Bar, Barrington's nucleus; RM, medial Raphé; RP, posterior Raphé; nPGi, nucleus paragigantocellularis; NTS, nucleus of the solitary tract.

protein, Arc, in the ventrolateral VMH. Surprisingly, although the short-term effects were not assessed, this treatment also leads to a reduction 5 days later in secondary dendrites in this area. In hamsters, sexual experience increases the density of dendritic spines (membranous protuberences from the dendrite that typically receive a single synaptic input) in the prefrontal cortex (PFC), while decreasing it in the NAc.[94] Corresponding neurochemical changes have also been reported as a function of copulatory experience, including sensitization of D1 DA receptors[212] and Fos expression[213] in the NAc. It is clear that environmental stimulation, including that from mating, causes structural changes in neuroanatomical areas involved in sexual behavior. It is currently unclear how these changes relate to behavioral responses.

Other Erogenous Zones

Nipple and lip stimulation can be highly erotic during sexual arousal in both men and women, and can stimulate further sexual activity.[214,215] Nipple self-stimulation in women activates the genital sensory cortex (as well as the thoracic) region of the homuncular map in sexually experienced women.[205] The nipple/areola complex in women is innervated by the anterior cutaneous branches of the 1st to 6th intercostal nerves and laterally from the lateral cutaneous branches of the 4th intercostal nerve with additional innervation by cutaneous branches of the 3rd and 5th intercostal nerves (reviewed in Ref. 214). Innervation of the nipple appears to follow a pyramidal hierarchy, with most cutaneous sensory input in most women coming from the 4th lateral cutaneous branch, followed by the 3rd and 4th anterior branches.

Sensory innervation of the upper and lower lips comes from the maxillary and mandibular branches of the trigeminal (5th) cranial nerve, respectively.[216] Primary neurons send input to the trigeminal sensory nucleus, which is actually a sensory ganglion in the brainstem. Second order neurons project from there to the ventroposterolateral thalamus, and from there tertiary neurons carry input to the secondary somatosensory cortex (SII), in the lip region of the homunculus. The blood supply to the lips comes from the external carotid system,[217] which is aroused during parasympathetic activation of the lingual nerve. In the case of vasodilation in general for all genital and erogenous tissues, antihypertensive drugs

like nifedipine and propranolol can diminish erectile capability in both men and women.

In female rats, cutaneous stimulation of the flanks and perineum induces lordosis. Estradiol increases the area where cutaneous stimulation around the flanks and perineum induces lordosis, essentially sensitizing the somatosensory inputs to induce the reflex. This occurs by actions of estradiol in the periphery and within CNS modules that alter the propensity for cutaneous stimulation to induce lordosis.[32] Estradiol induces neurochemical and ultrastructural changes in the hypothalamic module (discussed below), notably in the VMH in concert with actions in other hypothalamic nuclei like the mPOA, that lead to an altered activation of neurons in the midbrain module of the central gray, which in turn activate neurons of the lateral vestibular nucleus in the brainstem module. This outflow activates the lateral vestibulospinal and reticulospinal tracts that synapse on motor neurons of the spinal module in the lower spinal cord (L5, L6, S1) to contract the lateral longissimus and transversospinalis muscles of the back, producing the characteristic arch of lordosis. However, such contractions are produced only when somatosensory afferents from the skin of the rump, tailbase, and perineum are activated by the male during anogenital investigation and more extensively during mounts with pelvic thrusting. Thus, although lordosis is essentially a spinal reflex, it is normally under tonic inhibition during periods of sexual nonreceptivity. The action of steroid hormones in the hypothalamic module is to constrain the lordosis reflex to an extended periovulatory period and disinhibit it in response to competent somatosensory stimulation of critical erogenous zones in the skin.

Primary Visual and Olfactory Senses

Sexual stimulation is often defined in terms of genital and erogenous somatosensory inputs (as those are explicitly and directly sexual), but largely separate from the main sensory systems that detect sexual incentives at a distance. In primates, and especially sighted humans, such stimuli are visual and auditory in nature. In other animals like rodents, they are olfactory in nature. Some stimuli are unconditionally arousing to human females (e.g., erotic pictures of attractive nudes or copulating couples[218,219]) or female rats (e.g., the smell of male rat fur[220]). Those unconditioned stimuli evoke attention and sexual approach behaviors like nose-pokes in rats. Treatments that disrupt olfactory input in rats, e.g., olfactory bulbectomy, zinc sulfate lesions of the olfactory epithelium, or olfactory occlusion with a polyethylene tube inserted into the nose, severely disrupt appetitive solicitations in females, but do not alter lordosis if the female is mounted.[221] Such treatments block copulation in male rats if they are sexually naïve, but not experienced.[28] Male olfactory cues alone activate Fos in the main olfactory (piriform) cortex, mPOA, VMH, and MEA of sexually experienced OVX rats primed with estradiol and progesterone.[221]

Erotic visual cues are used in studies of sexual arousal and desire in women (and men), and include still pictures or videos of nudes or different types of sexually explicit heterosexual or homosexual interaction. These are also used in brain imaging studies to correlate brain activation using fMRI or PET. Women experience cyclic fluctuations in sexual attention and arousability. For example, Mass et al.[60] reported pre-menopausal women's self-reported sexual desire and electromyographic responses of the facial zygomasticus major muscle (used for smiling and expressing joy) changed across the menstrual cycle during their exposure to pictures of naked men, with increases in smiling during the follicular phase and decreases during the luteal phase. Notably, these responses co-varied with increases and decreases in plasma progesterone, respectively. Similarly, event related cortical potentials (ERPs) recorded by scalp electrodes attached to the head that correspond to attention and stimulus processing for working memory increase in women following the presentation of sexually arousing pictures, but not pictures of babies or body care products, during the ovulatory phase.[59] The same pictures do not activate those ERP components during other phases of the menstrual cycle, or in women taking oral contraceptives.[222] Another study used fMRI to compare brain activation of premenopausal women in mid-luteal or menstrual phases of the cycle in response to erotic video clips relative to neutral video clips.[58,223] Increased activation by the erotic clips was observed in the anterior cingulate cortex (ACC), left insula, orbitofrontal (OFC), and parietal cortices, NAc, and hypothalamus, during the mid-luteal phase relative to the menstrual phase. These are virtually identical to the areas activated in men exposed to similar stimuli. The OFC is also activated by pictures of male faces, and the degree of activation is correlated positively with estradiol and progesterone levels in blood. The augmentation of the OFC response predicts the perceived attractiveness of the faces.[61] The ability of erotic visual stimuli to activate limbic and cortical structures is reduced after menopause, but can be restored to premenopausal levels following combined estradiol and testosterone treatment.[138] Thus, timing, context, and hormonal milieu, are extremely important variables to bear in mind when studying sexual arousal and responses to visual sexual stimuli in women.

HORMONAL PRIMING AND CONTROL

Steroid hormone synthesis in the ovaries is under the control of follicle stimulating hormone (FSH) and LH that are released from the anterior pituitary in response

to gonadotropin releasing hormone (GnRH) (see Chapter 28). FSH stimulates the growth of the ovarian follicle (and egg). When the follicle reaches a certain level of maturation it begins to secrete estradiol. LH causes the rupturing of the mature follicle to release the egg. The follicle becomes the corpora lutea ("yellow body") which then synthesizes and releases progesterone. The peak in estradiol secretion by the ovaries is followed by a small but critical rise in circulating androgens, notably testosterone, which is tightly linked in time to ovulation, and which, either alone in some species or in concert with the actions of preovulatory progesterone (from the follicle), activates appetitive approach and solicitation behaviors in many species. In humans, such behaviors are consonant with an increase in sexual desire. In most species, the expression of female sexual behavior is tightly regulated by ovarian hormones and occurs only during the periovulatory period.

Lordosis is perhaps the most-characterized and studied model of the hormonal regulation of behavior. In fact, the hormonal and neuroendocrine regulation of lordosis is similar in many species, including rats, mice, guinea pigs, hamsters, and gerbils. During the estrous cycle of these species, the secretion of estradiol followed by progesterone from the ovaries results in a period of sexual behavior that is tightly linked to ovulation.[65,224–226] Removal of the ovaries results in the loss of expression of female sexual behaviors.[65,227] High levels of sexual behavior in estrous cycling or OVX animals require estradiol priming followed by progesterone.[226,228] After behavioral estrus ends, sexual receptivity is not expressed until the next proestrous stage of the estrous cycle at which time estradiol secretion followed by progesterone once again induces sexual behavior. Although females of each of these species[229–232] may respond to estradiol alone, estradiol followed by progesterone is typically necessary for the expression of the full suite of female sexual behaviors closely resembling that seen in estrus-cycling animals.[14,65,225,227,233,234] The increase in both lordosis and appetitive sexual behaviors also occurs in OVX rats primed repeatedly with estradiol alone[84] and in ovary-intact, aged rats treated with testosterone.[235]

In some cases, after exposure to progesterone, rats,[236] hamsters,[231] guinea pigs,[237] and mice[238] become refractory to further stimulation of sexual behavior by either progesterone alone or, in some cases, to estradiol and progesterone. Although progesterone is believed to cause heat termination in guinea pigs, the role of progesterone in termination of sexual behavior during the estrous cycle of rats[239] is unclear. Based on work in guinea pigs to be discussed later, it has been suggested that progesterone desensitizes its response to itself, leading to termination of sexual receptivity and subsequent facilitation requires additional exposure to estradiol. Estradiol priming of behavioral response to progesterone generally takes about a day,[240,241] However, an intravenous injection of progesterone may facilitate the expression of lordosis within an hour of injection in estradiol-primed rats.[242–245] Interestingly, latencies as brief as 10 min for progesterone facilitation have been reported.[246]

Steroid Hormone Receptors

Although other hormones are involved, estradiol and progesterone have been the most extensively studied in the regulation of female sexual behavior in a variety of rodent species. This regulation involves a now "classic" steroid hormone action on specific intracellular receptors that act as transcription factors to induce gene expression (see Chapter 9). In turn, this sets up the excitatory and regulatory functions of the sexual brain, altering neurotransmitter synthesis, release, binding and reuptake, and altering synaptic connections in critical regions. Because of their role in the regulation of sexual behavior, understanding the regulation of these receptors is essential. A basic principle is the homologous and heterologous regulation of the receptors, each essential to ensure the critical timing of behavioral events with ovulatory events. This, in turn, sets up different neurochemical actions that time the onset, duration, and offset (inhibition) of the behavior.

A wide variety of steroid binding proteins has been described in the brain. These include the classic (so-called, nuclear) receptors that have been most extensively studied: ERα, ERβ, progesterone receptor A (PR-A; often referred to as progestin receptor A), progesterone receptor B (PR-B), and androgen receptor (AR). In addition to these classic receptors, other receptors, notably cell-surface receptors, have been described more recently that also mediate the effects of steroid hormones in the brain. Although these novel receptors are of great interest, much less is known about their role in the regulation of sexual behavior. As models of the regulation of female sexual behavior that involve the novel receptors develop, they must also be able to account for the data demonstrating the importance of the classic steroid hormone receptors as well.[247]

Classic ERs and Their Distribution in Brain

As described above, several subtypes of ERs exist, including the well-characterized classic (primarily) cell nuclear ERα and ERβ. Although relatively little is known about the neuroanatomical distribution of membrane-based ERs, a good deal is known about the distribution of ERα and ERβ. The following description of the brain localization of ERs focuses on the pattern of ERα and ERβ expression.

Four independent techniques have convergently revealed the distribution of ovarian steroid receptors

in the mammalian brain: in vitro binding, receptor autoradiography, immunohistochemistry, and in situ hybridization. These techniques measure the anatomical distribution of receptor binding activity, protein or mRNA expression, respectively, providing compelling evidence for regionally specific expression of these receptors. Table 50.1 summarizes the brain regions with the highest levels of ERs, based on these studies. Although most mapping studies have used rats, mice and guinea pigs, partial mapping of either ER protein or mRNA has been done in many other species, ranging from fish[248] and lizards[249] to musk shrews,[250] sheep,[251] human,[252,253] and nonhuman primates,[254,255] and the overall pattern is conserved.

The first studies delineating the anatomical distribution of estradiol binding used steroid receptor autoradiography with 3H-estrogens injected systemically. Although the initial observation of binding of an estrogen in the brain was made in cats,[256,257] in more comprehensive experiments,[258,259] regions of the hypothalamus and amygdala were especially rich in ERs. With the later discovery of distinct α and β subtypes of nuclear ER, autoradiographic studies were performed in transgenic animals with a "KO" of each of the subtypes of the ER.[260] These studies confirmed distinct neuroanatomical distributions for ERα and ERβ.

A great deal of the early work on the role of ERs in female sexual behavior and the biochemical characterization of them relied on in vitro 3H-estradiol binding in cell nuclear preparations of homogenates of particular brain areas.[261] In microdissection studies, the areas with the greatest density of ERs were in close agreement with autoradiographic studies showing ERs most abundant in the mPOA, VMH, and MEA.[262]

The early autoradiography and ligand binding studies on neural ERs relied on the binding of ^{3}H-estradiol to receptor proteins. Unfortunately, these techniques did not distinguish binding to each of the ER subtypes. However, the molecular cloning of the ERα and ERβ led to development of antibodies that could be used in immunocytochemical procedures that were able to distinguish the two subtypes. Immunocytochemical procedures were developed for both ERα[263–265] and ERβ.[266] Although immunohistochemistry provides the opportunity for superb subcellular localization, it has the disadvantage of not being able to distinguish between bound vs unbound receptors. This differs from autoradiography, which depends upon the receptor binding ligand. Thus, in addition to confirming the general pattern of receptor expression that had been revealed by receptor autoradiography, immunohistochemistry uncovered ERs in axons, dendrites, and terminals.[267,268] The presence of ER immunoreactivity in these nonnuclear sites complemented other evidence for nongenomic actions of estrogens, which will be discussed later.

The mRNA for ERα and ERβ has been mapped with in situ hybridization techniques,[269] and the neuroanatomical distribution of mRNA for the ERα and ERβ isoforms has been completed.[270] The results are in agreement with mapping studies based on receptor autoradiography in KO mice and subtype-selective immunocytochemistry. As a testament to the functional significance of the receptors in these regions, mating behavior produces a pattern of immediate early gene expression in the brain, a marker of neuronal activity, that corresponds to the neuroanatomical pattern of many locations of ER density.[168,271] Thus, ERs are well positioned to modulate the activity of the neural circuits that control mating behavior.

In summary, the neuroanatomical distribution of nuclear ERs is well documented, with the pattern of ERα expression coinciding well with brain regions known to

TABLE 50.1 The Dominant Pattern of ER Subtype Expression in Brain Regions Involved in Reproductive Behavior

	ER-Alpha	ER-Beta	Both
Amygdala	Amygdalohippocampal area		Medial nucleus
			Cortical nucleus
Septum	Subfornical organ		Bed nucleus stria terminalis
Hypothalamus	Median preoptic nucleus	Paraventricular nucleus	Medial preoptic area
	Anteroventral periventricular nucleus		
	Arcuate nucleus		
	Posterodorsal preoptic nucleus		
	Ventromedial nucleus		
Mesencephalon	Periaqueductal gray	Dorsal raphé	
	Locus coeruleus		
	A1, A2		

promote female sexual behavior. In a later section, we discuss the concept that in many areas with high levels of ERα expression, such as the VMH and POA, estradiol treatment increases the expression of the two forms of the progestin receptor (PR-A and PR-B).

Necessity of ERs for Hormonal Induction of Female Sexual Behavior

The notion that ERs are *essential* in order for estradiol to prime mice to become sexually receptive has been demonstrated by a wide variety of techniques, including: (1) injection of estrogen antagonists, which block the binding of estradiol nonselectively to ERs, or estrogen agonists that selectively activate either ERα or ERβ, (2) ER gene-disrupted mice (ER knockouts; ERKOs), in which the ERα or ERβ gene has been disrupted, and (3) RNAi silencing of ERα in specific brain regions.[272] The results of each approach are consistent with the conclusion that ERα is essential for the effects of estradiol on the expression of sexual receptivity, and that ERβ may have a modulatory role.

Effects of Estrogen Antagonists and Agonists

Early studies using estrogen antagonists to block the binding of estradiol to ERs were unequivocal in demonstrating the absolute necessity of binding to ERs in the mechanisms by which estradiol primes rats for female sexual behavior.[273,274] However, those antagonists were not specific for ER subtype. More recently, experiments using the ER subtype-specific agonists, propyl-pyrazole triol (PPT; ERα agonist) and diarylpropionitrile (DPN; ERβ agonist) indicate that ERα mediates the effects of estradiol on both appetitive and consummatory aspects of sexual behavior in female rats. Although the ERβ agonist is without effect when administered alone, it reduces the effects of the ERα agonist, suggesting that ERβ has a modulatory role in damping the effects of ERα activation.[275]

KOs and Knockdowns

KO strains of mice have been developed in which the gene for each form[276,277] or both forms[278] of ER is disrupted. Targeted disruption of the ERα gene (ERKO) completely eliminates hormonal induction of female sexual behavior.[279,280] In contrast, disruption of the ERβ gene (BERKO) was reported to be without effect in OVX, hormone-injected mice,[281] although it extended the period of behavioral estrus and enhanced receptivity.[282] Double KO mice with disruption of both, ERα and ERβ, exhibit decreased levels of sexual receptivity supporting the critical role of ERα in the sexual behavior of female mice.[281] Further support for involvement of ERα in female sexual behavior, and specifically its role in the VMH comes from the use of RNAi silencing of this gene in the VMH.[283] However, in a more recent study using the more complete ERβ-null mouse ($ER\beta_{ST}^{L-/L-}$),[284] complete elimination of ERβ resulted in a decrease in lordosis and attractivity in homozygous female mice.[285] Taken together, these experiments point to a critical involvement of both forms of the ER in the regulation of female sexual behavior in mice.

Regulation of ERs and ER Action

Because of the importance of ERs in the induction of female sexual behavior, it is essential to understand the ways in which levels of ERs in cells are regulated by estradiol and other compounds. There is some inconsistency among studies on regulation of ERα protein and mRNA levels by estradiol. However, estradiol has typically been reported to down-regulate ERα in most neuroanatomical areas.[286–291] Most studies find that estradiol also down-regulates ERβ in some neuroanatomical areas but is without effect in others.[289,292–294] Besides the homologous down-regulation of ERs by estradiol, ERs are down-regulated by progesterone[295–298] under some circumstances. Down-regulation in specific neurons that results from either hormone would be expected to decrease hormonal responsiveness in those neurons. The inconsistencies that exist in the literature with respect to the regulation of ERs by estradiol are to be expected, because there are numerous, important methodological differences between studies, such as doses of estradiol used, duration of exposure to hormone, time since OVX, etc. Furthermore, there is heterogeneity in the regulation of each form of ER, not just among neuroanatomical areas, but even among the neurons in a neuroanatomical area.[289] Nevertheless, such receptor down-regulation may help to explain why a substantial proportion of pre-menopausal women taking synthetic estradiol-containing oral contraceptives experience a blunting of their sexual desire, and an uncoupling of desire around the time that ovulation would normally have occurred.[299,300]

Pattern of Estradiol Exposure Sufficient to Induce Sexual Behavior

Acute Administration

The pattern of hormonal exposure is another critical variable in determining response to hormones. OVX rats need not be exposed to estradiol continuously during the priming period in order to express sexual behavior. Two pulses of a low dose of estradiol (e.g., 5 μg of estradiol benzoate, EB) spaced 24 h apart are more effective in inducing female sexual behavior after subsequent progesterone administration than a single higher dose of EB (e.g., 10 μg) or continuous exposure to estradiol from a silastic capsule implanted subcutaneously (sc) for several hours.[301–303] The behavioral effects of each pulse can

be blocked by either a protein synthesis inhibitor[304] or pentobarbital anesthesia,[305] suggesting that both protein synthesis and neuronal activity are required for each of the pulses of estradiol to be effective. The potential roles of classic and membrane receptors in these processes are discussed below.

Although continuous exposure to estradiol is not essential for the expression of sexual behavior, the continued presence of estradiol-bound ERs seems to be a requirement; administration of an estrogen antagonist that displaces receptor-bound estradiol inhibits sexual behavior, even when administered just prior to progesterone injection within a few hours of testing.[306] This finding suggests that the down-regulation of ERs by progesterone, which would then be expected to reduce ER-dependent estradiol action, may be part of the mechanism by which the period of estrus terminates.

Chronic Administration

Priming regimens in the literature vary, as do the strains of OVX rats used. It is clear, however, that a low dose of estradiol (e.g., 5 μg of EB) injected sc induces a moderate lordosis, but no appetitive approach or solicitation behaviors. The addition of progesterone (e.g., 100–500 μg sc) can increase lordosis further, and stimulate appetitive behaviors. However, continuous EB exposure through a silastic capsule implanted sc results in high and continuous levels of both appetitive sexual behavior and lordosis, as do frequent injections of EB, which sensitize both lordosis and appetitive sexual behaviors in sexually experienced female rats. Maintenance of serum estradiol concentrations above 15 pg/ml over a 5-week period with silastic capsules maintain normal body weight along with normal patterns of appetitive and consummatory sexual behaviors in female rats.[307] Serum concentrations below this cause increases in body weight and a dramatic suppression of appetitive responses with smaller decreases in lordosis.

The progressive elevation of appetitive and consummatory sexual behaviors by sc injections of estradiol is dependent on the dose and injection interval (Figure 50.10), such that as EB dose increases, behavioral sensitivity increases.[84,301,308–314] Notably, the sensitization is not blocked by ADX in OVX rats,[84] making it unlikely to be induced by facilitated release of adrenal progesterone. This finding is important because it further demonstrates that hormones can override inhibitory mechanisms that would otherwise act to inhibit sexual behavior as estrus terminates. A function of this increased sensitivity to the hormones, or decreased sensitivity to VCS, may be to ensure the female receives sufficient stimulation to maximize reproductive success.[75,84]

Estrogen sensitization is an important consideration in pharmacological studies that investigate compounds that might facilitate sexual behavior. Typically, OVX females are primed with a dose of EB that produces low-to-moderate amounts of appetitive sexual behaviors and lordosis. This is done to prevent ceiling effects that occur when animals are primed with EB and progesterone. However, animals are often tested repeatedly to reduce the number of animals used or because long-term treatment effects are of interest. The sensitization of sexual behaviors with chronic EB is confounding and makes data interpretation complex. This is particularly problematic in studies where the facilitative actions of a drug seem to disappear over time. EB dosing regimens have been characterized for OVX rats to achieve desired baseline rates of female sexual behavior across time[84] which will help overcome these problems. Stable baselines are vitally important in preclinical models of female sexual function. For example, 5 μg EB administered every 7 days, induces a stable baseline[84,315,316]; and when given in combination with flibanserin, a mixed 5-HT1A agonist/5HT2A antagonist, appetitive sexual behaviors were increased significantly by the drug following 3 weeks of treatment,[316] whereas the control group did not manifest any increase in behavior.

ER Co-regulators

Another level of regulation of response of a cell to a hormone lies in steroid receptor coregulators; intracellular proteins that allow for efficient regulation of the transcription of steroid receptors (see Chapter 9).[317] These proteins bridge the receptor and the general transcriptional machinery and modify promoter regions of the receptor by a variety of mechanisms. Although there are over 300 steroid receptor coregulators,[318] and we have much to learn about how they modulate the action of ERs, perhaps fine-tuning responses to steroid hormones, we do understand the role of three coactivators in regulation of female sexual behavior. Work using intracerebroventricular infusion of antisense oligonucleotides directed against the mRNA for particular steroid receptor coactivators in rats suggests that steroid receptor coactivator-1 (SRC-1) and cAMP response element binding protein (CBP) act together to modulate the induction of sexual receptivity by estradiol,[319] as well as the induction of progestin receptors[319] and progesterone-facilitated sexual behavior in female rats.[320] Likewise, SRC-1 and SRC-2 play an important role in the cellular action of estradiol in the induction of female sexual behavior in rats and mice.[321]

In order for coactivators to influence the activity of steroid receptors, they must be coexpressed in the same neurons as the receptors. In fact, SRC-1 and SRC-2 are expressed in most cells expressing ovarian steroid hormone receptors in the VMH, mPOA, and arcuate nucleus (ArcN) of female rats and mice.[317] Although

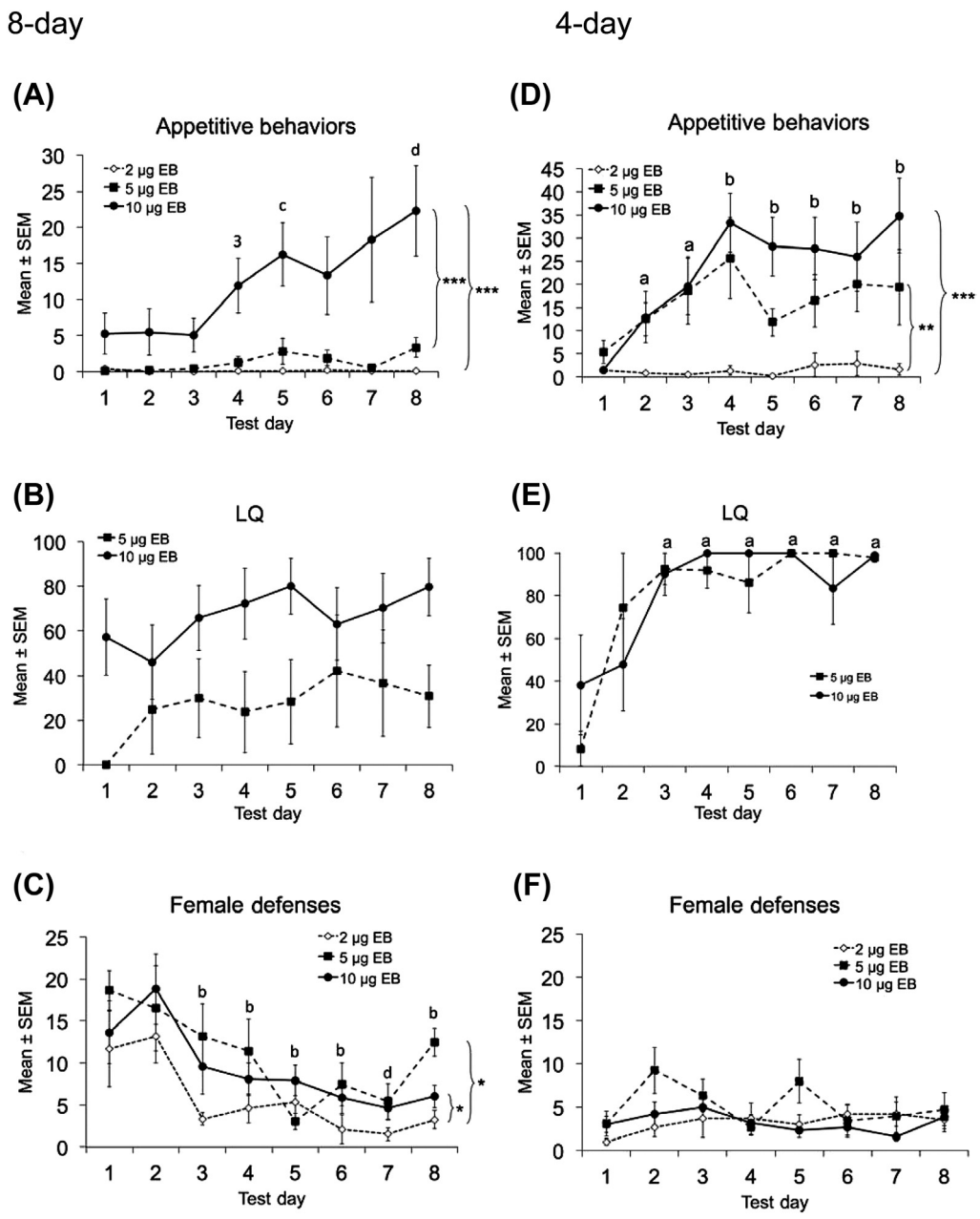

FIGURE 50.10 Sexual behaviors of OVX Long-Evans females treated with varying doses of estradiol benzoate (EB) at 8-day (left) or 4-day (right) intervals. (A) Appetitive sexual behaviors increased in females treated with 10 µg EB, but not 2 µg or 5 µg when treated at 8-day intervals. (B) Lordosis quotient (LQ) did not sensitize when treated with EB at 8-day intervals. (C) Collapsed across EB treatment group, females were less defensive toward males as of Test 3. (D) When treated at 4-day intervals sexually appetitive behaviors sensitized in females treated with 10 µg EB. (E) LQ sensitized in females treated with 5 and 10 µg EB when treated at 4-day intervals. (F) Defensive behaviors were unaffected when treated with EB at 4-day intervals. [a]Different from Test 1; [b]Different from Tests 1 and 2; [c]Different from Tests 1, 2, and 3. [d]Different from Tests 1–4. Numerical superscripts are used to indicate differences from specified test day. Brackets represent main effect of EB Group. $*p<0.05$, $**p<0.01$, $***p<0.001$. *Source: Reprinted from Jones et al.,[84] with permission of Elsevier.*

in vitro studies suggest that the relative expression levels of the coactivators and corepressors determine cell-specific, appropriate and graded responses to steroid hormones,[315] there has to date been no work on this subject in the brain and on behavior. It is however likely that this represents another level of fine-tuning of the hormonal regulation of female sexual behavior.

Is Binding of ERs to Estrogen Response Elements Essential?

Not all ER-mediated responses require the prototypical estrogen response elements on specific genes. A gene knock-in mouse model with a mutated ERα that does not bind to estrogen response elements (EREs)[322] expresses

negative, but not positive, feedback to estradiol on gonadotropin secretion, suggesting that negative feedback does not require binding of ERα to an ERE. While expression of masculine sexual behavior in males was shown to require binding to EREs,[323] results on female sexual behavior have not been reported. Nevertheless, this work suggests that some effects of estradiol that are relevant to female sexual behavior do not require binding the nuclear ER to an ERE, opening up the possibility that estradiol induces these effects through membrane ERs.

Role of Membrane ERs

Recent work has made clear that, while classic ERs are principal players in regulation of female sexual behavior by estradiol, membrane mechanisms are also involved. Although much less is known about membrane ERs than about the role of classic, so-called, nuclear ERs, there is now a good deal of evidence for the involvement of membrane mechanisms of action for the effects of estradiol on female sexual behavior. A variety of putative membrane ERs has been described,[324] including the classic ERα and ERβ, which can be translocated to plasma membranes,[325] ER-X, STX (a tamoxifen analogue that is estrogenic)-activated membrane ER, and GPR30 (G protein coupled estrogen receptor; GPER).

Although still in an early stage of research, the data argue strongly for the involvement of membrane ERs in the regulation of female sexual behavior by estradiol. For example, a biotinylated form of estradiol, which is impermeable to the cell membrane, interacts with metabotropic glutamate receptors in the mPOA, resulting in an increase in lordosis.[326] Specifically, membrane ERα[326] and/or the STX-activated membrane ER[327] is believed to interact with the metabotropic glutamate receptor 1a in the ArcN, resulting in the internalization of mu-opioid receptors in the mPOA and consequently an increase in lordosis via projections to the VMH. It should be noted, however, that while the STX compound facilitates the expression of lordosis in OVX rats, it only does so in rats administered a subthreshold dose of estradiol.[327] Therefore, the STX-activated mER participates in the process of estradiol priming of sexual behavior, but it is not sufficient to substitute for estradiol. As discussed earlier, administering estradiol in discrete pulses allows much lower doses of estradiol to be used to induce sexual behavior. However, little is known about the cellular basis for the enhanced response to pulsed exposure as opposed to bolus injection of estradiol. In some experiments, estradiol conjugated to bovine serum albumin (another form of estradiol which is impermeable to cell membranes), has been used.[328] The fact that this conjugated estrogen can substitute for either the first or later pulse of estradiol indicates that membrane ERs are capable of completing at least part of the priming for sexual behavior. Compounds that activate either protein kinase A (PKA) or PKC can also substitute for the conjugated estrogen,[328] suggesting involvement of these two intracellular signaling pathways in the priming action of estradiol on female sexual behavior. It should be noted that experiments using protein conjugates of steroid hormones must be interpreted very cautiously because of the possibility that the protein could be cleaved from the steroid molecule[329] and because the position of the protein on the steroid can have unexpected effects on function.[330] Nevertheless, the data argue for the ability of membrane ER mechanisms to substitute for interaction of estradiol with classic ERs.

With the exception of the work of Kow and others,[328] mechanistic studies have typically focused on the role of *either* nuclear ERs acting as transcriptional regulators or on the role of membrane receptors in regulation of sexual behavior. However in vitro, ERα and ERβ each may be processed to become associated with membranes, and are capable of signaling through the mitogen activated protein (MAP) kinase pathway.[331–333] This finding indicates that caution must be exercised in interpreting experiments which used hormone antagonists, antisense oligonucleotides or targeted gene disruption to test the involvement of ERs acting as transcription factors. If the ER genes that direct synthesis of the classic ERs also direct the synthesis of membrane receptors in the brain, then the manipulations that target the classic ER could disrupt membrane receptors as well as classic nuclear receptors.

As mentioned earlier, ERα immunoreactivity[267,334] has been observed in extranuclear locations within the guinea pig hypothalamus, including axon terminals and distal dendrites[267] (Figure 50.11). In some cases, this has been observed associated with synaptic densities and plasma membranes, which is consistent with the idea that classic ERs can be directed to membrane sites.

To summarize this section, in addition to actions of estradiol on classic ERs, estradiol may signal through a variety of membrane receptors to either substitute or perhaps, augment the behavioral effects of estradiol acting on classic ERs. There is still a great deal to be learned about the interplay of these various receptors in the fine-tuning of the hormonal induction of female sexual behaviors.

Classic PRs and Their Distribution in Brain

As with ERs, evidence from receptor autoradiography, immunohistochemistry, and in situ hybridization presents a coherent picture of the pattern of expression of nuclear PRs, although information about membrane receptors is still being gathered. Early receptor autoradiography experiments first revealed nuclear progesterone binding activity in the rodent brain.[335,336] Although progesterone binding was found in regions with an

abundance of ERs, such as the hypothalamus and amygdala, a few brain regions expressed PR in the absence of ER, suggesting separate functions. Double-label immunohistochemistry studies have shown that ERα and PR are often co-localized at the cellular level in brain regions known to control female sexual behavior.[289,337] The mapping of mRNA of the PR has been conducted using in situ hybridization techniques in rats, rabbits, lizards and fish.[338–341] Although there may be some species differences in regulation, the overall pattern of nuclear PR expression is conserved in regions associated with reproductive behavior, including the VMH, mPOA, median preoptic nucleus, anterior hypothalamic area, medial nucleus of the amygdala, anteroventral periventricular nucleus, lateral habenula, ArcN, and periaqueductal gray.[342,343] It should be noted many of the PR-immunoreactive cells in the VMH are located outside the Nissl-defined nucleus,[337] and these cells have been implicated in the facilitation of sexual behavior by progesterone, at least in guinea pigs.[344]

Although there is a great deal of overlap between neuroanatomical sites containing estradiol-induced PRs and those that contain ERs, immunocytochemical experiments demonstrated conclusively that estradiol-induced PRs are co-expressed in ER-ir cells in brain regions involved in regulation of female sexual behavior.[289,345] Virtually all cells expressing estradiol-induced PRs also express ERα (Figure 50.12).

The PR is synthesized from alternative, estrogen-inducible promoters on the PR gene, resulting in two isoforms of the receptor (PR-A and PR-B) with somewhat

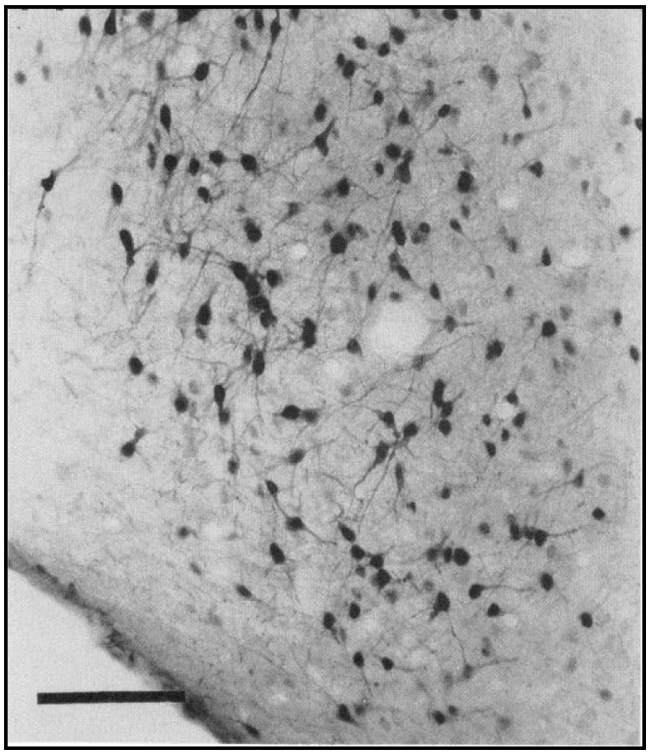

FIGURE 50.11 ER-immunoreactivity in the VMH of an OVX guinea pig, visualized by the silver-intensified, diaminobenzidine-peroxidase technique in vibratome-cut sections. Magnification bars = 100 μm. *Source: Reprinted from Blaustein et al.,[267] with permission of the Endocrine Society.*

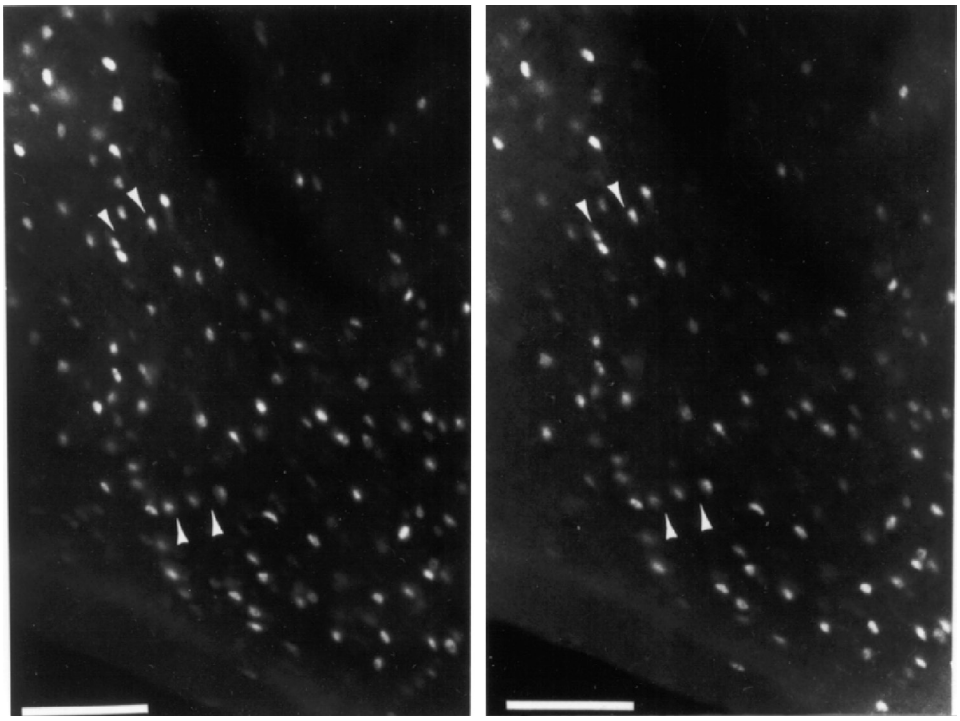

FIGURE 50.12 Photomicrographs of PR-immunoreactivity (right panel) and ERα-immunoreactivity (left panel) coexpression in the VMH of an estradiol-primed, OVX guinea pig showing that virtually all estradiol-induced PR cells also coexpress ERα-immunoreactivity. Arrowheads point to cells containing both estradiol-induced PR-immunoreactivity and ERα-immunoreactivity. *Source: Reprinted from Blaustein and Turcotte,[345] with permission of Karger.*

different transcriptional activities.[346,347] Although our understanding of the differential distributions of these PR subtypes remains incomplete, the two isoforms are expressed in the rat brain,[348] and the ratio of the two isoforms varies under different hormonal[349–352] and behavioral[353] conditions. Experiments using antisense oligonucleotides directed at the mRNA for each or both of the isoforms demonstrate differential regulation of particular genes by each isoform.[354]

Necessity of PRs for Progesterone Regulation of Female Sexual Behavior

The characterization of neural PRs suggested the hypothesis that PRs are essential for the facilitation of sexual behavior by progesterone.[355] This hypothesis predicted that sensitivity to progesterone is determined by the concentration of unoccupied PRs available in neurons involved in progesterone-facilitated sexual behavior, and response is dependent on an adequate concentration of activated PRs in those cells. An increased concentration of PRs (e.g., after estradiol priming) would be expected to increase the sensitivity of the neural substrate for progesterone, presumably by increasing the concentration of receptors that become activated in response to progesterone treatment. Likewise, a decreased concentration of unoccupied PRs would be expected to result in decreased sensitivity to progesterone. This PR hypothesis[356] was corroborated in later work on the regulation of PRs that will be discussed. A central mechanism involving neuroprogestin activation of PR, or ligand-independent activation of PR, has not been determined.

Upregulation of PRs

As discussed, estradiol increases the concentration of PRs in the hypothalamus, mPOA, and a number of other brain regions (Figure 50.13). Coincident with the increase in the hypothalamus, behavioral responsiveness to progesterone increases. The increased concentration of PRs and behavioral responsiveness to progesterone are both transient.[358,359] In gonadally intact, estrus-cycling rats, the concentration of unoccupied PRs in the hypothalamus increases during proestrus in response to estradiol.[244] Collectively, these experiments suggested that PRs are a critical aspect of the cellular mechanism by which progesterone facilitates sexual behavior.

The duration of sexual receptivity for each species is tightly regulated, lasting about 8 h in guinea pigs[360] and about 14 h in rats.[361] The timing of the *duration* of sexual receptivity is referable at least in part to the regulation of activated PRs in particular neurons.[356] The presence of activated PRs in particular neurons presumably leads to the expression of neuropeptides and neurotransmitters described below, but the presence of the activated

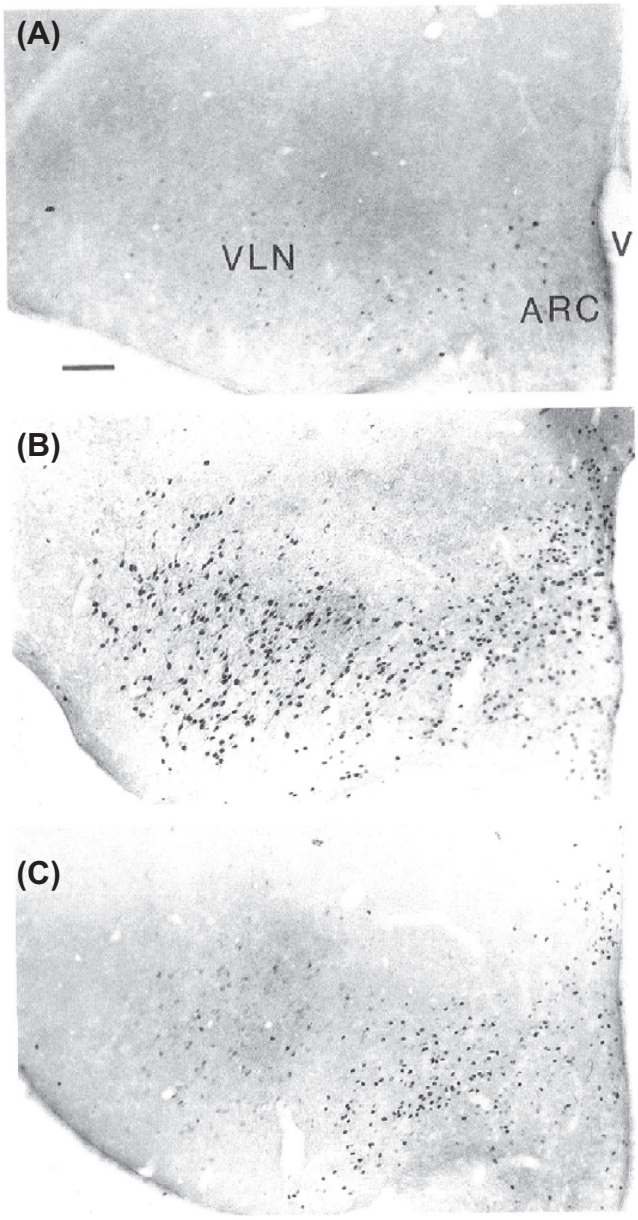

FIGURE 50.13 PR-immunoreactivity in the rostral aspect of the ventrolateral nucleus of the hypothalamus (VLN) and arcuate nucleus (ARC) of OVX guinea pigs injected with: (A) oil (0 h) and oil (42 h), perfused 24 h later, (B) estradiol benzoate (0 h) and oil vehicle (42 h), perfused 24 h later, or (C) estradiol benzoate (0 h) and progesterone, perfused 24 h later. Magnification bar = 100 μm. *Source: Reprinted from Blaustein and Turcotte,[357] with permission of Wiley.*

PR may act to gate the transcriptional activity of the relevant downstream genes. Progesterone injected in estrogen-primed, OVX guinea pigs and rats[244,362,363] or the preovulatory progesterone secreted during the estrous cycle[363] binds to and activates neural PRs. The presence of activated PRs in a pooled sample of the hypothalamus-preoptic area after progesterone injection correlates well with the ability of female rats to display lordosis.[362] Manipulations which prolong the period that

hypothalamic PRs remain occupied extend the duration of that period in female rats.[364,365] This temporal agreement between activated/occupied PRs and expression of lordosis suggests that it is maintained by elevated levels of occupied PRs, and that termination of lordosis is due at least in part to loss of these receptors.

Besides these correlational studies, a variety of techniques—injection of progestin antagonists, antisense oligonucleotides to PR mRNA and PR knockout (PRKO) strains of mice—has been used to demonstrate that PRs are *essential* for the facilitation of lordosis by progesterone. Systemic injection[366,367] or intracranial application[368] of a progestin antagonist inhibits the facilitation of lordosis by progesterone in rats and guinea pigs. However, because most antagonists are not completely specific, other techniques have been used to test the necessity of PRs for progesterone function in sexual behavior. Infusion of antisense oligonucleotides to PR mRNA, which inhibits PR synthesis, into the cerebral ventricles[369] or VMH[370,371] blocks facilitation of both appetitive sexual behaviors and lordosis by progesterone. Similarly a transgenic mouse strain with a targeted disruption of the PR gene (PRKO)[372] are completely unresponsive to progesterone for the facilitation of sexual behavior.[373] Using PR isoform-specific KO strains of mice to determine the relative contribution of each PR isoform to progesterone-facilitated female sexual behavior, Mani et al.[374] observed that progesterone-facilitated lordosis was completely eliminated in the PR-A null mutant mouse, and PR-B null mutant mice showed a trend of suppression of progesterone-facilitated sexual behavior. Although the specific function of PR-B is unclear, the data collectively suggest that PR-A is essential for progesterone-facilitated lordosis, and both isoforms are required for optimal facilitation by progesterone.

The question of which ER is involved in up-regulation of PRs has received some attention. The ERα selective agonist, PPT, induces PR mRNA, at least in the VMH and ArcN.[375] Although PR-ir induction in the brain by estradiol is dramatically reduced in ERαKO mice, KO of ERβ does not fully eliminate PR-ir induction.[281,376] Furthermore, genetic downregulation of ERβ (albeit in the incomplete ERβb KO mouse) was without effect on PR-immunoreactivity in the VMH. In subsequent work, in which it was confirmed that the ERα agonist, PPT, induces PR-ir in the VMH, it was also determined that the ERβ agonist, DPN does not. However, the sequential injection of an ERβ agonist after an ERα agonist induces PR immunoreactivity in more cells in the VMH than the ERα agonist alone.[377] Based on experiments using BSA-conjugates of estradiol, it has been suggested that membrane ERs are also involved in the induction of PRs in the VMH.[378] Collectively, these experiments point to a critical role for ERα, a possible role for a membrane ER, and a minor role for ERβ in the induction of PR immunoreactivity, at least in the VMH.

Membrane PRs and Nonclassic Mechanisms of P Action

The mechanism of action of progesterone is not as simple as activation of just PR-A and PR-B. Although the classic mechanism of hormone action plays an important role in the regulation of sexual behavior, membrane mechanisms are also involved.[379] Progesterone can also influence electrophysiology and facilitate sexual behavior within seconds and minutes, respectively. Some of these effects may be referable to activation of cell surface PRs, ion channels and cytoplasmic second messenger signaling cascades, and are independent of gene transcription.[380] Recently, membrane proteins unrelated to classic PRs have been characterized. The presence of membrane PRs (mPRs) and progesterone receptor membrane component 1 (PGRMC1) and PGRMC2 in the brain[342,381–384] provides a possible mechanism by which progesterone could have rapid effects on behavior and neurophysiology. mPRs are G-protein coupled receptor members of the seven trans-membrane adiponectin Q receptor family, and come in at least three subtypes.[385] In addition, PGRMC1 (also called 25Dx) is regulated by estradiol and progesterone in the VMH of female rats.[343,381] Although the direction of the regulation is not consistent between the two reports,[343,381] the hormonal treatments and methods used were quite different, which may explain the discrepancy.

PGRMC1, PGRMC2 and classic PR mRNAs are expressed at high levels and have a good deal of overlap in the mPOA and other hypothalamic nuclei and their projection sites.[342] Under some conditions, progesterone treatment results in an increase in PGRMC1 mRNA levels in the VMH and preoptic area.[343] Likewise, mPRα and mPRβ are present within the hypothalamus and preoptic areas, among other areas, and estradiol increases the expression of mPRβ[384] and the estrous cycle influences the expression of mPRα and mPRβ expression in some brain areas.[383] Although the functional role of each of these putative membrane receptors in hormonal regulation of sexual behavior remains to be determined, their presence in the brain suggests additional mechanisms by which progesterone could rapidly influence sexual behavior and possible interactions of mPRs and classic PRs within the same neurons.

Cross-Talk between Neurotransmitters and Steroid Hormone Receptors

Neurotransmitters Influence Concentrations of ERs and PRs

One of the most interesting aspects of the regulation of female sexual behavior is the interplay between external factors and the internal hormonal *milieu*. Because of the critical role of steroid hormone receptors in the

mechanisms of action of steroid hormones on female sexual behavior, studies of integration between afferent information and steroid-hormone sensitive systems have focused on the regulation of these receptors. The finding that catecholaminergic activity influences the concentrations of neural sex steroid receptors in rat and guinea pig brain[386] suggested that stimuli from the environment might regulate the concentration of steroid receptors in neurons involved in female sexual behavior, and consequently, behavioral response to hormones.

Drugs which either inhibit norepinephrine synthesis (dopamine-β-hydroxylase (DBH) inhibitors) or which block noradrenergic receptors (e.g., α-adrenergic antagonists) typically decrease the concentration of ERs in some neural areas[387,388] and/or inhibit induction of hypothalamic PRs by estradiol,[389–391] and α-adrenergic agonists reverse this suppression. Noradrenergic antagonists also decrease female sexual behavior in guinea pigs.[392] This, together with the finding that injection of an α_1-noradrenergic antagonist, which decreases ER concentrations in the hypothalamus, also decreases female sexual behavior,[393] suggests behavioral relevance of the neurotransmitter regulation of ERs. Finally, under some conditions, stimulation of DA receptors increases the concentration of ERs in the brain.[394,395]

A possible anatomical substrate for the integration between catecholaminergic neurons and steroid hormone-responsive neurons can be found in the catecholaminergic innervation of some ER-containing neurons,[396,397] and immunoreactivity for tyrosine hydroxylase and DBH, the enzyme that converts DA into norepinephrine (NE), varicosities are sometimes found closely associated with PR- or ER-immunoreactive neurons in the mPOA and hypothalamus.[398–400] The fact that those ER-immunoreactive cells with closely associated DBH-immunoreactive varicosities stain more darkly for ERs than other ER-immunoreactive neurons lacking this association suggests that noradrenergic input regulates the level of ERs in a population of these ER-immunoreactive cells,[400] and consequently, behavioral responsiveness to estradiol. It should be noted that neurotransmitter regulation of steroid receptor levels is not limited to the catecholamines. For example, muscarinic agonists and antagonists regulate the levels of neural ERs.[401]

Defined anatomical connections may influence steroid receptor levels and therefore, presumably, sensitivity to steroid hormones for their influence on sexual behavior. For example, anterior roof deafferentation using knife-cuts in female rats increases the behavioral response to estradiol, presumably referable to the resulting increase in the concentration of ERs in the mediobasal hypothalamus.[402] Conversely, olfactory bulb removal results in an increase in the concentration of ERs in the MEA in female rats, presumably related to the mechanism by which olfactory bulbectomy increases sexual behavioral response to estradiol.[403]

Input from the social environment also regulates steroid hormone receptors and hormonal response, presumably via neuronal pathways. The odor of male prairie voles induces estrous behavior in female prairie voles,[404] presumably in part due to the accompanying increase in the concentration of ERs in the mPOA.[405] Level of maternal care of pups induces long-term changes in the concentration of ERα in particular brain areas,[406] due to epigenetic changes (see Chapter 52) in the ERα gene promoter.[407] Likewise OT, injected systemically during the neonatal period, induces stable changes in the levels of ERα in prairie voles.[408,409] Therefore, regulation of steroid receptors by environmental stimuli working through neurotransmitters is another level of regulation of steroid hormone response in subsets of relevant neurons.

Ligand-Independent Activation of PRs

Steroid hormone receptors, such as ERs and PRs in addition to being activated by binding of their cognate ligand, steroid hormone receptors can be activated by a variety of intracellular signaling pathways.[410,411] Power et al.[412] first demonstrated in vitro that DA agonists also can activate PRs in vitro, which led to studies on alternate pathways to PR activation besides binding of progesterone, including pathways leading to the facilitation of sexual behavior in the absence of progesterone.

Intracerebroventricular infusion of D1 DA agonists substitute for progesterone in the facilitation of sexual behavior in estradiol-primed rats.[413] This facilitation by DA agonists is blocked by infusion of progesterone antagonists,[413] or antisense oligonucleotides directed at the PR mRNA,[369] or in PRKO mice,[373] providing strong evidence that DA facilitates the expression of sexual behavior by indirectly activating PRs in the brain in vivo.

Progesterone and DA both initiate second messenger signaling cascades involving increases in 3′-5′-cyclic adenosine mono phosphate (cAMP) levels, activation of PKA and phosphorylation of the neuronal phosphoprotein, DA and cAMP regulated phosphoprotein-32 (DARPP-32)[414,415] (Figure 50.14). These in turn result in alterations in phosphorylation of other proteins and activation of PRs and/or its coregulators in the hypothalamus. DARPP-32 KO mice express a decreased level of female sexual behavior in response to either progesterone or DA. This suggests that DARPP-32 is involved in ligand-independent activation of sexual behavior via PRs.[414] These results demonstrate the obligatory role that activation of DARPP-32 plays in the regulation of sexual receptivity by PRs, regardless of the route of activation of the receptors.

The mechanism of ligand-independent activation of steroid receptors by neurotransmitters and second messenger pathways provides a possible means by which afferent input from the male facilitates the expression

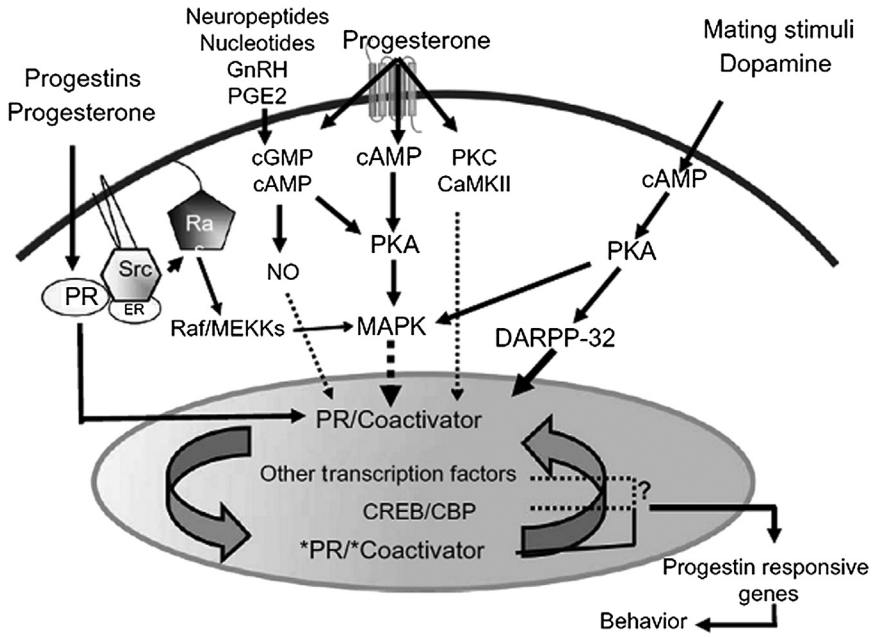

FIGURE 50.14 Cross-talk between progesterone and a variety of second messenger pathways converging on the PR leading to increases in female sexual behaviors. This schematic representation depicts a variety of interacting mechanisms. (1) Progesterone acts via a classic genomic mechanism of action mediated by classic (primarily) nuclear PRs. The ligands bind to their cognate receptors and activate PRs promoting interactions with coactivators; (2) Progesterone acts via second messengers (cAMP, cGMP) and signaling kinases (PKA, PKC, CaMKII), which then activate the MAPK signal transduction cascade, leading to phosphorylation of PRs and coactivators (CREB and/or its associated protein CBP shown here, as well as others); (3) Progesterone and progestins act via the Src kinase pathway to activate the MAPK cascade leading to activation of PR and coactivators. (4) Progesterone acts via the PKA/MAPK/DARPP-32 pathway to induce an increase in phosphorylation of PRs and/or its coactivators. (5) Mating stimuli and D1 agonists may stimulate PKA activation, which then phosphorylates DARPP-32 or MAPK leading to the activation of PR and/or its coactivators. (6) Neuropeptides, nucleotides, prostaglandin E2, and other molecules may act through various receptor- and/or second messenger pathways (cAMP, cGMP, NO) to activate nuclear PRs or other transcription factors. *PR/*Coactivator signifies activated progestin receptor and coactivators. Dashed lines indicate hypothesized, but as yet unproven, direct interactions. *Source: Reprinted from Mani and Portillo,[415] with permission of Elsevier.*

of sexual receptivity in the absence of progesterone. First some background information is necessary. When estradiol-injected, OVX or OVX/ADX rats are exposed intermittently to sexually active males, their level of sexual receptivity increases over the first few hours,[179–181,416–419] even if the ovaries and adrenal glands are both removed, presumably removing all sources of peripheral progesterone.[418] Progestin antagonists block mating-enhancement,[418] suggesting that the cellular mechanism by which the male's mating attempts facilitates sexual behavior is via ligand-independent activation of PRs.[418] It should also be noted that mating-related stimulation results in phosphorylation of DARPP-32 in areas associated with reproduction and reproductive behavior (mPOA, VMH, posterodorsal MEA, and BNST),[420] and a D1 DA antagonist eliminates Fos expression in response to mating-related stimulation in all areas investigated.[421] Although the evidence supports ligand-independent activation of PRs as the mechanism for mating-enhancement of sexual behavior, the possibility that neuroprogesterone is involved cannot be excluded at this time.[422]

Because sexual stimulation induces DA release,[423–426] immediate early gene response (Fos) in PR-containing neurons,[427] and DARPP-32 phosphorylation in relevant areas,[420] it is likely that mating-induced DA activates PRs via ligand-independent activation mechanism. The PR-dependent, ligand-independent relationship is involved in the mechanism by which a variety of compounds that substitute for progesterone facilitates sexual behavior. GnRH,[428] delta opioid receptors,[429] α_1-adrenergic receptors,[430] nitric oxide (NO),[431] MAP kinase,[429] PKA,[432] cAMP,[428] and cGMP[433] as well as prostaglandin E2[428] and epidermal growth factor[434] each facilitate sexual receptivity in estradiol-primed rats by a process that involves activation of PRs. Likewise, ligand-independent activation of PRs may be involved in the mechanism by which methamphetamine enhances the effects of ovarian hormones on female sexual behavior.[435] Thus, many of the numerous signaling pathways that induce female sexual behavior in estradiol-primed rats converge on ligand-independent activation of PRs.

Ring-A reduced metabolites of progesterone, 5α-dihydroprogesterone and allopregnanolone, as well as 5β-reduced progestins, in addition to GnRH, and prostaglandin E2, facilitate lordosis in OVX, estradiol-primed female rats.[436–440] Because these progestins and

hormones have relatively low or no affinity for PRs, it is unlikely that they facilitate lordosis by binding directly to PRs. However, facilitation of sexual behavior by these compounds is inhibited by a progestin antagonist, suggesting involvement of ligand-independent activation of PRs in the process by which they facilitate sexual behavior.[432,437,441] Further, it has been suggested that via ligand-independent activation of PRs, they, like progesterone,[442] then activate the Src/Raf/MAPK signaling pathway resulting in lordosis.[440] Therefore, even some progestins may facilitate the expression of sexual receptivity by ligand independent activation of PRs after which they converge on a signaling pathway in common with a pathway involved in facilitation by progesterone.

As mentioned earlier, progesterone facilitation of sexual behavior is dependent on the PR-A isoform. In contrast, ligand-independent activation of sexual behavior by a D1 DA agonist is reduced, but not eliminated in either PR-A or PR-B KO strains of mice.[374] Therefore ligand independent activation of sexual behavior is dependent on both isoforms. Sexual behavior facilitated by the PKA activator, 8-bromo-cAMP, is eliminated in PR-A KO mice. Therefore, each isoform is involved in the facilitation of sexual behavior, but the importance of each isoform may depend on the mode of activation. Although PR-A seems to have the primary role in most situations, PR-B has a larger role in ligand-independent activation of sexual behavior.

The fact that many neurotransmitters can both influence steroid receptor levels and activate steroid receptors raises questions in the interpretation of many pharmacological studies. Pharmacological experiments performed with the assumption that the drugs are stimulating or antagonizing output of steroid hormone-sensitive neurons. However, it is likely that some of the drugs are influencing steroid receptor-containing neurons, either activating steroid hormone receptors directly or regulating the concentration of receptors.

Ultrastructural Changes Induced by Estradiol and Progesterone

Hormonal Regulation of VMH Structure

The VMH is well established as a key site in the control of female sexual behavior by ovarian steroid hormones, as it has been extensively studied in rats, as discussed above. It should be noted that the borders of the VMH as defined by Nissl stain may not perfectly match the distribution of typical VMH markers, such as ERs, in some species. Nevertheless, the term "VMH" is useful as referring to a brain region with functional similarities across vertebrates, even if its borders and connectivity differ somewhat in various species.

Hormone-induced changes in VMH structure must be understood within the context of the VMH local circuit, which comprises several cell types. Unfortunately, phenotypic markers for these cell types are only partially defined (Table 50.2), and the direction of information flow between these cell types is not yet known. One cell type expresses nuclear ERα and estradiol-induced PR. Given that many studies have used nuclear labeling to identify the ERα-expressing cells, it remains unclear whether these same neurons also mediate the membrane-based effects of estradiol discussed above. Given that these neurons express OT receptors, their long dendrites manifest an estradiol-induced increase in synapses, as discussed below.

A second cell type, are neurons with axonal connections to the periaqueductal gray,[443,444] a critical relay in the control of the lordosis reflex. A small subset of PAG-projecting neurons expresses steroid receptors (15–25%). These neurons have a more elaborate dendritic tree, and estradiol treatment reduces spines on the long dendrites of these neurons.

A third cell type within the VMH is defined in part by the lack of ERα and axonal projections to the midbrain, and the presence of mating-induced Fos expression[427,444,445] These neurons exhibit a robust estradiol-induced increase in dendritic spines. In summary, the VMH microcircuit includes at least three cell types that exhibit differential patterns of neural plasticity after estradiol treatment.

Recent insights into the ovarian hormone-induced remodeling of neural circuits within the VMH in adult female rats provide insights into the neurological control of this behavior. It is worth noting that estradiol-induced synaptic changes have been observed in a number of

TABLE 50.2 Phenotypic Markers of Three Types of Neurons in the VMH Local Circuit

Cell Type	Features
ER/PR expressing neurons	Glutamatergic Co-express enkephalin Dendrite arbor not known Weakly activated by mating Express oxytocin receptors Estradiol increases synapses on LPDs
PAG projecting	Transmitter not known Five to six dendrites Weakly activated by mating Estradiol reduces LPD spines (length)
"Unidentified" (lack ER/PR and no projections to the PAG)	Transmitter not known Three dendrites Activated by mating Estradiol increases spines on short dendrites Estradiol reduces LPD length

other brain systems that are not directly tied to sexual behavior. These include the hippocampus[446] and the PFC.[447] Thus, it seems that estradiol's effect on synaptic organization in the VMH represents a typical mode of action for this hormone.

Estradiol has global structural effects on neurons in the VMH. Hours after OVX animals are treated with estradiol the size of neuronal cell bodies is increased, based in part on the hypertrophy of the nucleus, rough endoplasmic reticulum, and Golgi apparatus.[448] In addition, estradiol changes several parameters within the dendritic arbor (Figure 50.15). The size and shape of a dendritic tree are indicative of its ability to sample and weight afferent inputs. Neurons in the VMH exhibit

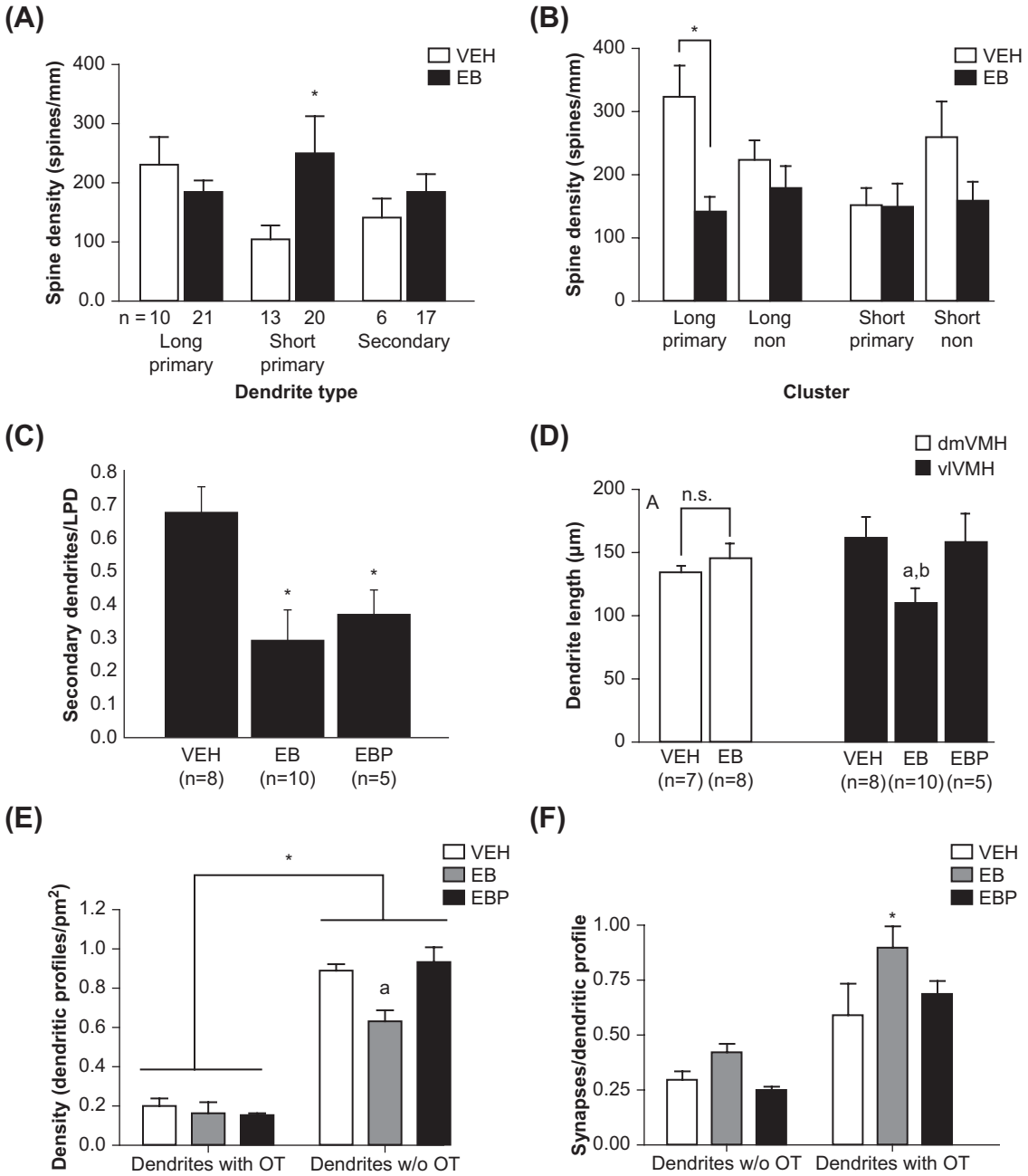

FIGURE 50.15 Complex effects of vehicle (VEH), estradiol (EB), or EB and progesterone (EBP) on the dendritic arbor of VMH neurons. Golgi impregnation and electron microscopy studies show that estradiol increases spines on short primary dendrites (Panel A); reduces dendritic spines on long primary dendrites of projection neurons (Panel B); decreases branches on long primary dendrites (LPD) (Panel C); retracts long primary dendrites (Panel D); selectively removes oxytocin (OT)-negative dendrites from the lateral fiber plexus (Panel E); and increases innervation on oxytocin-positive dendrites in lateral fiber complex (Panel F). *(Source: Adapted from Calizo and Flanagan-Cato,[443] Calizo and Flanagan-Cato,[449] Griffin and Flanagan-Cato,[450] and Griffin et al.[451])* vlVMH, ventrolateral VMH; dlVMH, dorsolateral VMH.

simple dendritic arbors, usually with three or four dendrites. Based on studies using various techniques to quantify neuronal morphology in intact estrous cycling rats and OVX females given ovarian hormone replacement, the number of dendritic spines correlates with hormonal states that increase reproductive behavior.[449,452–454] Thus, estradiol treatment increases local excitatory input onto VMH dendrites (Figure 50.16).

At the same time, long dendrites retract from the lateral afferent zone, a process that is reversed with subsequent progesterone treatment. This regulation of VMH dendrite length was first observed with Golgi analysis[450] and subsequently confirmed with electron microscopy analysis.[451] Ultrastructural evidence suggests that non-OT receptor-bearing dendrites retract, allowing for the transfer of afferent inputs to the OT-bearing dendrites.[455] Thus, estradiol treatment appears to simultaneously heighten local excitatory input and direct extranuclear afferent input to the OT-sensitive neurons, suggesting a substantial hormone-dependent shift in the computational activities of VMH neurons and their outputs that control sexual behavior (Figure 50.16, bottom).

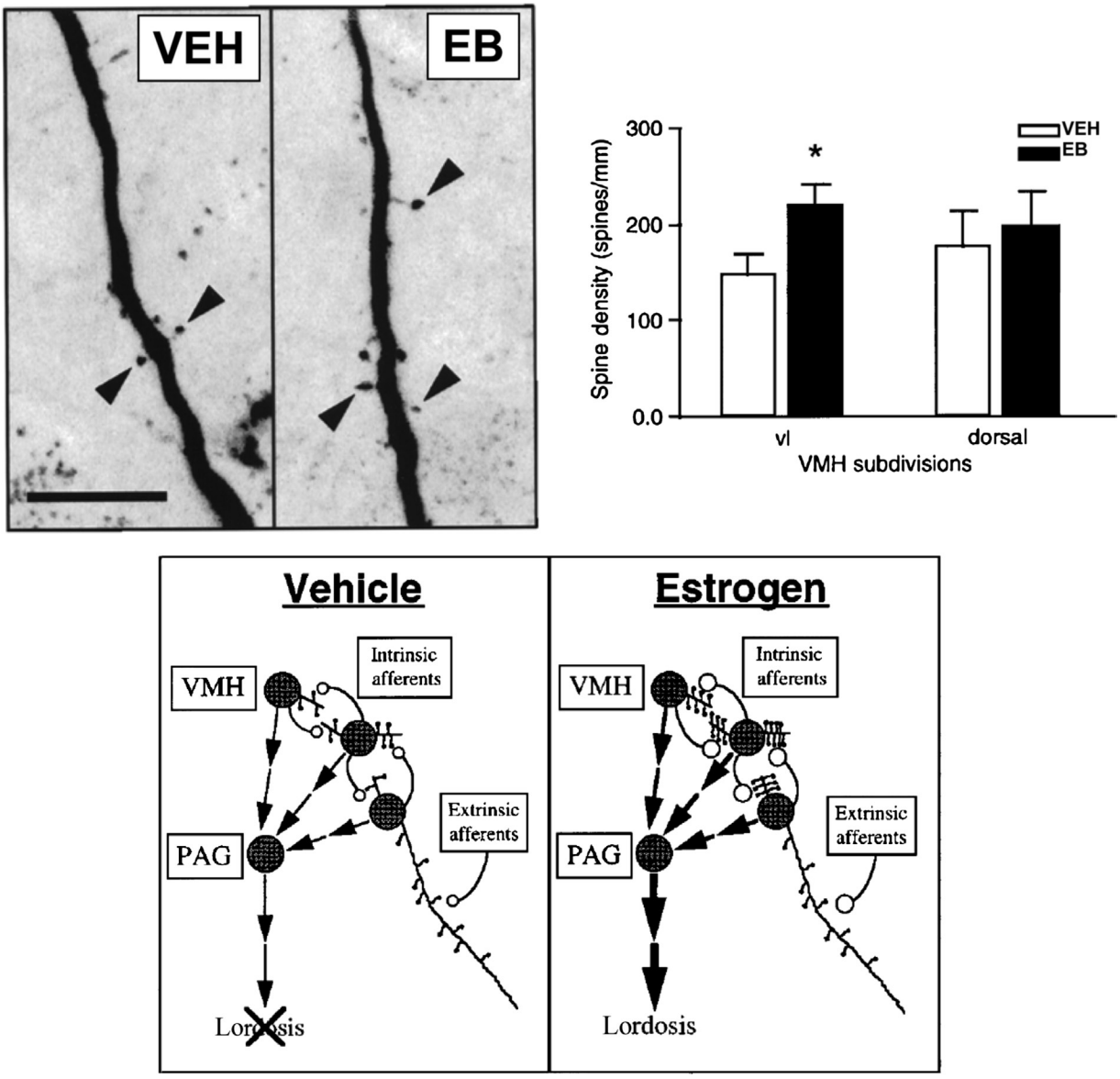

FIGURE 50.16 Top left: Confocal projections of representative female rat VMH dendrites illustrating the effect of estradiol treatment to increase the number of spines. Arrows indicate the locations of representative spines. Scale bar = 10 μm. Abbreviations: VEH, vehicle; EB, estradiol benzoate. Top right: Mean spine densities from vehicle- and EB-treated rats in the ventrolateral (vl) and dorsal regions of the VMH. *$p < 0.05$. Bottom: Model of possible mechanisms of spine induction by estrogen on short primary dendrites in the VMHvl. The output of the increased action of intrinsic afferents would be to increase the activation of the periaqueductal gray (PAG) to reach a critical threshold for lordosis to occur. *Source: Reprinted from Calizo and Flanagan-Cato,[449] with permission from the Society for Neuroscience.*

An important projection from the VMH terminates in the ventrolateral PAG,[456] a critical relay in the motor control of lordosis behavior. Estradiol treatment induced remodeling of synaptic organization in this region as well.[457] In particular, estradiol treatment increases the number of synapses and the number of dense-core vesicles per synapse. As mentioned above, ERα is abundant in the PAG. To date, little is known about hormone-induced plasticity in the medullary reticular formation or spinal motor neurons, although in the cat estradiol increases the innervation of the nucleus retroambiguus to the lumbar spinal cord.[458] It would not be surprising if dendrite structure in the lumbar ventral horn were also changed. Thus, ovarian hormones may promote lordosis by modifying the connections between several nodes in the descending lordosis pathway.

The relevance of synaptic reorganization of the VMH in human sexual behavior has been not been studied due to obvious technical and ethical limitations. However, hormone-induced regulation of VMH dendrite length has been documented in other rodents, namely hamsters[459] and prairie voles,[460] which indicates that it is not a phenomenon unique to rats. Studies in nonhuman primates would be helpful to assess the possible relevance to sexual behavior in women. The synaptic reorganization that has been observed with structural studies is associated with altered activity of VMH neurons (discussed below).

Genitosensory Stimulation Activates Cells with Steroid Hormone Receptors

Many of the cells that respond to mating-related stimulation also contain ERα-ir[444,445] and/or PR-ir[427] suggesting that they are part of the neuronal substrate for integration of hormonal signals with afferent input from the social environment. Mating- or VCS-induced Fos is extensively coexpressed with ERα-ir in the mPOA, bed nucleus of stria terminalis, posterodorsal MEA, midbrain central gray[445] and VMH.[444,445]

Although other areas were not investigated, extensive coexpression of Fos with PR-ir is seen in the mPOA, parts of the VMH, and the ArcN.[427,461] Within the VMH (primarily rostral), mating induces Fos-ir in a number of PR-ir cells that project to the midbrain central gray[462] consistent with a role for these neurons in female sexual behavior. The fact that they are responsive to mating stimulation and express PRs suggests that they may be a substrate for interaction between mating stimulation and PRs.

In some, but not all cells, VCS-induced Fos expression in estradiol-primed, OVX rats requires PRs, suggesting that ligand-independent activation of PRs is an obligatory step in the activation of the c-fos gene.[418,461] Although progestin antagonists block VCS-induced Fos expression in the mPOA, medial bed nucleus of stria terminalis, and caudal VMH, they were without effect in other areas studied, including the MEA, dorsomedial hypothalamus and PVN. Interestingly, a progestin antagonist blocks VCS-induced Fos expression in the rostral, but not caudal, mPOA.[461] Collectively, the data support the interpretation that in cells expressing PRs, VCS induction of Fos expression requires functional PRs; that is to say, neuronal response to particular stimuli seems to be gated by PRs. The mechanism is likely to be ligand independent activation of those PRs by the afferent input. Although olfactory stimuli are of great importance to reproduction and sexual behavior, unlike VCS-induced Fos expression[427] the neurons in which exposure to bedding soiled by male rats induces Fos expression do not contain PR-ir.[463]

In a study of responses of ERα and ERβ cells to mounts with and without intromission, Gréco et al.[464] reported that either mounts alone or mounts with intromissions induced Fos in ERα-ir cells within the rostral mPOA. However, Fos expression in ERα/ERβ coexpressing cells was induced only by mounts with intromission. The pattern in the amygdala was different. In the dorsal posterodorsal MEA, only mounts that included intromission induced Fos expression, and it did so only in ERα or in ERα/ERβ coexpressing cells, not in cells expressing only ERβ. In the ventral posterodorsal MEA, only mounts with intromission induced Fos expression, but the response was restricted to ERβ cells, but not cells expressing ERα. Although work in ERKO mice suggests primary involvement of ERα in female sexual behavior, these results suggest roles for both forms of ERs in integration of hormonal information and information relating to specific types of sexual or genital stimulation.

ARs and Aromatase

Androgens, principally testosterone and 5α-DHT, exert their effects by activating a specific AR. Thus far, a single subtype of AR has been described, a classic member of the steroid receptor superfamily, a hormone-activated transcription factor, with the canonical functional domains of proteins in this superfamily. Thus, upon ligand binding, AR undergoes phosphorylation, homodimerizes, interacts with DNA, and binds to androgen response elements. Transcriptional machinery and cofactors are then recruited to the site to promote or repress gene expression. In addition to this standard genomic mechanism, AR is found in dendrites and axons, suggesting short-term effects on neurotransmission.[465,466] Some brain regions respond to testosterone by converting it to estradiol, via the enzyme aromatase, also known as cytochrome P450 enzyme. Aromatase is found in the smooth endoplasmic reticulum of some populations of neurons. Thus, aromatase activity would direct

TABLE 50.3 Brain Regions Involved in Female Sexual Behavior that Contain Dominant ER Subtypes, AR, and/or Aromatase Expression

	ER-Alpha	ER-Beta	Both	AR	Aromatase
Amygdala	Amygdalohippocampal area		Medial nuclei	Medial nuclei	Medial nuclei
			Cortical nuclei	Cortical nuclei	Cortical nuclei
Septum	Subfornical organ		BNST	BNST	BNST
				Lateral septum	
Hypothalamus	mPOA	PVN	mPOA	mPOA	mPOA
	AVPV				
	ARC			ARC	
				PVN	PVN
	VMH			VMH	VMH

circulating testosterone to act via ERs rather than AR. Therefore, the expression of both AR and aromatase will be considered for a full view of testosterone action in the female brain.

Multiple techniques and animal models have helped describe the distribution of ARs in the vertebrate brain, including rats, mice, hamsters, and nonhuman primates; however, there is surprisingly limited information focused on adult female mammals. Available evidence suggests that females and males have similar neuroanatomical distributions of AR, with somewhat different levels in females. Table 50.3 includes a listing of the brain regions with the highest levels of ARs, based on these studies.

The first studies delineating the anatomical distribution of testosterone binding used steroid receptor autoradiography with 3H-testosterone injected systemically. Abundant testosterone binding was reported in the preoptic area, amygdala, and especially the VMH, in female rats[467,468] and female rhesus monkeys.[469] The molecular cloning of AR led to the development of antibodies that could be used in immunocytochemical procedures, although initial reagents were plagued with conflicting results. The messenger RNA for AR has been mapped with in situ hybridization techniques,[269] and these results nicely complement the distribution described with autoradiography. Studies in juvenile females indicate that AR mRNA is abundant in several brain regions associated with female reproductive behavior, including the lateral septum, BNST, mPOA, and VMH.[470] Although it has not been studied extensively, it appears that in some brain regions that co-express AR and ER, many neurons express AR only, but most neurons that express ER co-express AR.[471]

As with AR, most studies on aromatase in the brain have focused on developing males rather than adult females, but some basic information exists. Aromatase has been documented with in vitro enzyme activity assays of discrete brain regions, including the BNST, mPOA, MEA, and VMH.[472] This analysis also compared the levels of aromatase and AR in adult females. A striking finding was that AR binding activity is highest in the VMH, where aromatase activity is the lowest. The relatively low level of aromatase activity in the VMH is reflected in relatively low levels of aromatase messenger RNA.[473] This finding suggests that in the VMH testosterone may be more likely to act through the AR than the ER in females. Ovarian hormones do not regulate aromatase activity in female rat brains.[474] Overall, transcripts for aromatase are found in the BNST, the mPOA, the MEA, and the VMH in females.[475]

In summary, ARs are expressed in the female brain, including in brain regions that promote female sexual behavior. Although some brain regions may convert testosterone to estradiol, androgen-specific actions that affect female sexual behavior seem likely, especially in the VMH. Although not well studied yet, it is possible that 5-alpha reductase is also expressed in the VMH in female mammals, as it is in green anole lizards.[476] This enzyme converts testosterone to the more potent androgen, DHT. If so, females would have the potential to compensate for their relatively low circulating levels of testosterone by locally enhancing androgen action. Although there is little information about the role of androgens in female sexual behavior, ARs appear to be poised to contribute.

Hormone Replacement Therapy in Women

A decline in sexual arousal, desire, and/or activity occurs following surgical and natural menopause. Surgically menopausal women, induced by bilateral oophorectomy with or without hysterectomy experience a sudden and drastic decline in sexual desire and arousal.[126–129] Significant improvement in desire and arousal occur following adequate hormone replacement regimens,

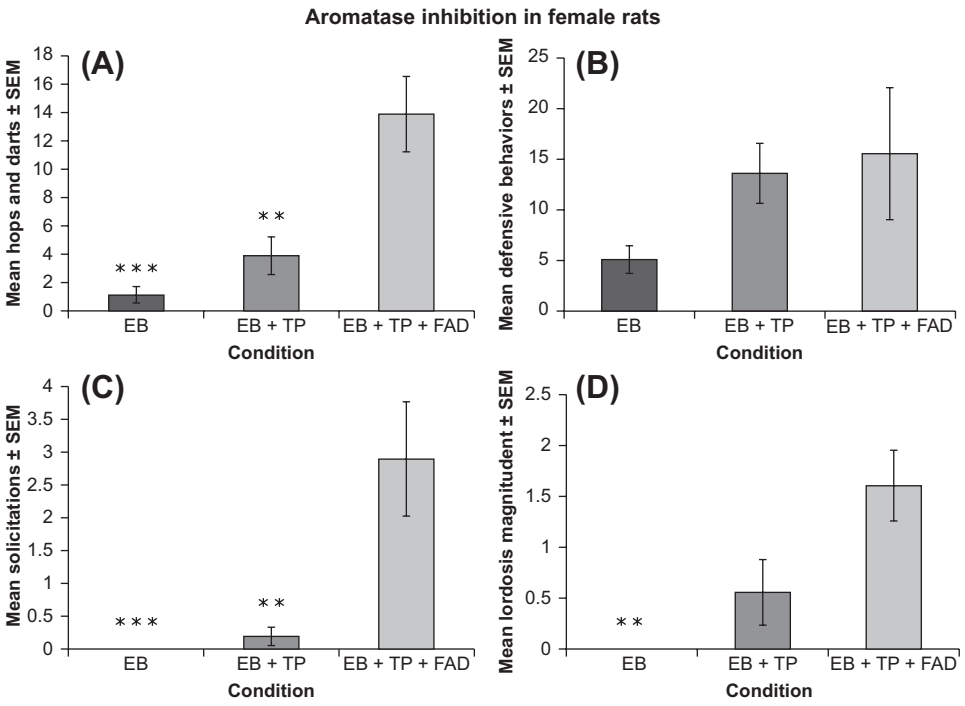

FIGURE 50.17 Hops and darts (A), solicitations (C), defensive responses (B), and lordosis reflex magnitudes (D) of OVX rats primed with estradiol benzoate (EB) alone, EB + testosterone propionate (TP), or the combination of EB + TP along with the aromatase inhibitor fadrazole to block conversion of testosterone to estradiol, thus augmenting the effect of the exogenously-administered TP on behavior. *(Source: From Rosenbaum, Jones, and Pfaus.[478])* $^{**}p < 0.05$, $^{***}p < 0.01$, from the EB + TP + FAD-treated rats.

particularly replacement with exogenous estrogens in combination with testosterone[126,127,131,133,135–139] relative to estrogen treatments alone. Although co-administration of estrogens and progestins (notably medroxyprogesterone acetate) also increased sexual arousal and desire, this formulation increased overall risk for secondary disease factors in the Women's Health Initiative randomized trials.[477]

An unresolved question is how co-therapy with estrogens and androgens works and why it offers greater therapeutic value over estrogen replacement alone. There are at least three possible scenarios. The first is that the induction of SHBGs by exogenous estrogens would likely decrease free estrogen concentrations, thereby limiting their ameliorative action in the brain and periphery. The addition of androgens like testosterone to this would compensate by binding to SHBGs with higher affinity, thus allowing more free estrogens to get to target tissues. The second possibility is that testosterone induces actions on its own through binding to ARs in the brain and periphery. Indeed, a significant proportion of untreated postmenopausal women reported increases in sexual desire and activity following double-blind administration of testosterone for 6 months through a transdermal patch that released approximately 300 μg/day of testostereone.[137] This treatment increased free testosterone to within normal physiological premenopausal levels without any concomitant increases in plasma estradiol or SHBG levels. Placebo control conditions did not induce this increase. These data suggest that testosterone alone is able to reverse the decline in sexual arousal and behavior in postmenopausal women. However, the presence of aromatase in the brain could convert exogenous testosterone into estradiol locally in different hypothalamic or midbrain regions, despite no increases in plasma estrogens. Long-term OVX rats treated systemically with the aromatase inhibitor fadrozole and given hormone replacement with the combination of estradiol and testosterone displayed significantly greater numbers of appetitive solicitations and lordosis responses compared to females given the combination of estradiol and testosterone alone (Figure 50.17). Females treated with estradiol alone displayed low numbers of solicitations and lordosis responses.[478] Thus, blocking the aromatization of testosterone into estradiol induces a greater facilitation by testosterone of both appetitive and consummatory aspects of female sexual behavior relative to estradiol alone or the combination of estradiol and testosterone. This suggests strongly that testosterone acting at ARs facilitates female sexual behavior. The final possibility of course is that both testosterone and estradiol exert distinct actions on ARs and ERs, respectively, that combine to facilitate sexual desire and behavior.

NEURAL ORGANIZATION

As mentioned above, sexual behavior has a beginning, middle, and end, each of which is controlled by different, but integrated, brain systems. The function of many of those systems is altered by steroid hormones, such that sexual incentive stimuli come to the foreground

of attention and action. These neural systems must be able to process rudimentary sensory components of sexual stimulation (e.g., olfactory, visual, somatosensory, auditory), and sum them into Gestalts or "wholes" that represent contextual and/or discrete unconditioned incentives (e.g., suitable receptive partners, partner odors, individual facial features, etc.) or unconditioned inhibitors (nonreceptive partners, predators, or parents, or competing incentives like food if hungry), and do the same for conditional incentives or inhibitors (e.g., neutral cues that become predictors of the unconditioned incentives, e.g., a favorite sex toy, odor or place cue associated with sexual reward, or inhibitors, e.g., same stimuli associated with thwarted sexual behavior or sexual nonreward). They must be able to compute competent responses to those cues so that sexual behavior is timed correctly and appropriate to the circumstances, and automate those responses so that they become more optimal with experience (e.g., faster initiation of solicitations in the presence of highly valued partner cues). At each level of analysis, there are excitatory and inhibitory neurochemical systems that regulate the intensity of the perceived incentive or inhibitory stimuli and that modulate the timing and intensity of the responses.[35] These are also modulated by steroid hormones and by feedback from positive and negative sexual experiences (see Section Consequences of Sexual Stimulation).

Excitation, Inhibition, and Disinhibition of Sexual Responses

The notion of separate, but interactive, neural systems for behavioral excitation and inhibition (Figure 50.18) goes back to the work of early neurophysiologists like Sechenov, Sherrington, and Pavlov, and more modern psychologists like Gray, who applied the idea to the study of fear and anxiety.[480] It has important implications for sexual behavior because it posits that behavior can commence either due to direct excitation or through a process of disinhibition. This concept was advanced further by Bancroft and Janssen[481] and Perelman,[479] who presented dual control models of human sexual response in which the net expression of sexual behavior is based on the influence of excitatory and inhibitory mechanisms in the brain and periphery, set around a "sexual tipping point". As in Gray's theory, this model stressed the adaptive nature of both excitatory and inhibitory processes. For example, the adaptive nature of sexual excitement would drive individuals to seek out sex partners for reproductive or reward purposes. The adaptive nature of sexual inhibition would guard against situations that threaten the individual, including chronically stressful life events. It would also be important to keep the optimal expression of behavior constrained to the "right time", as in the case of females that display sexual behavior only during a periovulatory period, when they are most likely to become pregnant. Bancroft and Janssen viewed the propensity for sexual excitement or inhibition as an individual tendency based on the genetic makeup and/or behavioral expectations of the individual: Those whose propensity for central inhibition of sexual response is too high have increased vulnerability to sexual dysfunction, whereas those whose inhibitory propensity is too low would be more likely to engage in hypersexual or high risk sexual behavior. Indeed, the study of sexual inhibition is also critical if we are to understand how certain events or drugs like alcohol, cocaine, or amphetamine, may induce sexual disinhibition and the propensity to engage in risky sexual behaviors.[482]

Excitation

We may view excitation from autonomic arousal and genitosensory/erogenous stimulation as a "bottom-up" phenomenon in which individual sensory modules come together at higher levels of processing. This occurs in the thalamus, but also in each domain of the hypothalamus, limbic, system, and cortex, that make up the excitatory sexual system of the brain.[35] At the hypothalamic level, genitosensory and olfactory information is integrated in both the mPOA and VMH, which have outputs to the PVN, SON, and ArcN of the hypothalamus. In turn, those regions control the release of OT, vasopressin both in brain and from the posterior pituitary, and the release of melanocortins (MCs), opioids, and corticotrophin releasing factor (CRF) from the anterior pituitary. The mPOA also sends lateral efferents to the VTA, which stimulate DA neurons and DA release in mesolimbic and mesocortical terminals, such as the NAc, ACC, lateral septum, corticomedial amygdala, and mPFC. Thus, the mPOA is well situated to "drive" the DA-mediated incentive motivational system in the presence of salient unconditional and conditional external sexual cues, and also to register and perhaps link those cues to genitosensory and autonomic input. In addition, noradrenergic inputs to the hypothalamus coming from the locus coeruleus are themselves stimulated by the reticular activating system, which is stimulated by general autonomic and somatic inputs from the spinal cord. For females, sensitivity to sexual arousal is enhanced during ovulation, and thus excitatory systems in the CNS appear to require steroid hormone priming.

Estradiol regulates a variety of neurotransmitter and second messenger systems in brain areas involved in sexual behavior.[483,484] Estradiol priming induces the synthesis of α_{1B}-adrenergic receptors in the VMH[485,486] and augments the release of DA in the striatum, NAc, and mPOA during copulation.[90,425,487,488] Extracellular DOPAC (3,4-dihydroxyphenylacetic acid), a DA metabolite, increases in the mPOA in the afternoon to the early evening of proestrus, around the time that sexual

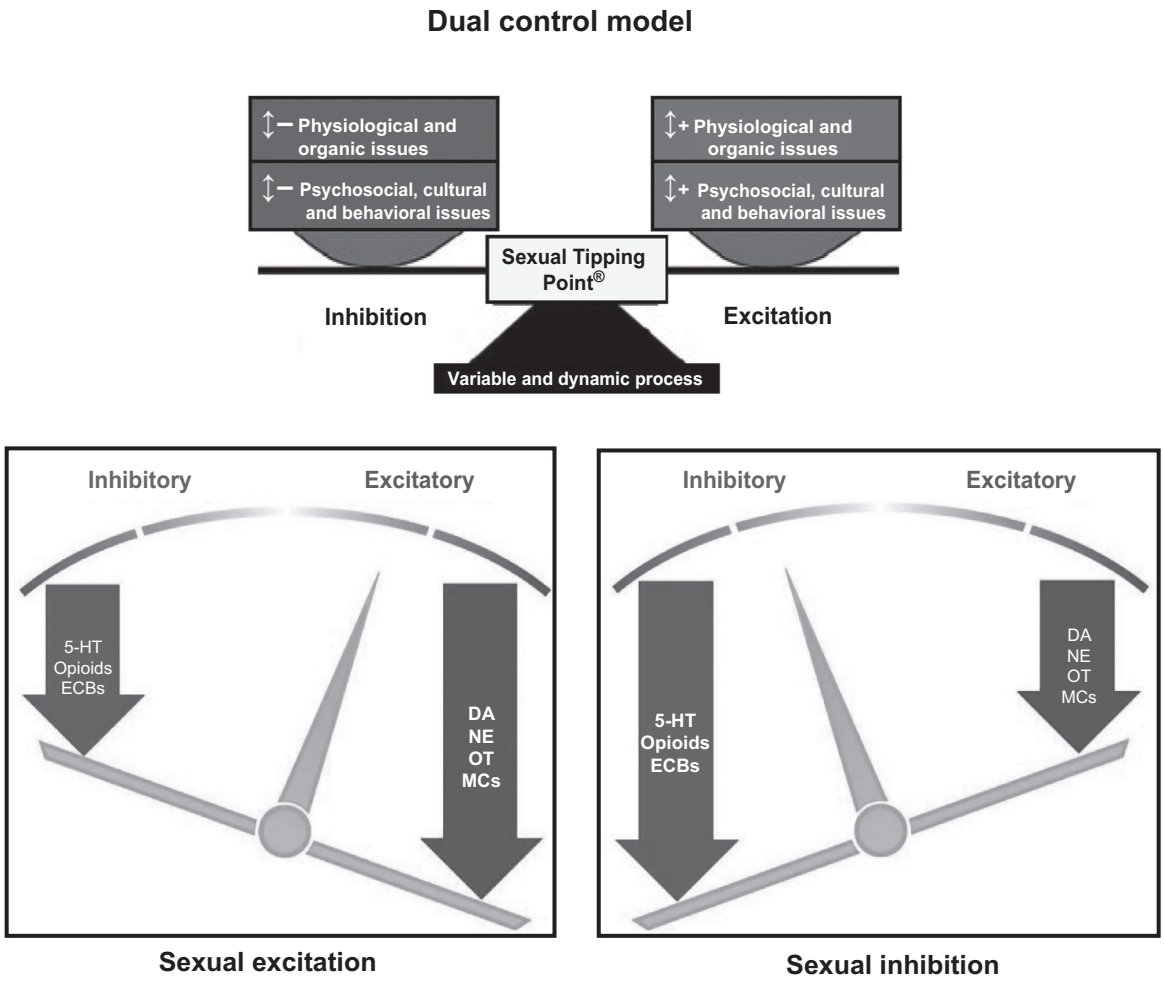

FIGURE 50.18 **Dual control model of sexual excitation and inhibition around a "sexual tipping point".** Activation of excitatory neurotransmitters, such as dopamine (DA), norepinephrine (NE), oxytocin (OT), and melanocortins (MCs), within midbrain, hypothalamic, and limbic regions occurs in response to steroid hormones, sexual incentive cues, and sexual stimulation. Activation of inhibitory neurotransmitters, such as serotonin (5-HT), opioids, and endocannabinoids (ECBs) in cortical, limbic, hypothalamic, and brainstem regions occurs during and after orgasm or in response to stress or aversion, resulting in a net decrease in excitatory tone. *Source: Adapted from Perelman*[479] *and Pfaus.*[35]

behavior is activated.[482,489] DA is also released within the mPOA in OVX females treated with EB and progesterone, however this release is not detected in females treated with EB alone.[424,490] Hormone dependent differences also occur with the application of DA agonists to the mPOA, such that in females primed with EB-alone, D2 receptor subtype agonists facilitate sexual behavior, whereas in EB + progesterone primed females D1 receptor subtype activation facilitates sexual behavior.[482,491,492] Tonic DA release activates D2 receptors, whereas phasic DA release stimulates D1 receptors. Thus, priming with EB-alone may tonically activate D2 receptors, whereas subsequent progesterone administration may stimulate phasic DA release within the mPOA, and activate D1 receptors, to facilitate sexual behaviors. Estradiol also stimulates the synthesis of MC type four receptors in the hypothalamus[493] and synthesis of proopiomelanocortin (POMC) in ArcN neurons[494] and stimulates the synthesis of cholinergic receptors and enkephalin within the VMH.[495,496] GABA and glutamate activation are also stimulated by estradiol in these regions (see below).

Inhibition

Inhibitory synapses make up a large part of the CNS, and local inhibitory networks can hone a response by eliminating competing responses that would interfere with it (e.g., as happens in the visual system with lateral inhibition by amacrine cells[497]). Such inhibition can be observed when behavior comes in bouts or phases, or in Masters and Johnson's EPOR model would be consonant with the "R" phase, a period of postorgasmic refractoriness in which further sexual interest is diminished.[21] Local inhibition can also play a role in the timing of behavior to make it occur only during optimal periods. Female desire and lordosis behavior are obvious examples, and the role of steroid hormones may well be

to suppress the behavior during reproductively nonoptimal (nonovulatory) periods.

General behavioral inhibition, however, is typically viewed as a "top-down" phenomenon involving the cognitive process of "executive function".[498] Animals always have to choose among different drives and motivations (e.g., between feeding and copulation), and indeed between several possibilities per motivational system, to achieve an optimal outcome. The mPFC organizes this by creating behavioral hierarchies based on expectancies, planned actions, and calculations. The mPFC, (and likely other cortical areas) therefore, must inhibit a complex and ongoing interplay of motor tendencies to arrive at planned and sustained actions. People or rats with disrupted prefrontal function, either due to lesions or neurochemical imbalance, have great difficulty focusing attention on tasks, are unable to inhibit competing responses, and experience retroactive and proactive interference.[498] With regard to sexual behavior, such top-down inhibition can be activated as "morality" and would be based on a cultural value system that imposes "right" and "wrong" on certain behaviors, such that some that feel good are "right" and can be experienced without guilt, whereas others are "wrong" and carry the weight of guilt and/or rule of law against them.[28,35] This type of inhibition gives rise to the classic "approach-avoidance" conflict, where the expectation of reward drives the desire, but the inhibition imposed by the real or perceived aversive consequences of engaging in sexual activity blunts the initiation of behavior. Such inhibition may well lie at the root of the inhibited sexual response experienced by women who are not taught to express their sexual desires without some form of guilt. Such inhibition would likely be reinforced if women experienced sexual nonreward during copulation, and such reinforced inhibition would likely overlay itself on desire components to suppress them directly. Some women may be more susceptible to this type of inhibition than others. Accordingly, the "prosexual" nature of drugs such as alcohol, cocaine, and methamphetamine may be a function of their ability to disinhibit such suppressed sexual responding.

Refractory inhibition that comes after orgasm involves the activation of at least three neurochemical systems: opioids that induce pleasure, euphoria, and ecstasy; endocannabinoids that induce sedation; and serotonin that induces satiety.[35] The reward states induced by CLS, VCS, or paced mating in rats appear to be independent of steroid hormone priming, although the priming is necessary to activate the excitatory systems that bring about the behavior in the first place.[28,162] At present it is not known where such inhibition actually takes place. Indeed, the mPOA and VMH have receptors for all three transmitter systems, indeed for all three opioid systems. Activation of delta opioid receptors in the mPOA inhibit lordosis,[499] whereas activation of mu opioid receptors in the VMH inhibits lordosis.[485] Inhibition also comes in the form of estrus termination. This is discussed in more detail below.

Disinhibition

As noted above, certain prosexual drugs can disinhibit sexual responding, but only in individuals with sexual inhibition. This has been modeled in male rats.[90] Male rats trained not to copulate with sexually nonreceptive females will attempt copulation with them under the influence of alcohol. Cocaine, amphetamine, and methamphetamine can do the same in males,[482] and Frohmader et al.[490] provided evidence that methamphetamine could do this after males were inhibited from making sexual approach responses toward scented, sexually receptive females using LiCl-induced gastrointestinal distress as the inhibitory outcome. Alcohol and cocaine stimulate appetitive behaviors in females primed with estradiol alone.[482] Intermittent amphetamine or methamphetamine administration sensitizes sexual approaches and solicitations in female rats[435,500] and increases Fos activation in the MEA and VMH following copulation.[435] However, methamphetamine treatment also makes female rats less selective in preference for particular males.[501]

Disinhibition may also occur as a function of hormone priming. Systems that normally maintain inhibition over female sexual behavior during nonovulatory periods likely must be inhibited to allow the behavior to occur. This appears to be the case for some systems in the VMH that help to time the behavior and that help to bring about estrus termination (see below). Thus the brain is set up with modules that gather and interpret sensory input and generate competent motor outputs at optimal times. This allows reward systems to be activated when females engage in the right behaviors at the right times, and allows such behavior to optimize the reproductive outcomes. The fact that paced copulation is both rewarding to females and results in stronger copulatory stimulation from males, leading to a greater chance of successful impregnation, is a good example of the intricate timing systems that blend the two. Far more is known about the neural and neurochemical control of lordosis than appetitive sexual approach, solicitation, or pacing behaviors. These are outlined below.

Sexual Approach Behaviors

Mesolimbic DA is involved in the sensitization and crystallization of incentive responding,[38] especially within terminals in the NAc. Microdialysis studies have shown that DA in the NAc increases during copulation in OVX female rats or hamsters primed fully with EB and progesterone. In rats the increase is approximately 150%

of baseline when females are presented with a gonadally intact, sexually vigorous male rat behind a screen, and increases to approximately 180% when the screen is removed and copulation ensues.[488] The temporal resolution of microdialysis (e.g., 10-min samples), however, does not permit specific behaviors to be correlated with the rise in DA especially during copulation. However, the degree of release is positively correlated with the number of attempts made by the female to nose-poke through the wire-mesh divider. DA release increases more in the NAc and dorsal striatum in hormonally-primed females that must make an operant response to gain access to males compared to females that do not,[487] suggesting that mesolimbic and striatal DA release helps orient the female toward a sexually active male and make approach responses in anticipation of sexual stimulation and reward.

Solicitations

The pioneering work of Wallen et al.,[57] McClintock,[50] and Erskine[49] refined Beach's[39] general category of "proceptive" behaviors in female macaques and rats into species-specific descriptions of sexual approach, solicitation, and pacing. In rats, solicitations could be defined as full (headwise orientation to the male followed by a runaway) or partial (hops and darts, and perhaps also ear-wiggles) depending on how close in proximity the female was to the male, and whether she had room to run away (e.g., Figure 50.5). Pacing could be operationalized as an intermount or inter-intromission interval imposed by the female, and could be assessed in open fields or as exits and returns to the male's side in a unilevel pacing chamber (e.g., Ref. 49), or level changes per mount or intromission in bilevel pacing chambers (e.g., Ref. 75). Female mounting of sexually sluggish or naïve males was considered by Beach[106] to be a "super-solicitation behavior" that would allow females to "show" the male what they wanted. Such behavior has been studied extensively in rats using sexually experienced OVX females primed with EB alone or EB + progesterone and given access to castrated male rats.[221,502–504]

Hoshina et al.[505] were the first to show that axon-sparing excitotoxic lesions of the mPOA abolished both full and partial solicitations, but enhanced lordosis, in sexually-experienced OVX rats primed with EB and progesterone. Lesions of the mPFC inhibited the temporal patterning of full solicitations, disrupting the runaway component.[506] In contrast, lesions of the lateral septum or VTA did not alter the frequency of hops and darts.[507,508] Lesions of the mPOA, VMH, or MEA, abolished the mounting of sexually sluggish males in OVX rats primed with EB + progesterone, whereas crystalline implants of estradiol to the VMH, but not the mPOA or MEA, induced the behavior in OVX rats.[221]

Role of Incertohypothalamic DA

As with lesions of the mPOA, systemic administration of DA antagonists such as haloperidol abolish solicitations but augment lordosis.[509,510] This effect does not appear to be mediated by mesolimbic DA, as 6-OHDA lesions of VTA DA neurons projecting to the NAc did not alter solicitations in small chambers.[511] However, DA in the mPOA plays a key role in the stimulation of solicitations, with D2 receptor activation facilitating solicitations in OVX rats treated with EB alone, and D1 receptor activation facilitating solicitations in OVX rats treated with EB and progesterone.[491,492] DA projections to the mPOA originate in the zona incerta (ZI). DA turnover in the ZI increases with estradiol administration.[512] Extracellular DOPAC, a DA metabolite, increases in the mPOA in the afternoon to the early evening of proestrus, around the time that sexual behavior is activated.[489] DA is also released within the mPOA in OVX rats treated with EB and progesterone, but not in rats treated with EB alone.[81,160,424,513] Infusions of SCH-23390, a D1 antagonist, to the mPOA of OVX rats primed with EB + progesterone significantly reduced solicitations selectively.[492]

Role of MCs

MCs like α-melanocyte stimulating hormone (α-MSH) are derived from POMC, a precursor peptide made largely in the ArcN from which is also derived the opioid β-endorphin and ACTH.[514] α-MSH synthesis is stimulated by estradiol[515] within ArcN neurons. Projections of those neurons terminate in the mPOA and secrete α-MSH. Two MC receptors exist in the brain, MC3 and MC4, of which the latter is found in the mPOA. Bremelanotide (formerly PT-141) is a synthetic analogue of α-MSH and is the active metabolite of melanotan-II (MT-II). Systemic administration of bremelanotide stimulates solicitations selectively in female rats primed with EB or EB + progesterone.[516] MT-II produces a weaker effect,[517] although five consecutive days of MT-II administration produces an effect similar to bremelanotide in magnitude.[518] The enhancement of solicitations by bremelanotide is duplicated by infusions to the lateral ventricles or mPOA, but not the VMH.[30] Systemic bremelanotide also stimulates DA release in the mPOA, but not NAc or dorsal striatum of OVX rats treated with EB and progesterone. Finally, the stimulation of solicitations by systemic bremelanotide can be reversed by infusions of a selective MC4 antagonist (HS019) to the mPOA, but not VMH.[30] It can also be reversed by infusions of the D1 antagonist SCH-23390 to the mPOA,[30] suggesting that incertohypothalamic DA terminals in the mPOA contain MC4 receptors that drive DA release which in turn stimulates solicitations by acting on D1 receptors in this brain region. Thus the integration between MC and DA systems in the mPOA is a critical component in the regulation of solicitations.

Glutamate in the Ventrolateral VMH

Full and partial solicitations are also under the control of the VMH. In a series of studies, Georgescu et al.[519,520] investigated the inhibitory role of glutamate neurons in the ventrolateral VMH on the sexual behavior of female rats. Both full and partial solicitations were inhibited dramatically by infusions of glutamate, and also by infusions of the selective ionotrophic receptor agonists AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainite to sexually experienced OVX rats primed with EB and progesterone.[519] Conversely, infusions of the dual AMPA/kainite receptor antagonists CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and DNQX (6,7-dinitroquinoxaline-2,3-dione) to the ventrolateral VMH of sexually experienced OVX rats primed with EB alone increased full and partial solicitations.[520]

Differential Effect of Opioid Receptors

Pfaus and Pfaff[521] reported that infusion of the delta opioid agonist DPDPE, but not the kappa opioid agonist U50-488h, or the mu opioid agonist DAMGO, to the lateral ventricles of sexually experienced OVX rats primed with EB alone or EB and a low dose of progesterone (to induce moderate lordosis and low solicitations) increased solicitations in bilevel chambers significantly over control infusions. It is not known at present where in the brain this facilitation of solicitations may occur.

Taken together, these data suggest that the connections between the VMH and mPOA are critical in the regulation of solicitations relative to lordosis. This is evident from the fact that mPOA lesions suppress solicitations but augment lordosis, and is an example of the kind of mutually-exclusive behavioral patterns discussed by Konorski.[522] Females cannot hold a lordosis posture while making a forward-directed solicitation and vice-versa. Mutually interactive inhibitory subsystems in the two regions likely regulate the timing of solicitations and lordosis.

Pacing Behavior

The ability of female rats to pace the copulatory contact is critical for the timing of intromissions. This timing leads to distributed stimulation of the clitoris, vagina, and possibly also the cervix, stimulation that female rats find rewarding,[81,160,513] and that facilitates pregnancy or the induction of pseudopregnancy,[49,523] and partner preference.[77,78] Female rats pace at a faster rate early in the copulatory interaction, but with successive ejaculations, the number of level changes per mount in bilevel chambers (Figure 50.5) increases dramatically, thereby increasing male inter-intromission intervals.[75] A similar effect is also observed in large open fields, and is more pronounced in wild rats compared to domesticated laboratory rats.[50] In unilevel pacing chambers, the latency to return to the male's side after mounts, intromissions, and ejaculations shows a progressive increase in time, which increases with successive ejaculatory series.[49,74] Low, steady rates of pacing are induced in OVX rats by EB and progesterone, whereas OVX rats administered EB alone show higher rates of pacing and rejection responses. Fully primed female rats tested in small chambers that offer no escape from the male also display rejection responses (e.g., rearing and boxing postures[524]) at a higher rate than females tested in chambers where they can escape. It would appear that females use rejection responses to pace the copulatory contact if they cannot do so otherwise. However, in those conditions, copulation does not induce reward and does not facilitate pregnancy or pseudopregnancy (see below).

Very little work has been done examining the neural control of pacing. However, Xiao, Kondo, and Sakuma[525] found that bilateral radiofrequency lesions of the lateral septum, but not the mPOA, disrupted the pattern of female exits from the male side of a 3-hole unilevel pacing chamber. In general, females with lateral septal lesions did not leave the male side after mounts, and took significantly more time than sham-lesioned females to leave the male side after intromissions or ejaculations. This suggests that activation of the lateral septum by copulatory stimulation is an important component of the regulation of pacing. It is not known whether such lesions would facilitate or inhibit the development of place or partner preferences. Guarraci, Megroz and Clark[526] found that cell body lesions of the mPOA, but not MEA or BNST, increased the intromission and ejaculation contact-return latencies of females in pacing chambers, and increased the number of withdrawals from the male's side following intromissions, suggesting that mPOA activation is critically involved in keeping females with males, consistent with its role in solicitations. Interestingly, clitoral anesthesia induced by lidocaine injections also increased the number of exits and returns displayed by OVX, EB and progesterone-primed rats in a 4-hold unilevel pacing chamber, decreased the amount of time spent with males, and increased the ejaculation return latency.[163] This indicates that CLS maintains low rates of pacing, which increase if the stimulation is blunted. As noted above, polysynaptic clitoral afferents project to the mPOA and CLS activates Fos in medial regions of the mPOA of female rats. It is not known if the reward induced by CLS is eliminated by mPOA lesions, although such lesions disrupt the reward induced by VCS[527] (see below).

Lordosis

More is known about lordosis than any other behaviorally relevant spinal reflex with supraspinal control, except perhaps the control of penile erection[172] and the conditioned eye-blink response.[528,529] The seminal work

of Pfaff[32,62] merged electrophysiology with anatomy and pharmacology and behavioral neuroendocrinology with molecular biology to determine the modular supraspinal components of the lordosis reflex and its control by ovarian hormones acting on receptors in the brain (Figure 50.19). The action of steroids on those receptors alters the brain's neurochemistry and activates the excitatory sexual systems reviewed above. This activation then alters the reaction of the female to incentive sexual stimuli, which leads her to being attracted to male cues. Similarly, it results in approaches and solicitation of sex from the male and, upon simple palpation of the flanks and perineum, the female no longer reacts with violent intense rejection but rather with ear wiggles and sexual receptivity. Thus, the behavioral reflex is linked by the mechanics of gene transcription and translation in critical hypothalamic circuits to the timing of ovulation so that the two can co-occur.

Coordinating physiological responses with behavior requires timing. Hormone priming essentially sets up a timing system for lordosis onset and offset. Although some of the neurochemical systems involved in onset are part of the excitatory system, others are actually inhibitory and keep lordosis from occurring too soon. Likewise, activation of the excitatory neurochemical systems keeps the potential for lordosis on long after the female has taken herself out of the mating game. In general, the hypothalamic targets of estradiol include neurosecretory neurons such as GnRH and DA neurons that affect both pituitary secretion and sexual behavior, and local circuitry neurons such as POMC, GABA, and glutamate.

Given the pivotal role that the VMH and the surrounding area plays in the control of lordosis behavior, our discussion will focus on this region, with the understanding that estradiol also affects the neurochemistry within the broader network of brain regions with ERs. Based on the global effects of estradiol and progesterone on VMH activity, as described below, ovarian hormones clearly transform neural processing in this brain region.

Lordosis Onset

Activation of Excitatory Systems

Estradiol and progesterone activate gene expression for a number of neurochemicals systems in the hypothalamus, most notably in the mPOA and VMH, which stimulate female sexual behaviors including lordosis (Figure 50.20). This includes an upregulation of specific neurotransmitter receptors by estradiol, e.g., progestin preceptors, OT receptors, adrenergic α_1 receptors, muscarinic receptors, MC three and four receptors, and delta opioid receptors, GnRH receptors, GABA-A receptors, and D1 DA receptors.[62,484] This changes how the circuit between the two regions operates. Enzymes are also upregulated in this system, including NO synthase, prostaglandin-D synthase, and DBH, leading to an upregulation of the end products. NO in particular is a critical and ubiquitous player in neurotransmitter release, so its upregulation is important in helping to set the stage for the upregulated neurochemical systems to play a functional role in the generation of the behavior. Pharmacological studies help to confirm the role played by these neurochemical substrates:

GABA AND GLUTAMATE

Throughout the central nervous system, many synapses include excitatory or inhibitory amino acids as neurotransmitters, namely glutamate and GABA, which bind to ionotropic receptors, allowing brisk changes in the postsynaptic potential. Both glutamate and GABA, and their ionotropic receptors, are present in the VMH.[531–538] Several methodological approaches have demonstrated ovarian hormone regulation of these neurotransmitter systems and implicated their actions in the VMH in the control of lordosis behavior (reviewed in Refs 536,539–541), providing insight into the neurological control of mating behavior.

Glutamate neurotransmission in the VMH has been studied with many technical approaches. Double-label immunohistochemical studies indicate that the ERα-expressing neurons in the VMH are glutamatergic, based on the colocalization of the vesicular glutamate transporter-2.[542] Ultrastructural analysis of the VMH has revealed abundant excitatory inputs, including axospinous synapses, which comprise approximately one third of the total VMH synapses. Based on hormone replacement regimens, estradiol treatment increases glutamate levels in the VMH, and this is reversed with subsequent progesterone treatment.[543] The number of AMPA and NMDA (N-methyl-D-aspartate) receptor subunits are likely to increase with estradiol treatment.[538,544] Although the behavioral pharmacological evidence is not complete, glutamate action in the VMH acutely inhibits female sexual behavior during the time window of receptivity.[541] This may be mediated by a combination of kainite, AMPA, and NMDA receptors.[519,520] Thus, it appears that female reproductive behavior depends on the inhibition of glutamate neurons in the VMH.

Whereas glutamatergic neurons in the VMH are the direct target of ovarian hormone action, the specific functions of GABAergic cells in the VMH are not yet clear. Nevertheless, GABAergic activity in the VMH is clearly modulated by estradiol. Estradiol treatment increases GABA levels in the VMH, based on hormone replacement regimens, and subsequent progesterone treatment returns GABA back to vehicle-treated levels.[545,546] Similarly, estradiol treatment may also increase GABA turnover.[546] The estradiol-induced increase in GABA production may be based on transcriptional regulation of GAD65, an enzyme involved in GABA biosynthesis,

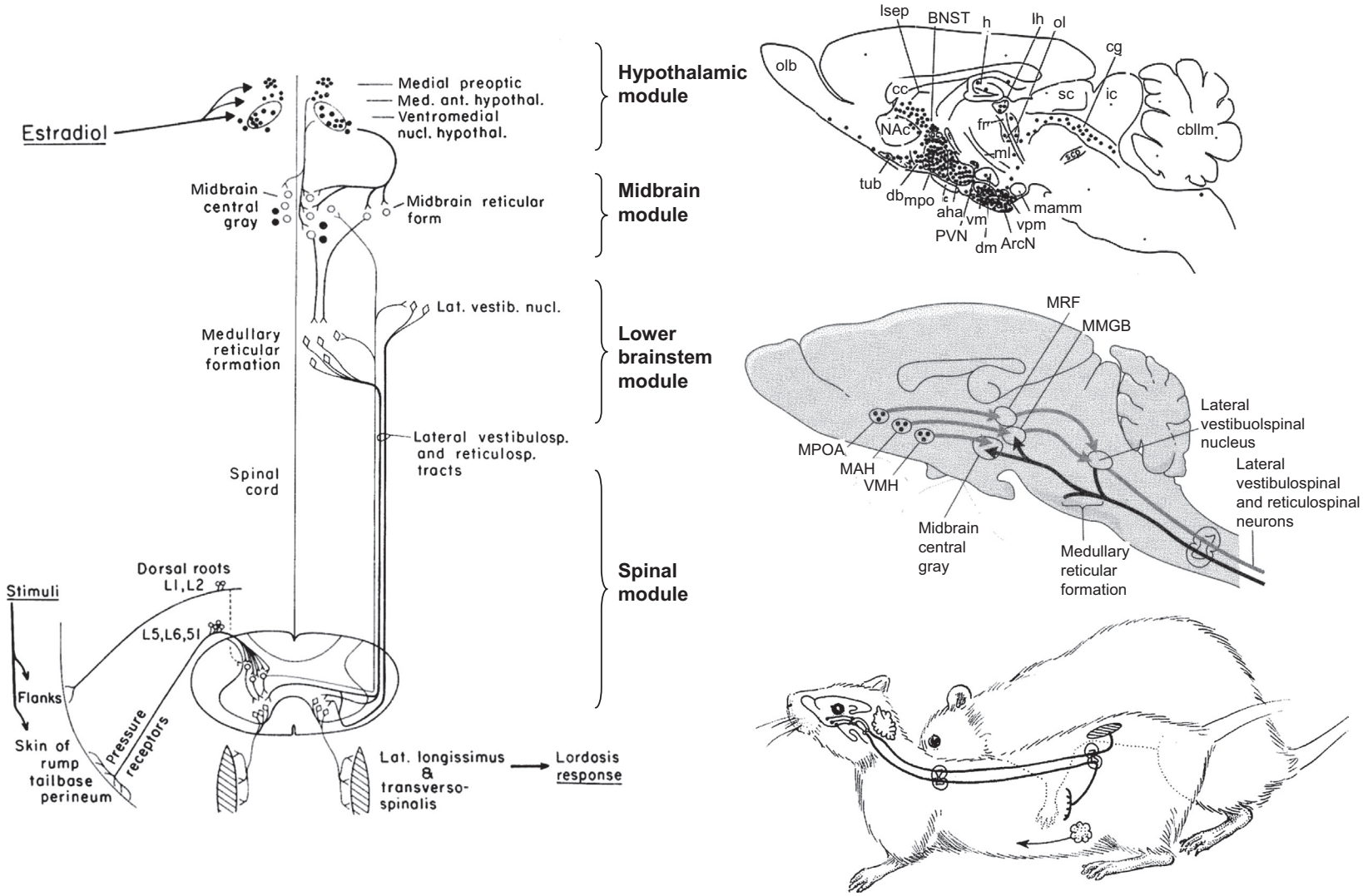

FIGURE 50.19 **Modular control of lordosis.** Left: Estradiol binds at the level of the hypothalamic and midbrain modules, altering the way that incoming somatosensory inputs from flank and perineal stimulation via the spinal cord to brainstem are processed and responded to. This alters the response of the female to palpation of the flanks, switching her from fighting to lordosis (bottom right). Right top: estradiol binding in the mediobasal hypothalamus, and midbrain of female rats. Right middle: critical elements of the lordosis circuit depicting regions of estradiol binding in the medial preoptic area (mPOA), ventromedial hypothalamus (VMH), and anterior hypothalamus. Motor pathways for the circuit include the lateral vestibular nucleus (for postural orientation) and lateral vestibulospinal and reticulospinal neurons that control contractions of the lateral longissimus and transversospinalis muscles of the back, which raises the base of the tail and head. Estradiol activates both nuclear and membrane-bound receptors in the hypothalamus to induce these changes, which include the augmentation of neurochemical systems that are excitatory for sexual behavior (see text). Abbreviations: aha, anterior hypothalamic area; ArcN, arcuate nucleus; BNST, bed nucleus of the stria terminalis; cblm, cerebellum; cc, corpus callosum; cg, midbrain periaqueductal gray; db, diagonal band of Broca; dm, dorsomedial hypothalamus; fr, fasciculus retroflexus; h, hippocampus; ic, inferior colliculus; lh, lateral habenula; lsep, lateral septum; MAH, medial anterior hypothalamus; mamm, mammillary bodies; MMGB, medial region of the medial geniculate body; mpo, medial preoptic area; ml, medial lemniscus; NAc, nucleus accumbens; ol, nucleus of the lateral olfactory tract; olb, olfactory bulb; PVN, paraventricular nucleus; tub, olfactory tubercle; vm, ventromedial hypothalamus; vpm, ventral premammillary nucleus. *Source: Modified from Pfaff.*[32]

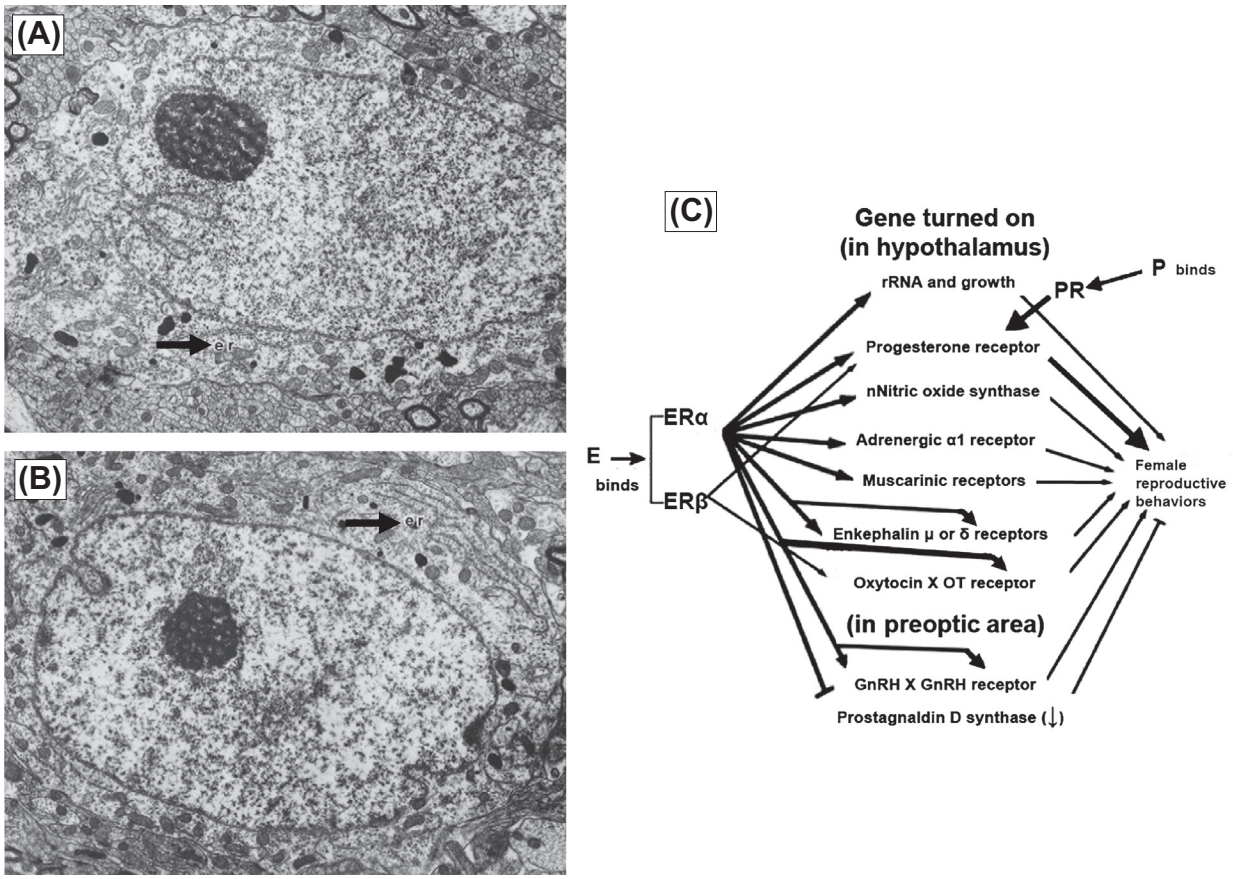

FIGURE 50.20 (A) Electron micrograph of a cell body in the VMH from an OVX rat treated with oil. (B) Electron micrograph of a cell body in the VMH from an OVX rat treated with estradiol (E) for 3 days. Note the accumulation of rough stacked endoplasmic reticulum (e r shown by arrow) indicative of protein synthesis in B compared to the smooth and unstacked, e r in A. *(Source: Adapted from Meisel and Pfaff.[448])* (C) Activation of excitatory neurotransmission in the lordosis circuit by E and progesterone (P) acting on ERs and PRs to modify gene expression. *Source: Adapted from Kow and Pfaff.[530]*

by ERα and ERβ.[547] Behavioral pharmacology experiments suggest that GABA activity may facilitate female sexual behavior,[543] thus exerting disinhibition.[539] GABA may work, in part, by inhibiting local serotonin release, which tonically inhibits lordosis behavior in the VMH,[543] and also by inhibiting glutamate neurons (see below).

These neurotransmitter systems also work in concert to exert opposite effects in the mPOA and VMH on lordosis. In the VMH, infusions of the GABA-A receptor agonist muscimol to OVX, EB-primed rats facilitates lordosis,[548,549] whereas infusions to the POA inhibit lordosis.[549] Infusions of muscimol also reduce extracellular 5-HT concentrations in the VMH.[548] Conversely, infusions of the GABA-A receptor antagonist bicuculline to the mediobasal hypothalamus resulted in an inhibition of lordosis. VMH infusions of antisense oligodeoxynucleotides that flank the start codon for two isoforms of the GABA synthesizing enzyme glutamic acid decarboxylase (GAD; GAD65 and GAD67) inhibited lordosis in rats primed with continuous diffusion of crystalline estradiol in sc silastic capsule implants.[550] These data suggest that GABA in the mPOA inhibits lordosis by an action at GABA-A receptors, but facilitates lordosis in the VMH.

Glutamate was first reported by Kow et al.[540] to inhibit lordosis in OVX, EB-primed rats following infusions to the VMH. This effect was replicated and extended by Georgescu and Pfaus[519,520] who showed that infusions of glutamate or its ionotrophic receptor agonists AMPA, NMDA, and kainate, to OVX rats primed with EB and progesterone inhibited lordosis. NMDA and kainate infusions also increase the display of rejection responses. In contrast, infusions of the NMDA antagonist AP-5 or the AMPA/kainate antagonists, CNQX or DNQX, each facilitate lordosis in OVX rats primed with EB alone.[520] Interestingly, an electrophysiological depolarization signature due to glutamate release in VMH slices was shown by Booth, Weyman, and Jackson[551] to be inhibited by the same treatments. Moreover, pharmacological agents known to inhibit lordosis systemically, such as the 5-HT1A agonist 8-OH DPAT, the mu opioid agonist DAMGO, bicuculline, and CRF all potentiate the in vitro VMH glutamate EPSP (excitatory postsynaptic potential) signature.

The local VMH GABA and glutamate circuits are modulated, in part, by major afferents arriving from the hindbrain somatosensory-arousal systems providing serotonergic, noradrenergic, histaminergic and cholinergic projections to the area lateral to the VMH proper,[426,552–559] innervating the long primary dendrites that extend into the lateral fiber plexus. The brainstem nuclei of origin for these neurotransmitters all express estrogen receptors.[269,397,560,561] In summary, the key neurotransmitters of fast synaptic neurotransmission, glutamate and GABA, are regulated by estradiol within the VMH and have opposing effects on female sexual behavior.

DOPAMINE

Systemic administration of DA agonists can facilitate or inhibit lordosis behavior in OVX rats primed with EB and progesterone or EB alone. Paradoxically, systemic administration of a range of doses of DA antagonists also facilitates lordosis, although the behavioral signature of the effect is different. As mentioned above, whereas DA agonists produce a small increase in lordosis, but a large increase in solicitation behaviors, DA antagonists produce a large increase in lordosis quotients but abolish solicitations. Both the inhibitory and facilitatory effects of systemic DA agonists appear to act through D2 receptors in OVX, EB primed rats, but on D1 receptors in OVX rats primed with EB and progesterone. DA release in the VMH facilitates lordosis. Infusions of apomorphine or DA to either the mPOA or VMH facilitate lordosis behavior in OVX rats primed with low doses of estrone, whereas infusions of the DA receptor antagonists haloperidol or α-flupenthixol to these regions inhibit lordosis, but only in OVX rats made highly receptive with high doses of estrone. DA release is also increased in the VMH during copulation.[426] As discussed above, crosstalk between D1 receptors and steroid hormone receptors in the VMH appears to play a role in the facilitation of lordosis by a cellular process of ligand-independent activation of steroid hormone receptors. Mani et al.[413] reported that the D1 agonist SKF-38393 facilitates lordosis in OVX, EB-primed rats following infusions to the lateral ventricles, and that this effect is blocked by the progesterone receptor antagonist RU-38486 or by infusions of and antisense oligodeoxynucleotide directed against the start codon of the PR-A. These data indicate that activating D1 receptors in the VMH is capable of activating PR, possibly by altering the phosphorylation of the PR or a specific transcription coregulator. Thus, in addition to progesterone altering the activity of diencephalic DA systems, DA in the VMH appears to facilitate lordosis by the indirect activation of PR.

NOREPINEPHRINE

There is considerable evidence that the hormonal changes that underlie lordosis behavior and certain neuroendocrine reflexes, such as the preovulatory LH surge and pseudopregnancy, are associated with altered norepinephrine transmission. Early studies suggested that hindbrain noradrenergic projections to the hypothalamus provide critical visceral and somatosensory cues for female sexual behavior.[555] Extensive work by the Etgen laboratory has demonstrated that estradiol and progesterone enhance norepinephrine release in the vicinity of the VMH.[562] From a postsynaptic perspective, ovarian hormones reconfigure the populations of subtypes of noradrenergic receptors in the VMH, substantially altering signal transduction pathways and neurophysiological responses.[563] These changes in responsiveness and signal transduction channels appear critical for allowing the relevant sensory information to the VMH to promote the lordosis reflex.

Although systemic treatment with α or β noradrenergic receptor agonists and antagonists modulate lordosis, no clear picture emerges. Central effects of adrenergic drugs on female sexual behavior have not been studied in detail. Infusions of the α_1 antagonist prazocin into the VMH, but not the mPOA, inhibit lordosis, whereas infusions of the α_2 antagonist idazoxan or the β antagonist metoprolol to the VMH have only small inhibitory effects in some animals. Infusions of metoprolol to the mPOA inhibit lordosis in most rats. These results indicate that stimulation of α_1 receptors in the VMH facilitates lordosis, whereas stimulation of β receptors in the mPOA may inhibit lordosis. Consistent with this, in vivo microdialysis studies have shown that copulation with intromission increases extracellular norepinephrine concentrations in the VMH.[426]

ACH AND HISTAMINE

Both Ach and histamine have similarly been implicated as arousal neurotransmitters acting in the VMH to promote female sexual behavior, based on behavioral pharmacology studies.[564,565] As with serotonin and norepinephrine, specific subtypes of receptors for both of these signals are regulated by ovarian hormones.[566,567] Electrophysiological studies indicate that during estradiol exposure these signals have an enhanced excitatory effect on VMH neurons.[530,567] Histamine, in part, acts through H3 receptors to inhibit spontaneous GABA release.[532] The overall pattern that arousal transmitters that facilitate lordosis are also excitatory to VMH neurons would seem paradoxical, given that glutamate, a major excitatory transmitter, appears inhibitory for female sexual behavior within the VMH. In this regard, it is important to remember that the VMH includes several cell types that may be differentially regulated and exert excitatory vs inhibitory effects on behavior.[444] In addition, the effect of glutamate on neurotransmission may not be as straightforward as "excitatory". The net effect of glutamate on the membrane potential may be

dependent on the general state of excitability.[551] Nevertheless, there is a strikingly consistent pattern for hindbrain arousal pathways to converge on the VMH, with effects being modulated by estradiol.

OXYTOCIN

One of the most studied neuropeptides in female sexual behavior is OT, which acts in both the mPOA and VMH to promote female sexual behavior.[568,569] OT immunoreactivity is present in the lateral fiber complex, in axons arising from the PVN, colocalized with glutamate.[451] These OT axons innervate the long primary dendrites that extend into the lateral fiber complex.[451] Receptors for OT in the VMH undergo striking regulation by estradiol and progesterone in the VMH.[570] In general, ovarian hormones increase OT signaling by increasing levels of the receptor in the vicinity of the OT fibers. Electrophysiological studies suggest that OT has excitatory effects on VMH neurons.[571,572] Thus, OT may be co-released with glutamate in the area surrounding the VMH to signal social and sexual cues, and ovarian hormones sensitize specific VMH neurons to this information.

Within the VMH, estradiol stimulates OT receptor transcription. Lordosis is facilitated dramatically following systemic and intracerebroventricular administration of OT, and OT receptors in the mPOA and VMH appear to facilitate the frequency and duration of lordosis, respectively.[573] OT infusions to the VMH of OVX, estrogen-primed prarie voles reduces rates of aggression and increases the amount of physical contact that the females made with males, although it also leads to a faster termination of estrus. Copulation with intromission induces Fos within OT neurons of the paraventricular hypothalamus, but not the SON,[574] suggesting that endogenous OT systems are activated during copulation and may participate in the facilitation of lordosis following intromission. Interestingly, masturbation to orgasm increases plasma OT levels in women.[575]

GnRH, PROLACTIN, AND CHOLECYSTOKININ

Like OT, GnRH and prolactin have various effects that promote reproduction in females, including the promotion of female sexual behavior.[576–579] GnRH-labeled neurons reside in more anterior regions of the rat hypothalamus, but axonal fibers surround the VMH[580] and GnRH receptors are expressed in the VMH.[581] Likewise, prolactin receptors are expressed in the ventrolateral VMH,[582] and prolactin-immunoreactive axons are found in the mediobasal hypothalamus.[583]

GnRH produces a dramatic facilitation of lordosis in OVX, estradiol-primed rats following systemic or ventricular administration. Infusions of GnRH into the anterior POA, arcuate, and PAG, also facilitate lordosis in estradiol-primed rats, whereas infusions of a GnRH antibody into the MCG reduce lordosis quotients. Copulation with intromission, or manual VCS induces Fos within GnRH neurons of the anterior POA,[584] indicating that these neurons are activated during copulation. Interestingly, estradiol enhances the proportion of GnRH cells that express Fos following VCS, possibly through long-term noradrenergic activation. These results suggest that the activation of OT and GnRH neurons may form part of the system that facilitates lordosis following VCS. The behavioral effects of prolactin have not received as much experimental attention as GnRH, although there is a perception that sexual satiety is induced by prolactin in both men and women because prolactin release from the anterior pituitary into the general circulation occurs with orgasm.[585] Recently, it was found that women's self-reported satisfaction from orgasm is correlated positively and significantly with the concentration of postorgasmic prolactin found in serum.[586] Additional studies are needed to understand the neural circuitry, neurochemical coding, and neurophysiological targets of both GnRH and prolactin.

Another peptide that robustly innervates the VMH and mPOA is cholecystokinin (CCK). Immunoreactive terminals for CCK innervate VMH soma and dendrites, arising from the lateral parabrachial nucleus,[587–589] a region with strong ERα expression, and making symmetric synapses suggestive of inhibition.[590] Likewise, CCK receptors are found in the VMH.[591,592] However, the significance of CCK action in the VMH for lordosis and the type of information being relayed remains unclear.

DELTA OPIOIDS AND SUBSTANCE P

Two peptides intrinsic to VMH neurons are enkephalin and substance P. Enkephalin immunoreactivity is present in inhibitory axons terminating on GABA-containing VMH neurons. Thus, enkephalin may modulate these intrinsic GABAergic VMH neurons, thereby disinhibiting VMH output.[534] Enkephalin itself is upregulated by estradiol and progesterone in the VMH,[496] like its apparent co-transmitter GABA. Electrophysiological studies suggest that enkephalin has excitatory effects on VMH neurons. As with GABA, the delta opioid receptor agonist DPDPE inhibits or facilitates lordosis following infusions to the mPOA or VMH. Infusions of DPDPE to the lateral or third ventricles facilitates lordosis in OVX rats primed with EB alone or EB and progesterone.[521,593] A similar action was found when low doses of DPDPE were infused directly to the VMH.[593] However, higher doses inhibit lordosis. These effects were blocked by the delta opioid receptor antagonist naltrindole, and infusions of naltrindole alone to the VMH inhibited lordosis. Infusions of antisense oligodeoxynucleotides that flank the start codon for pre-proenkephalin mRNA to the VMH reduced lordosis quotients in OVX rats primed with estradiol.[594] As mentioned above, estradiol stimulates the synthesis of pre-proenkephalin in the VMH

which could act as an endogenous ligand for delta receptors there.[595] However, infusions of DPDPE to the lateral mPOA inhibit lordosis.[499]

Substance P is intrinsic to VMH neurons, and many substance P-labeled neurons express ER.[596,597] These substance P neurons are thought to project to the periaqueductal gray to promote sexual behavior.[598,599] Substance P-labeled terminals are likely to be excitatory, based on ultrastructure.[600] Thus, although both enkephalin and substance P are present in the VMH and promote lordosis, evidence suggests that they comprise distinct elements within the lordosis circuitry, with enkephalin modulating local inhibitory connections and substance P modulating the descending excitatory outflow.

In sum, the local VMH circuit includes glutamatergic and GABAergic neurons, which are directly and indirectly responsive to estradiol, and which have acute effects on lordosis behavior. The neuropeptides substance P and enkephalin are co-localized with these amino acids, and are likely to modulate their actions. The VMH also receives ascending influences from the hindbrain carrying arousal and sensory information, which is also modulated by ovarian hormones. Although the neurochemistry of many hypothalamic connections is not known, peptides associated with reproduction, such as OT, GnRH and prolactin may also affect lordosis by acting on VMH neurons. Taken together, the VMH is transformed by exposure to estradiol to change its internal pattern of activity, and at the same time, the salience of various inputs to the VMH is drastically recalibrated. As we better define the neurochemistry that controls the lordosis circuit, it will be important to understand how these neurotransmitters and neuromodulators work in concert to affect neurophysiology and behavior.

Inhibition of Inhibitory Systems

Tonic inhibitory systems exist for lordosis in the mPOA and VMH that must be overridden to activate lordosis. This action then constrains lordosis to the periovulatory period. Local actions of both mu and delta opioids, and glutamate, appear to keep lordosis constrained until such time as those systems are either inhibited directly, or the action of excitatory neurochemical systems overcomes the inhibitory tone.[35]

Serotonin is the most intensely studied transmitter in this category, and as might be expected, projections from the raphé to the VMH inhibit female sexual behavior.[601,602] Treatment of OVX rats with behaviorally effective doses of estradiol plus progesterone significantly reduces the turnover of serotonin in the VMH in a manner that correlates with changes in lordosis behavior.[603] Decreased serotonin in parallel with increased sexual behavior is also seen across the estrous cycle.[489] This inhibitory effect of serotonin on female sexual behavior is associated with an inhibitory effect on VMH neuronal activity, although the affected cell types have not been described. The serotonin receptor subtype 2C has been localized to the VMH and its neuropil.[533] Acute systemic treatment with the selective serotonin reuptake inhibitor fluoxetine disrupts estrous cyclicity, and reduces lordosis and the amount of time OVX rats primed with EB and progesterone spend with males, with Sprague-Dawley rats being less sensitive to these effects than Fischer rats.[604–606] The reduction in lordosis by fluoxetine was attenuated by progesterone,[607] and by chronic daily exposure to males.[606]

Timing Mechanisms

There are at least two timing mechanisms that have been explored, one that keeps the onset of sexual behavior in females tightly linked to ovulation, and another that allows females to receive a requisite amount of genitosensory stimulation before they fall out of heat. Sinchak et al.[608] have carefully provided evidence for a hypothalamic timing microcircuit in which estradiol acting at ERα in the ArcN stimulates neuropeptide Y release locally within the arcuate that in turn stimulates the activation of POMC neurons that project to the mPOA. These neurons release β-endorphin which activates mu opioid receptors in the mPOA, internalizing them and effectively inhibiting lordosis for the duration of its action. The maintenance of the mu opioid receptor internalization is provided by the activation of GABA-B receptors in the arcuate. This mechanism would also be expected to inhibit appetitive solicitations derived from activation of DA in the mPOA. However, this transient inhibition is itself inhibited by subsequent progesterone actions at PRs, and/or by higher doses of free estradiol acting at membrane receptors in the ArcN (linked to Gq-coupled activation of Srx) and/or in the mPOA (linked to GPER-30 receptors) to deactivate mu opioid receptors in the same membrane.[609,610] Together, these mechanisms create a biochemical timer that keeps sexual behavior from occurring until requisite physiological changes have taken place to support pregnancy. It may also be an important mechanism to investigate for estradiol-induced negative feedback, which may augment opioid actions in the mPOA while reducing the GPER-30 linked disinhibition.

Lordosis must also be maintained long enough for females to receive sufficient CLS for reward and potentially cervical stimulation to induce the neuroendocrine reflexes (e.g., upsuck for sperm transport, increased prolactin surges) that facilitate pregnancy and the progestational state. Glutamate neurons and glutamate transmission in the VMH are activated by VCS and inhibit lordosis as part of a local hypothalamic circuit that terminates estrus (see below). Estradiol augments GABA turnover in the VMH, and glutamate neurons

have GABA-A receptors, making them a target of inhibition by GABA. Such a mechanism likely extends the period of sexual receptivity before glutamate release in the VMH reaches a critical threshold for estrus termination.

It is tantalizing to consider that estradiol tips a balance between energy regulation and sexual behavior by modulating hypothalamic systems that promote feeding, utilizing them to inhibit both appetitive solicitations and lordosis until ovulation, at which time mechanisms that normally inhibit feeding (e.g., MCs) are activated that stimulate sexual behavior (e.g., Refs 611,612). For example, infusions of antisense oligodeoxynucleotides to GAD, which stimulate lordosis, inhibit food intake in rats.[613] This contrasts dramatically with the ability of food and sex to activate the central nucleus of the amygdala and stimulate mesolimbic DA release similarly in the NAc.[614,615] This suggests a fundamental difference in the way that hypothalamic regulatory systems and more general mesolimbic incentive motivational systems regulate different motivational states. Hypothalamic systems appear to regulate different motivations as if each possesses its own separate and mutually exclusive drive state. In contrast, the mesolimbic incentive system treats external stimuli associated with those drive states as essentially similar in the induction of forward-directed locomotion. However, it is also the case that glutamate in the VMH inhibits both feeding[616] and sex,[519,520,540] and may be involved in satiety-related mechanisms that terminate both motivational systems.

Estrus Termination

The offset of appetitive sexual responses and lordosis occurs as the period of sexual receptivity terminates. This is typically referred to as "estrus termination". Part of this process accompanies the natural decline in hormonal titers, whereas more immediate inhibition stems from an abbreviation of the sexually appetitive and receptive period brought about by VCS from intromissions and ejaculations.[617] Abbreviated estrus induced by mating stimulation or VCS has been shown to occur in rats,[75,617,618] guinea pigs,[619] and hamsters.[620] Indeed, allowing females to pace sexual contacts increases the inhibitory effect of intromissive stimulation on estrus duration in estrus-cycling rats[74,523] presumably because males make deeper and more powerful thrusts that could stimulate the cervix. In fact, OVX rats primed with EB and progesterone and given 50 distributed VCSs with a glass rod (that approximates the number of intromissions they would receive in an hour from a male) display no solicitations, low levels of lordosis, and high numbers of rejection responses when tested 12 h later.[75] In that study, rats given sham stimulation 12 h before testing displayed normal rates of solicitations and lordosis, and no rejection responses. Females allowed to copulate freely with males in bilevel chambers show changes in the intensity of appetitive and consummatory behaviors, such that by the fourth ejaculatory series, fully primed female rats show few solicitations, lower lordosis frequencies and reflex magnitudes, longer pacing intervals, and higher numbers of rejection responses, compared to the first ejaculatory series.[70] It is around this time that naturally-cycling female rats in large semi-natural environments will take themselves out of the open area and back into the burrow system, effectively ending their participation in sexual activity. However, in experimental settings where sexual stimulation is not controlled adequately by the female, she displays longer periods of sexual receptivity than would be predicted from studies in the wild or in semi-natural environments. This is similar to the observation by Wallen et al.[621] of female rhesus macaques displaying nearly constant receptivity when paired in small enclosures with a single male, relative to females in large natal groups that take themselves out of a sexual interaction by retreating to the female territory.

Role of PRs

Inhibition of protein synthesis by infusion of a protein synthesis inhibitor into the mPOA in hamsters[622] or following systemic administration to guinea pigs[619] blocks mating-induced abbreviation of the period of sexual receptivity. Likewise, inhibition of protein synthesis delays heat termination.[623] Recent work suggests that heat termination that is hastened by mating stimulation is referable to more rapid down-regulation of PRs (specifically PR-B)[354] than the down-regulation in response to progesterone. Thus, although there is still much that is not known about the role of PR-A and PR-B in particular neurons, it is clear that PRs play a key role in the timing of sexual receptivity in a variety of circumstances by serving as a gatekeeper for the transcriptional processes within those neurons involved in sexual behavior.

Although, there is a temporal correlation between decreased blood levels of progesterone and termination of behavioral estrus,[362] the two events are not causally related. That is to say, the period of sexual receptivity ends even when levels of progesterone are maintained.[624,625] Because of the importance of PRs to the facilitation and maintenance of sexual behavior, the cellular basis for heat termination requires looking at the regulation of PRs. Progesterone down-regulates its own receptors. Loss of behavioral response can typically be attributed to either a declining concentration of activated/occupied hypothalamic PRs[356] or the absence of a sufficient level of progesterone to interact with the particular level of unoccupied receptors. The decline in concentration of unoccupied PRs can come about in a variety of ways; a decrease in estradiol levels results in the loss of induction of PRs, and exposure to

progesterone down-regulates PRs. Both processes typically occur in tandem.

It has been suggested[356,626] that the refractory period, which follows termination of sexual receptivity[236,627,628] comes about as a result of the same mechanism that causes heat termination—down-regulation of PRs by progesterone. During the refractory period, the concentration of hypothalamic PRs is depressed in relevant brain areas,[357,358,629,630] and progesterone treatment results in low levels of activated PRs.[362,631] In addition, a supplemental estradiol injection, which offsets the decrease in the concentration of unoccupied PRs, resulting in high levels of occupied PRs in response to progesterone,[364] causes the animals to regain response to a second progesterone injection.[236,628,632] The refractory period can also be overcome by injection of a large dose of progesterone,[631,633] which, unlike a lower dose, results in a large increase in progesterone-occupied PRs in the hypothalamus.[631] Therefore, with a variety of conditions, there is a strong concordance between the level of activated PRs and the expression of lordosis.

There have been conflicting reports of progesterone involvement in estrus termination in rats. However, although rats may not become completely insensitive to progesterone after estrus termination, they do in fact become hyposensitive to it[634] The hypothesis that estrus termination and the refractory period are both due to loss of activated PRs may explain the conflicting opinions concerning progesterone's role in estrus termination.[224,635,636] Perhaps behavioral response is critically dependent on an adequate concentration of PRs, rather than progesterone per se.

Role of Disinhibited VMH Glutamate

As mentioned above, VCS that induces estrus termination activates a population of glutamate neurons in the ventrolateral VMH[637] (Figure 50.21). When OVX rats are given 0, 1, 5, 10, 20, 30, 40, or 50 distributed VCSs, the activation of Fos in those neurons is delayed by prior treatment with EB or EB and progesterone, relative to the oil-treated control, such that fewer neurons reach a threshold for activation until animals receive between 10 and 20 VCSs.[169] This blunted activation indicates that steroid hormones have suppressed the ability of VCS to activate these inhibitory glutamate neurons, although by 50 VCSs there is no difference in the number activated between steroid-treated and control groups. How might estradiol with or without progesterone do this?

Virtually all the glutamate neurons that co-express Fos after VCS also contain GABA-A receptors which are up-regulated by EB treatment.[638] The stimulation of GABA synthesis by EB in GABA neurons that project to the VMH would be expected to bind to GABA-A receptors on the inhibitory glutamate neurons and induce IPSPs for an extended period of time, long enough perhaps for the female to receive enough intromissions and ejaculations to ensure pregnancy, or at least the progestational state consonant with pseudopregnancy. Extracellular concentrations of glutamate in the VMH during copulation in OVX females are very high in females injected only with oil (which reject male advances), and lowest in females primed with EB and progesterone who display full appetitive and consummatory sexual responses.[638] Indeed, infusions of the AMPA/kainate receptor antagonist DNQX to the VMH prior to the application of 50 distributed VCSs delayed the induction of estrus termination observed in saline-treated controls 12 h later.[639] The source of the GABA input to glutamate neurons has not been identified, although preliminary findings suggest it comes largely from the mPOA.[640] Thus blocking glutamate receptors in the ventrolateral VMH blocks the abbreviation of estrus induced by VCS, an effect that may occur naturally by estradiol-induced inhibition of glutamate neurons. The net effect would be for females to remain sexually receptive long enough to receive a requisite number of intromissions and ejaculations to ensure pregnancy.

Disorders of Sexual Desire or Interest in Women

An important example of how basic research translates into clinical treatments comes from the study of the neurochemistry of sexual desire. At least three potential treatments for disorders of sexual desire or interest in women are being considered, including the MC agonist bremelanotide, the serotonergic mixed 5-HT1A agonist/5-HT2A antagonist flibanserin, and a combined pill containing testosterone and a phosphodiesterase-5 (PDE-5) inhibitor called Lybrido®. Acute bremelanotide increases solicitations selectively in preclinical models using OVX rats primed with low doses of EB, or low EB and progesterone.[30,516] Likewise chronic flibanserin increases solicitations and reduces rejection responses in OVX rats primed with EB or EB and progesterone.[316] Microdialysis samples from the mPFC, NAc, and mPOA showed that acute flibanserin increased basal levels of NE in all areas, along with DA in the mPFC and mPOA, but not the NAc. Acute flibanserin also decreased serotonin levels in all areas. However, chronic flibanserin increased DA and NE significantly in the mPFC, but did not alter serotonin, glutamate, or GABA relative to chronically injected controls. Finally, acute treatment of OVX rats primed with low EB with testosterone and a PDE-5 inhibitor increased solicitations and hops and darts.[641] All three drugs have shown significant efficacy in increasing self-reported sexual desire in pre- and post-menopausal women diagnosed with hypoactive desire disorder.[30,642–646] The ability of these three drugs to stimulate solicitations in a rat model of hypoactive sexual desire predicts their functional application in women with hypoactive sexual desire. This suggests strongly that the neurochemical systems underlying

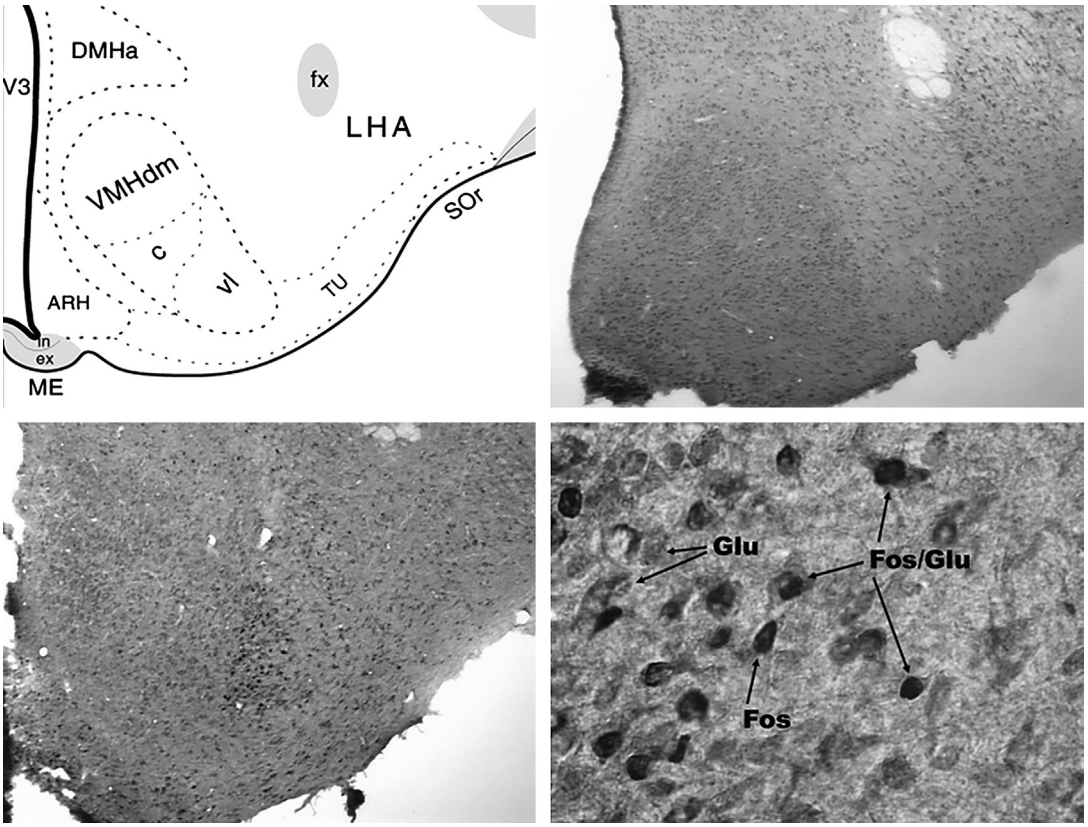

FIGURE 50.21 Activation of glutamate neurons in the VMH by 50 artificial vaginocervical stimulations over the course of 1 h. Top left: schematic drawing of the VMH and its substructures. Top right: glutamate staining in the VMH. Bottom left: Fos induction within glutamate neurons in the ventrolateral VMH. Gray cytoplasmic staining is for glutamate (Glu) whereas black nuclear staining is for Fos. Bottom right: close up of double labeling of Fos within Glu neurons, Fos alone (non-Glu neurons), and Glu neurons without Fos. *Source: Reprinted from Georgescu et al.,*[637] *with permission of Elsevier.*

appetitive sexual behavior are conserved between at least rats and humans, and that translational work on the neural and hormonal systems that mediate sexual responses in women can be derived from basic and clinical research in other species.

CONSEQUENCES OF SEXUAL STIMULATION

In addition to estrus termination and the activation of neuroendocrine reflexes associated with pregnancy and pseudopregnancy, stimulation of the clitoris and possibly also vagina and cervix induce a state of pleasure or reward. As with many rewarding stimuli, a neurobiological "preference" can be established for physical elements associated with the original pleasure-inducing event. For things such as pleasure-inducing drugs this can include the place in which the drug effect is experienced. In animal models this can be manipulated so that an individual's preference for one distinctive environment in which the drug effect was experienced, vs a different environment in which the effect of vehicle or placebo was experienced, can be quantified and used as an indicator of how rewarding the drug is.[647] This same principle can be applied to sexual stimulation received in one distinctive environment vs no stimulation in another environment. In both cases the location in which the rewarding event occurred becomes a conditioned stimulus in the classic sense and thus this phenomenon (or behavioral assay) is referred to as conditioned place preference (CPP). Specifically in the context of sexual reward experienced with a distinctive partner, the same principle can be applied such that salient partner-related cues become conditioned stimuli that induce a partner preference. Research into the role of conditioning as a result of sexual stimulation brings together three important observations in females (and males) of a variety of species.

First is the phenomenon of mate choice, observed naturally in female prairie voles that display monogamous social and sexual partner preferences with the first male they mate with (see Chapter 48). This phenomenon traces its cause in large part to the particular way that OT is activated in regions of the limbic system and hypothalamus that create an incentive sexual preference for a particular

male with whom the female has mated with.[648,649] The male of this species also displays monogamous social and sexual preferences,[650,651] and the two will form a partnership around care and raising the young. It has been assumed that most other species are polygamous, and in particular, sexually promiscuous. If there are preferences at all (especially in males) it is for a different partner every time in order to spread the genes far and wide in the pool. Both male and female rodents are assumed to be polygamous and promiscuous, with both showing "Coolidge effects" in which sexual activity is more vigorous with new compared to familiar partners.[72,652]

The second phenomenon is sexually CPP, as referred to above. In these studies rats or hamsters are placed into the start chamber of a CPP box (Figure 50.22) that contains two distinctive environments to either side that vary by floor pattern or some other distinctive characteristic but one that will not, in and of itself, invoke a preference. The sequential pairing of sexual stimulation or a reward state induced by sexual interaction with a partner is then experienced in one side, and no stimulation is experienced in the other side, and occurs for several trials after which the rat is placed into the start chamber and allowed to roam freely between the two distinctive environments. If the sexual stimulation has positive reinforcing (i.e., rewarding) properties then the rat will spend significantly more time on the side of the chamber where it previously experienced the rewarding sexual stimulation (UCS (unconditioned stimulus)).

Seminal work by Paredes and colleagues starting in the mid-1990s asked if female rats "liked" sex. Prior to this work, lordosis and other sexual behaviors were seen as being "driven" by estradiol and progesterone, but given the existence of penile spines it was far from clear that female rats found sexual interaction with males rewarding. And given the anesthetic properties of progesterone, it was indeed possible that they did not, but that the potential pain of sex was merely reduced to endurance levels by hormone action. In fact, in some

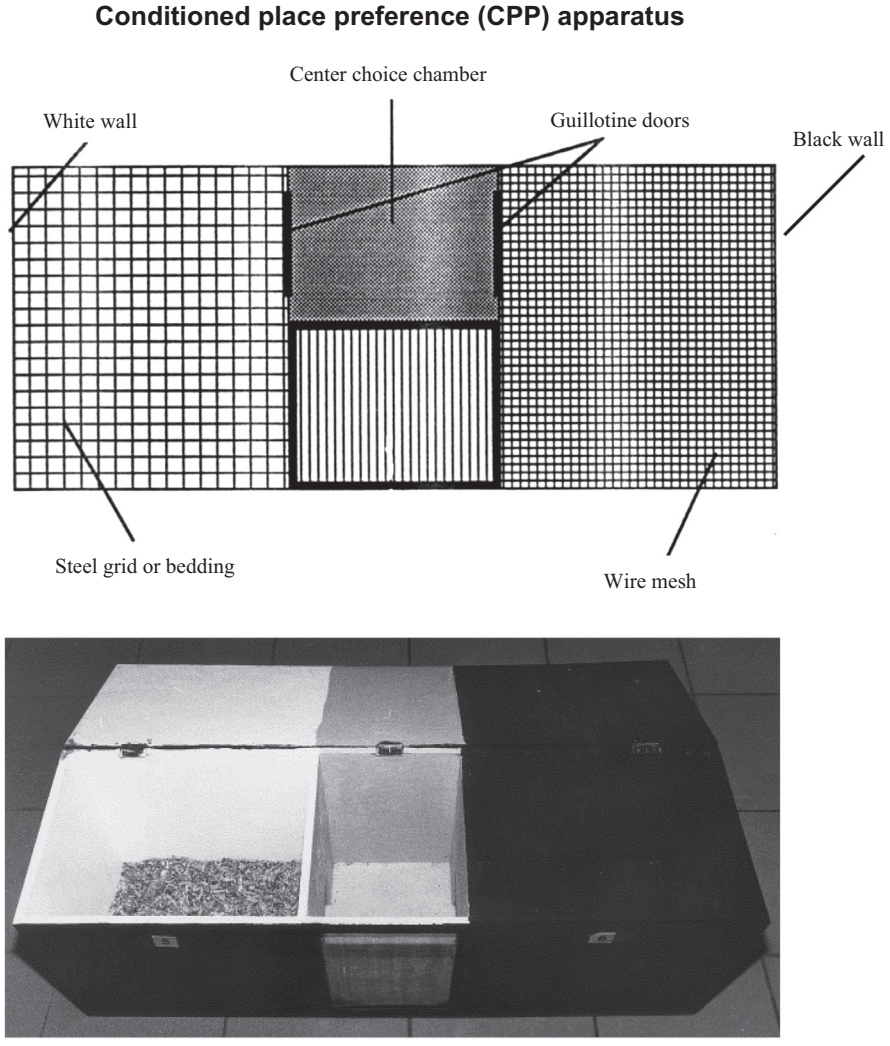

FIGURE 50.22 Typical conditioned place preference (CPP) apparatus used to determine the rewarding properties of drugs or sexual stimulation. *Source: Adapted from Paredes and Vazquez.*[81]

studies prior to Paredes' work, female rats were observed to run away from the male after ejaculation, or to choose to spend more time with a castrated male relative to an intact male.[653] Two important papers by Paredes' group changed that perception (see below).[81,513]

The third phenomenon merged the first two, showing that polygamous and promiscuous female rats are capable of developing sexually conditioned partner and mate preferences based on the degree of reward experienced during their first sexual encounters, and whether this sexual reward had been paired with a discrete odor cue (e.g., almond) on the male they had their first rewarding sexual experience with.[28,78] This effect was also demonstrated for the strain of the male with which the female had her first sexual experience.[77] Female rats also display mate guarding behavior, just as female prairie voles do, if they are sexually receptive and placed into a situation with their male and another competitor female who is sexually receptive. Together, these phenomena link reward and reproduction, and bring them to a more cognitive level of analysis that translates extremely well to human sexual behavior. One significant advantage to the use of neutral stimuli such as an odor of a place being paired with a sexual reward state is that the stimuli can be presented alone—as a priming cue—and the activation of brain areas in response to the conditioning cue in the absence of the actual rewarding stimulus can be identified. This has led to important insights into the neurological underpinnings of reward, both in response to pleasure inducing drugs and sexual stimulation.[63]

Sexual experience in females also changes cellular morphology in critical regions of the reward circuitry. In female golden hamsters, for example, copulatory experience increases dendritic spine density in the NAc, but decreases it in the PFC[94] (Figure 50.23). An augmentation of synapses in the NAc, concomitant with a decrease in the PFC, would be expected to enhance the ability of distal sexual incentives to focus a female's attention and activate excitatory appetitive sexual responses. Thus, the ability of sexual experience to augment the activation of sexual reward, and in turn sexual desire, is most likely rooted in molecular, structural, and neurochemical changes that sensitize females to competent sexual incentives and cues that predict sexual reward or pleasure.

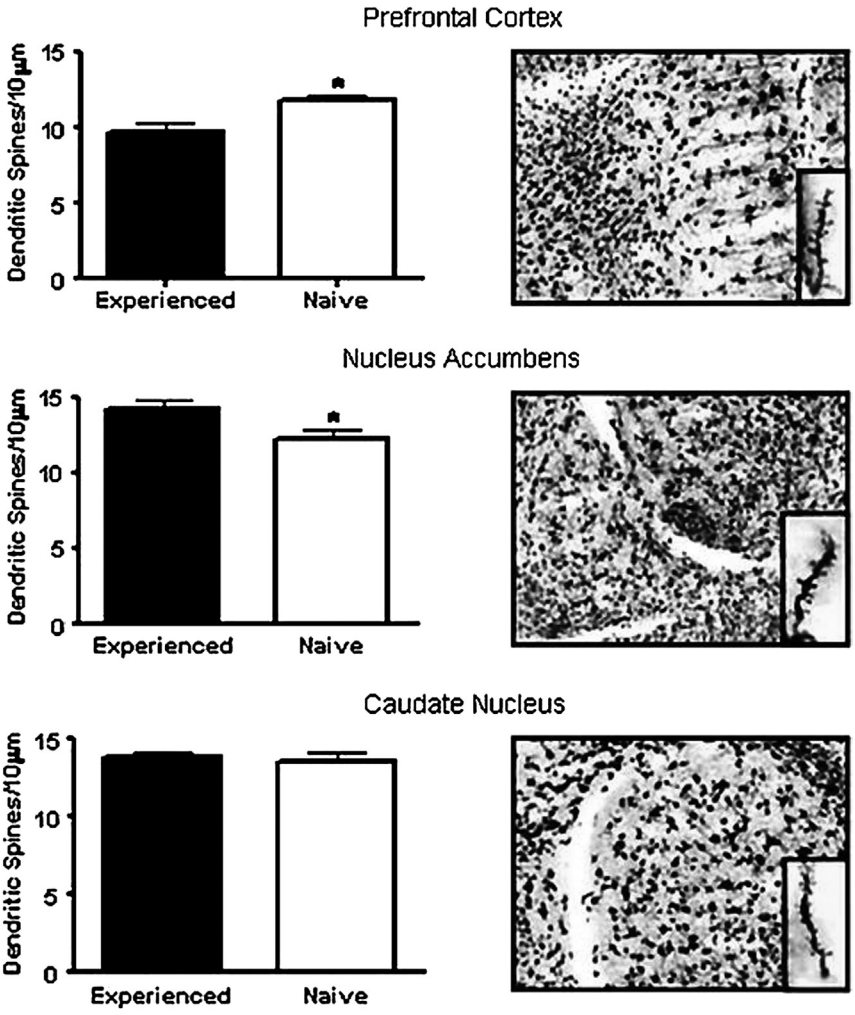

FIGURE 50.23 Spine densities of Golgi-stained neurons from the prefrontal cortex (PFC), NAc, or caudate nucleus (dorsal striatum) in sexually experienced or naïve female hamsters. Sexual experience induces a significant reduction in spine density in the PFC, but a significant increase in the NAc. This pattern of synaptic alteration would be expected to enhance reactivity to both unconditioned and conditioned sexual incentives. *Source: Reprinted from Meisel and Mullins,[94] with permission of Elsevier.*

Sexual CPPs in Females

Oldenburger et al.[654] found that when copulation occurred within one of the distinctive compartments of a CPP apparatus, female rats showed a weak preference for the chamber in which mating occurred. Subsequently, Paredes and Alonso[513] and Paredes and Vazquez[81] demonstrated a robust place preference in female rats when they were able to pace the rate of copulation without having to employ defensive behaviors. This was accomplished using unilevel pacing chambers bisected by a Plexiglas divider with one or more small holes that only the female can pass through.[74,81,513,523] The male was sequestered on one side of the chamber and the female was then free to pace the copulatory contact by running from side to side. Like males, females acquired a strong preference for a distinctive environment only if they were placed into the CPP box after paced copulation. No preference was found if the copulation was unpaced prior to placement in the CPP box (meaning that it had occurred in the same pacing chamber but without the divider). Thus, for a female rat, place preference develops only if she has been able to control the initiation and rate of copulation freely without having to use defensive behaviors.

There is an alternative interpretation of the place preference induced by controlled paced mating in females. Rather than paced mating being rewarding per se, it may be that under conditions in which the female has little control over the interaction, such as is usually the case in tests of animal mating behavior performed in small constrained arenas such as glass aquaria, that these interactions are actually aversive. To examine this possibility, Afonso, Woehrling, and Pfaus[504] allowed female rats to copulate in two unilevel pacing conditions using Plexiglas dividers that had either four holes or one hole. This was done to eliminate the possibility of an "aversive" state resulting from unpaced copulation. Trials were conducted sequentially at 4-day intervals and each pacing condition was paired with one of the distinctive sides of a CPP apparatus, in a counterbalanced fashion. Control groups contrasted the 4-hole or 1-hole condition with a no-divider condition (as was done by Paredes and Alonso[513]). Control females developed significant CPP for either the 1-hole or 4-hole condition, relative to unpaced copulation with no divider. These data replicate the findings of Paredes and Alonso[513] and indicate that both the 4-hole and 1-hole condition were rewarding relative to the unpaced (no divider) condition. However, they do not rule out the possibility that the real distinction being made was between an aversive condition (unpaced copulation) and a rewarding condition (paced copulation). This was addressed in the group allowed to contrast the 4-hole vs 1-hole condition, in other words females that could control the pace of the sexual interaction freely (in the 4-hole chamber, females can move freely from side-to-side) vs those that could not (in the 1-hole chamber, males often obstruct the hole with their heads, forcing the females to wait longer to get to the male's side). Females developed significant CPP for the 4-hole relative to the 1-hole paced copulation experience. Thus free control over the rate of copulation appears to be a crucial variable in the rewarding aspects of pacing. Similarly, Jenkins and Becker[655] found that female rats developed significant CPP for paced relative to unpaced mating, but also for unpaced mating in which the experimenter removed the male for a period that approximated the female's imposed interintromission interval, relative to unpaced mating in which male removal did not occur. Thus, female rats develop CPP for sex at their own preferred intervals. Taken together with the results of Matthews et al.,[656] these data suggest that reward comes from the sexual stimulation that females receive, namely mounts with intromission, so long as that stimulation occurs at the desired time intervals.

What is it about paced copulation that leads to CPP in females? Meerts and Clark[657] reported that VCS applied with a 1 ml syringe plunger at 200 g of pressure for 2 s at 30-s intervals, for a total of 15 stimulations, induces a reliable CPP in OVX females primed with estradiol and progesterone. Given that VCS could stimulate the internal clitoris as well as the cervix, we asked whether external CLS could induce CPP.[160,658] As mentioned above, in these studies CLS was administered either with a lubricated paintbrush or a small cotton-tipped vibrator at preferred intervals for 10–15 min over five to six reinforced sessions. Both types of stimulation induce robust CPP. Importantly, reward as a consequence of CLS can be induced in OVX females with or without hormone priming,[162] indicating that sexual reward is independent of steroid priming, although such priming would normally be required for females to experience CLS from mounts with pelvic thrusting, as it would be necessary to induce lordosis which exposes the clitoris to the male's perineum during pelvic thrusts.[164] Indeed, females primed sequentially with EB and progesterone, or its ring A-reduced metabolites, show enhanced CPP from paced copulation relative to females primed with EB alone, presumably due to the greater degree and frequency of lordosis induced in females receiving progesterone in addition to estradiol.[659] OVX rats primed with low doses of EB that induce low to moderate lordosis do not develop CPP,[660] OVX, hormone-primed rats given exitotoxic lesions of the nucleus paragigantocellularis of the brainstem have attenuated lordosis and appetitive behaviors.[661] Interestingly these females also do not develop CPP to artificially-applied VCS.

Maintenance of the memory of paced sexual reward does not require exposure to hormones. Parada et al.[162] attempted to extinguish paced copulation-induced CPP

by exposing OVX rats in three priming conditions (oil, EB alone, and EB+progesterone) to the CPP box without prior copulation. Only females primed fully with EB+progesterone shifted their preference back to the original preconditioning side. At first glance, this would appear to be counterintuitive, given that the reward state induced by distributed CLS (and possibly also paced copulation) is not hormone dependent. However, *desire* is hormone-dependent, and only the females that had hormone-induced activation of appetitive sexual motivation, but did not receive sexual stimulation, showed extinction of the CPP. Nonprimed, or EB-primed, females did not extinguish the CPP. This makes sense from a conditioning viewpoint: animals that are not in a state of desire do not need that state satisfied, whereas animals in a state of desire seek satisfaction of that desire. Extinction thus occurs only when a state of desire or need exists and there is no satisfaction.

Finally, the CPP induced by paced copulation in females can be blocked by systemic injections of naloxone,[662] or following infusions of naloxone to the mPOA, VMH, or MEA, but not the NAc.[663] Similar data have been reported for males, suggesting that common opioid systems in the brains of male and female rats are activated by sex-related cues[662] and constitute a primary reward signal. Bilateral lesions of the nucleus paragigantocellularis in the brainstem decrease the amount of time females spend with males, which in turn, attenuates the CPP induced by paced copulation.[621] Such lesions also attenuate the CPP induced by artificial VCS, suggesting both a behavioral and sensory deficit induced by the loss of the nucleus paragigantocellularis.

Conditioned Sexual Partner Preferences

As outlined above, female rats also show olfactory conditioned partner preference for males associated with a pacing-induced reward state.[78] This was accomplished in unilevel pacing chambers in which the paced condition involved the placement of either a 1-hole or 4-hole Plexiglas divider through which the female could regulate the initiation and rate of copulation. The nonpaced condition involved copulation in the same chamber but without the divider. Females in the paired group were given paced copulation with males that had almond odor applied to their necks and anogenital area vs nonpaced copulation with males that had distilled water applied to the same areas. After four paced vs nonpaced trials, females were placed into a large open field with two tethered males, one scented and the other unscented, and choice of male to solicit, copulate with, and receive ejaculations from, was recorded. Females for which the odor was paired with the paced condition selectively solicited, copulated with, and received ejaculations from the scented male. Females that had the odor explicitly unpaired or paired randomly with pacing did not display a preference (Figure 50.24).

As with males, females showed a similar preference for strain cues associated with paced copulation,[77] although it was stronger if the strain associated with paced copulation was their own. Interestingly, in that study, pigmented or albino females solicited whichever strain of male was associated with paced copulation, but received ejaculations preferentially from males of their own strain and only if that male had been associated with paced copulation. This also revealed a degree of assortative choice, especially for mating, and showed that females, like males, can differentiate copulation from mating. Finally, female rats that experienced manual distributed CLS in the presence of a cotton gauze pad soaked in almond extract chose to copulate selectively with almond-scented males over unscented males during their first sexual experience in a large open field with both males.[161] Interestingly, they did not show a preference to receive the scented male's ejaculations, suggesting that the VCS received from males during paced copulation induced a further reproductive or mate choice. It is not yet known whether this stems from specific stimulation of the cervix (and pelvic nerve) or from full stimulation of internal and external aspects of the clitoris,[166] or other sensory regions inside the vagina. Experience with paced, relative to nonpaced, copulation in unilevel chambers induces significant neurogenesis in the granular layer of the accessory olfactory bulbs,[664] a region known to contain intrinsic memory systems related to pheromonal stimulation and recognition of conspecifics.[665,666]

Female rats also learn inhibitory associations. Coria-Avila et al.[83] found that administration of the opioid receptor antagonist naloxone blocks the development of sexually conditioned partner preference in OVX female rats primed with estradiol and progesterone. Subsequent analysis of the naloxone training sessions revealed that by the 6th or 7th trial, most females display significantly fewer, if any, solicitations, a low frequency and magnitude of lordosis, and a far higher number of rejection responses, as if they were in a state of estrus termination (Figure 50.25). In some females this occurred before any intromissions were achieved by the males, although such stimulation was often reacted to violently by the females who would box and push the males onto their sides or backs. *And this was despite full hormonal priming with estradiol and progesterone*.

In addition to the inhibitory effect of sex without opioid reward induced by naloxone, thwarted sexual activity in the presence of an inaccessible male can also induce an inhibitory state. Parada et al.[161] gave sexually naïve female rats five trials of CLS in the presence of a sexually active male scented with almond behind a screen. On alternating days, the females received sham CLS in the presence of an unscented male behind the screen. During

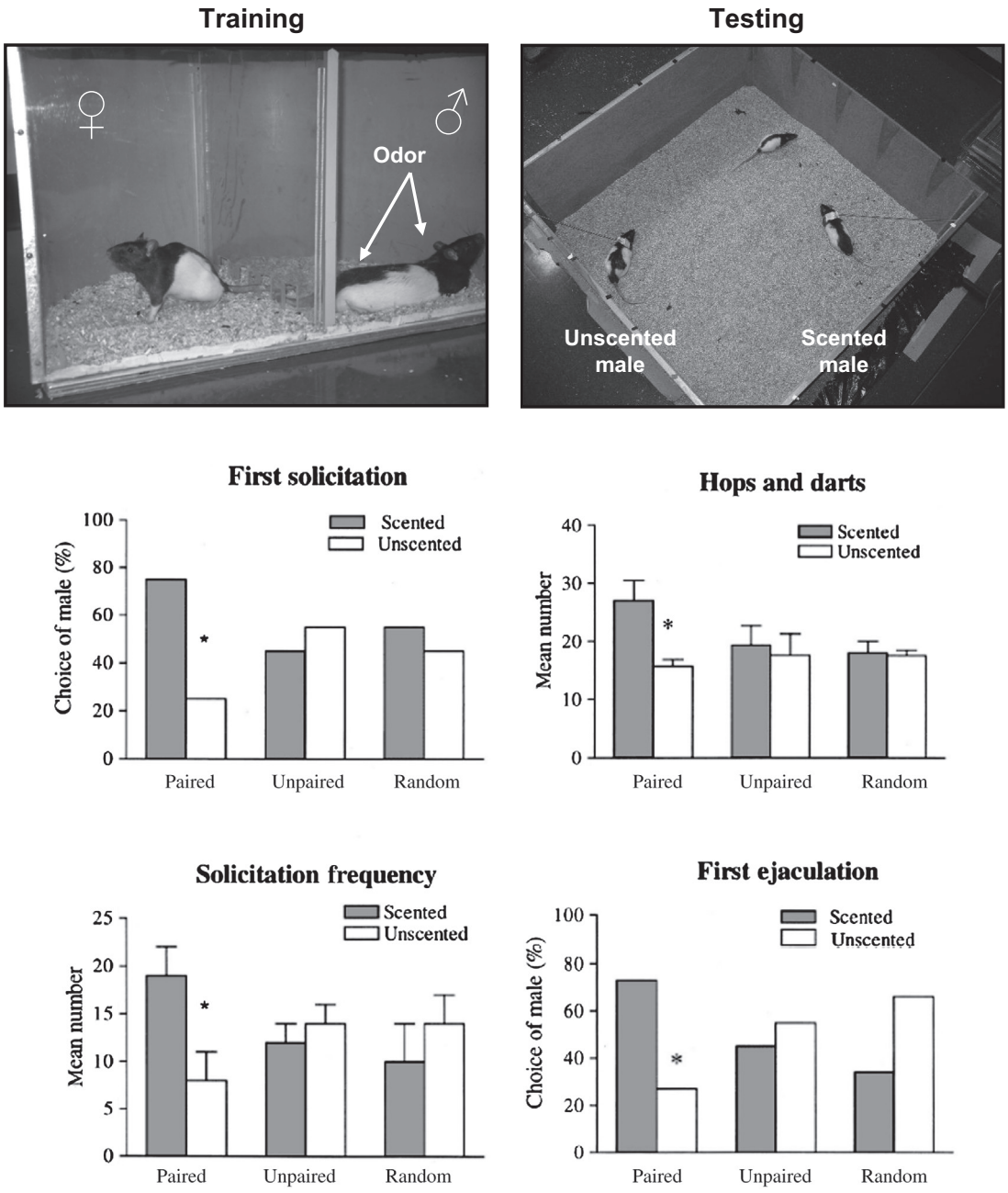

FIGURE 50.24 Conditioned partner preference in the female rat. Top: Paired females are given their first sexual experiences in a unilevel pacing chamber with a male behind a 1- or 4-hole divider scented with a neutral odor (e.g., almond). This is followed 4 days later by exposure to an unscented male but with the divider removed. After several sequential exposures of scent and paced copulation and no scent and unpaced copulation, females are tested in a large open field with two tethered males, one scented and one unscented. The choice of male for solicitations, hops and darts, mounts, intromissions, and ejaculations is recorded. Unpaired females are given the opposite order of association during training, no scent with paced copulation and scent with unpaced copulation. Random paired females are given scented and unscented males randomly associated with paced and unpaced copulation. Bottom: Choice of male for first solicitation, frequency of solicitations, hops and darts, and choice of male for first ejaculation, from paired, unpaired, or random-paired groups. *Source: Adapted from Coria-Avila et al.*[78]

the final open field test with two males, one scented and the other unscented, females solicited selectively the *unscented male* and showed a trend to receive that male's ejaculations preferentially. At first glance, these data seem at odds with the fact that CLS induces a reward state. However, it was noted that females attempted to solicit the males behind the screen following CLS during the training trials, which, of course, were not successful because the male was behind a screen. Thus, it is likely that the female was in a state of thwarted sexual nonreward that she associated with the odor and generalized to the choice of male for her first sexual experience.

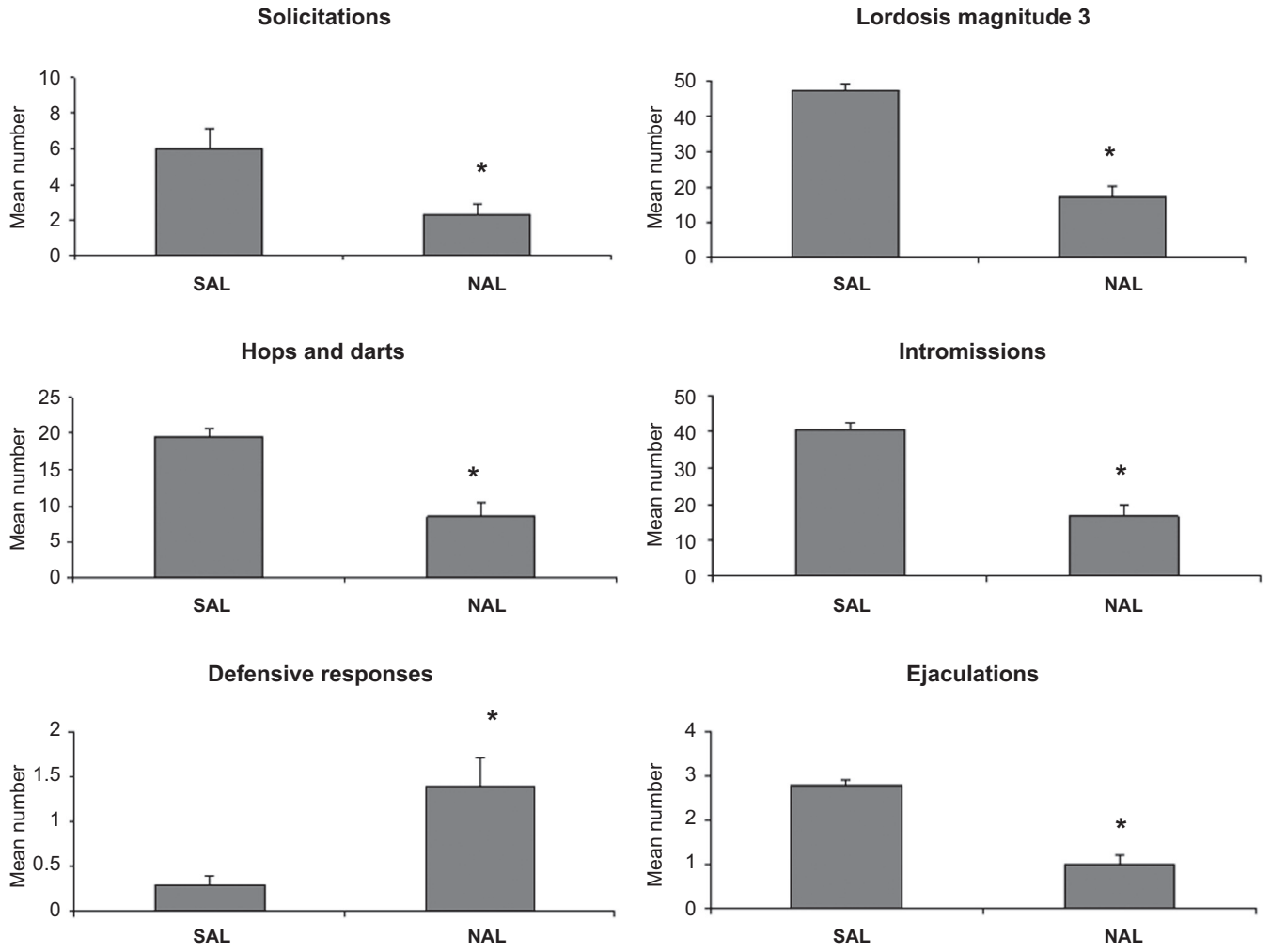

FIGURE 50.25 Effects of acquiring sexual experience under the influence of saline or naloxone (5 mg/kg, ip) on appetitive and consummatory sexual behaviors in OVX female rats primed fully with estradiol and progesterone. Females received six multiejaculatory experiences at 4-day intervals prior to the final test in which all rats received an injection of saline. *Source: Adapted from Pfaus et al.*[28]

After conditioning, the odor cue can be presented alone to examine its priming effect in the brain. Relative to the unpaired group, the odor presented to females in the paired group activates Fos (Figure 50.26) in a circuit that is strikingly similar to that observed in fMRI brain scans of women during the presentation of erotic pictures, and especially pictures of their partners.[64] These regions include: olfactory tubercle, piriform cortex, ACC, insula, NAc, dorsal striatum, lateral septum, mPOA, PVN of the hypothalamus, ArcN, VMH (scattered), and VTA.[76] Presentation of strain cues behind a wire-mesh screen also activated Fos. Relative to unpaired rats, Fos protein was activated in significantly more cells in the piriform cortex, mPOA, VMH, and VTA.[76] Areas of common activation by odor and strain related cues associated with paced copulation are shown in Figure 50.27.

Finally, OVX, hormone-primed female rats given their first 10 multiejaculatory sexual experiences with the same unscented male display agonistic behavior toward an OVX, hormone-primed competitor female (CF) when the three are placed into an open field. Sexually receptive female rats have generally been observed to compete with one another in large mating arenas[50]; however this behavior is reminiscent of the mate guarding displayed by Prairie voles.[667] The partner female (GF) typically mounts the competitor female (HO) and pushes her into corners of the open field, sometimes with aggressive postures, prior to running back to the partner male with her ears wiggling. Occasionally she positions herself between the male and the competitor, making it nearly impossible for the male to gain access to the other female.[668] These observations have been replicated and extended,[669] and brain activation by the encounter compared between the GF and the HO. In both cases, Fos activation of CLS and VCS zones in the hypothalamus and limbic system have been detected; however in GFs that position themselves more frequently between the male and the HO, Fos protein was induced in a greater number of cells by the copulatory stimulation.

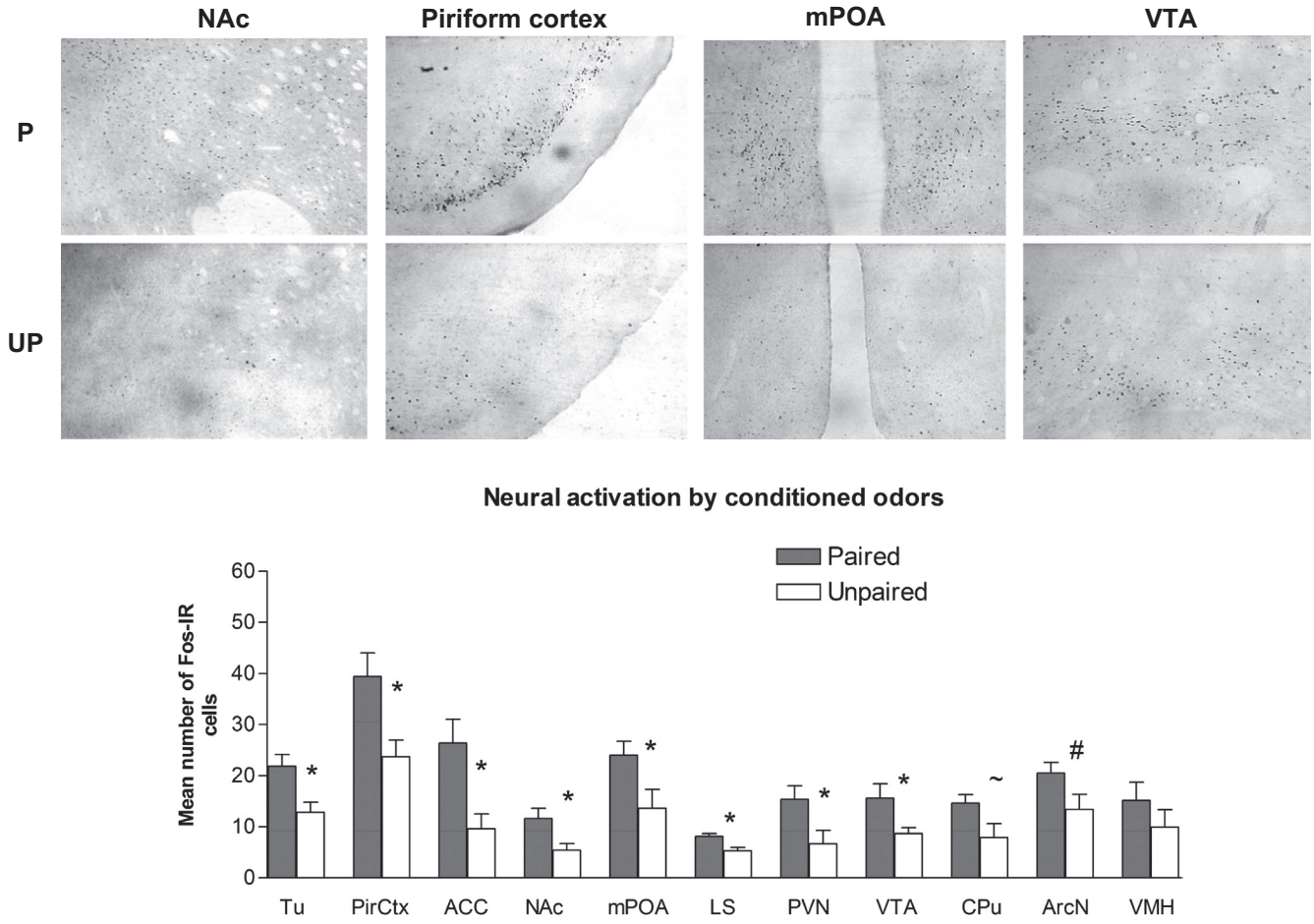

FIGURE 50.26 Selective activation of Fos by an almond odor paired (P) or unpaired (UP) with paced copulation. Note the activation of the central DA cell body-rich region of the VTA by the odor cue. Abbreviations: Tu, olfactory tubercle; PirCtx, piriform cortex; ACC, anterior cingulate cortex; NAc, nucleus accumbens; mPOA, medial preoptic area; LS, lateral septum; PVN, paraventricular nucleus; VTA, ventral tegmental area; CPu, caudate-putamen; ArcN, arcuate nucleus, VMH, ventromedial hypothalamus. * $p<0.05$; # $p = 0–.06$; ~ $p = 0.08$. *Source: Reprinted from Coria-Avila and Pfaus,[76] with permission of Elsevier.*

However, only the GF shows significant activation of Fos within OT and vasopressin neurons of the paraventricular and supraoptic nuclei, and within regions of the hippocampus and corticomedial amygdala that could indicate an additional stress response.

It would appear that female rats possess the ability to show behavioral and neuronal rudiments of either selective or promiscuous mating depending on their early sexual experience. Such experience seems to crystallize sexual response patterns and preferred sexual stimuli by sensitizing a circuit similar to that activated in monogamous prairie voles during their formative sexual experiences[96,651,670] and following parturition.[671] The results of Aragona et al.[96] are particularly instructive, as the sexual bond formation was inhibited by activation of D1 receptors, but facilitated by the activation of D2 receptors. This suggests a neural reorganization in mesolimbic terminals after formative sexual experiences that "seals the bond", making it less likely for other stimuli to acquire associative strength.

Such an effect is consistent with modern theories of learning (e.g., Refs 672,673) and has been implicated in the susceptibility to drug addiction, especially in terms of responding to cues that predict drug reward,[674] and more generally in response to food-related cues.[614,675] The interaction of OT and DA in the PVN, mPOA, VTA, and NAc of male rats induces penile erections and links them to appropriate appetitive sexual behaviors.[676] Thus, opioid reward states may form the rudimentary mechanism of bonding because they sensitize DA release in the presence of reward-related cues compelling animals to focus their attention and goal-directed behavior toward those cues. Activation of brain OT systems (by DA or other means) adds a reduced social distance and bonding to this neurochemical reward state. Given that pharmacological activation of opioid receptors induces a direct suppression of both hypothalamic and pituitary OT secretion,[677] sensitized and potentially reorganized mesolimbic and hypothalamic DA systems must be a necessary intermediary. This is consistent with a

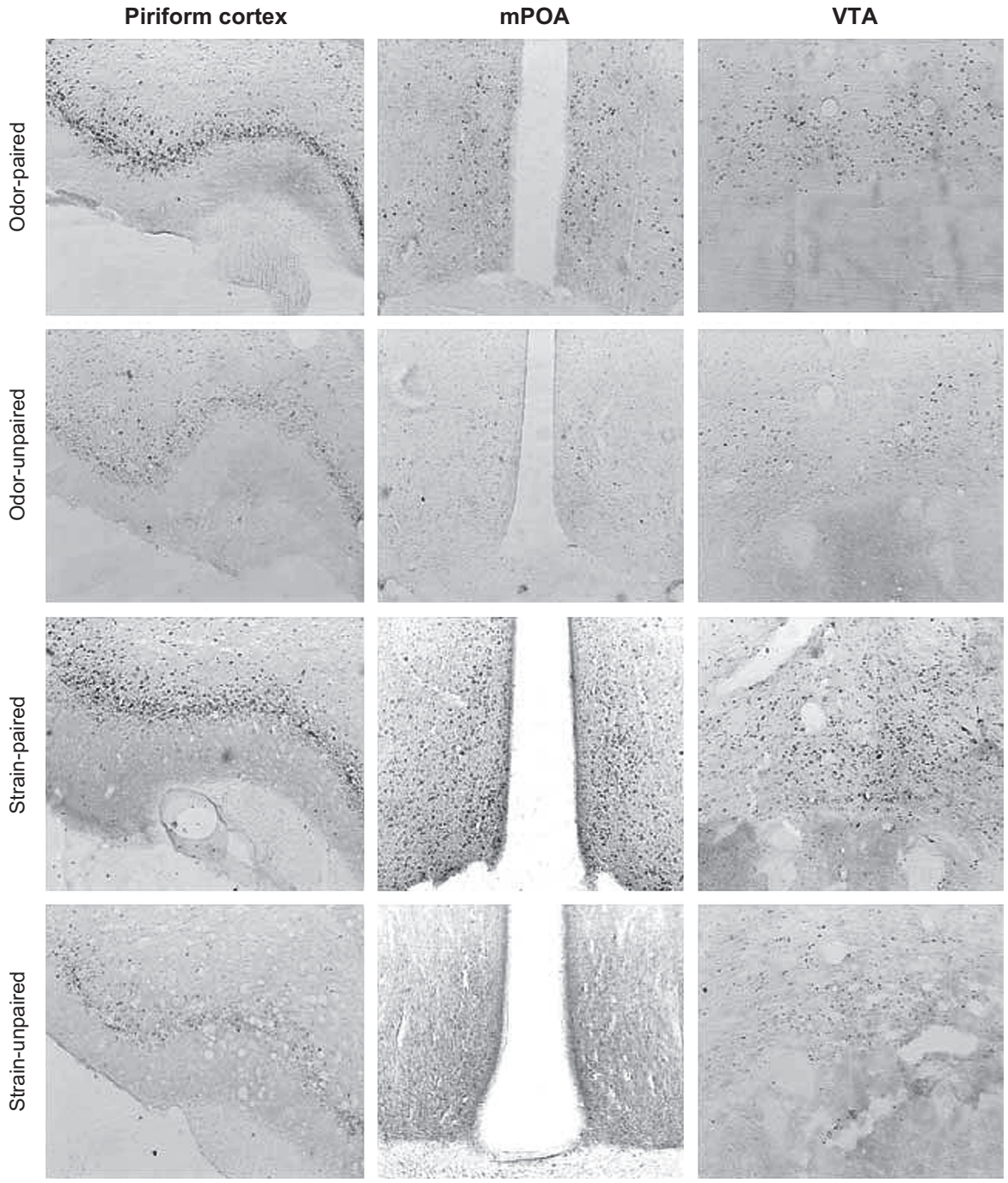

FIGURE 50.27 Comparison of Fos in the piriform cortex, mPOA, or VTA, by almond odor or strain cues (pigmented vs albino) paired with paced copulation. *Source: Reprinted from Coria-Avila and Pfaus,[76] with permission of Elsevier.*

multifaceted role of mesolimbic DA in incentive salience and response initiation.[38]

Conditioned Sexual Arousal in Women

It is difficult to condition sexual arousal or desire in adult humans as they have likely already had their sexual preferences set by experience long before. This is especially true in women when using erotic films or pictures as the UCS that are rated as mildly or moderately stimulating (e.g., Ref. 678). More recent attempts have been more successful using UCSs or CSs of higher incentive quality. For example, Both et al.[679,680] found that neutral pictures of male headshots paired with 2 s of intensely pleasurable vibrotactile CLS produced greater vaginal pulse amplitude (VPA) during extinction in the

paired vs unpaired groups. In another study, stimuli were presented briefly (30 ms).[681] Although only the paired group showed increased VPA to the CS during the first extinction trial, there was no increase in the conscious affective value of the stimulus. Finally, Hoffmann, Janssen, and Turner[682] varied the duration and relevance of a CS (abdominal area vs a gun) that was paired with short erotic film clips in both men and women. Interestingly, when the stimuli were presented subliminally for brief durations, the relevant abdominal stimulus increased arousal in both men and women. However, when the stimuli were presented for longer periods prior to the erotic film clips, a sex difference emerged in which the relevant CS alone (abdominal area) induced genital arousal in men, but the presumably irrelevant stimulus alone (gun) induced genital arousal in women. This latter effect may indicate that women require CSs that increase autonomic arousal to a higher extent than men, a potential corollary of the "discordance" experienced by women, but not men, between genital and subjective sexual arousal.[151] It may also be the case that anything that activates the sympathetic nervous system sufficiently in women may generate a genital blood flow response, regardless of whether the stimulus is sexual in nature. This may occur at anytime during the menstrual cycle, but may be interpreted as more "sexual" when women are periovulatory.

An Integrative Model of Conditioned Sexual Responding

The model proposed by Pfaus, Ismail, and Coria-Avila[683] and shown in Figure 50.28 accounts for both unconditioned and conditioned sexual responding, integrating a number of important regions that regulate female sexual responses by the hypothalamus, limbic system, and cortex. With reference to conditioning, CSs associated with sexual reward state UCSs act as "priming

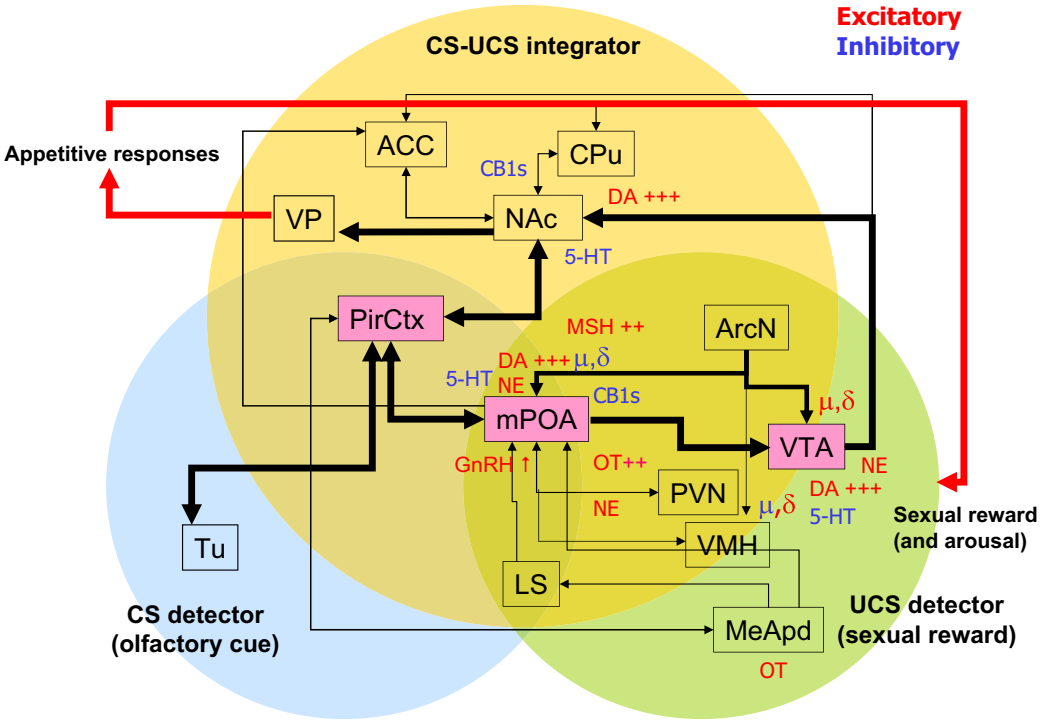

FIGURE 50.28 Neural systems critical for the display of sexual behavior in the rat. This figure is reproduced in color in the color plate section. Appetitive behaviors made toward unconditioned or conditioned sexual incentive stimuli lead to sexual reward that is processed by three interactive systems. Two systems process olfactory stimuli and sexual reward relatively independently, whereas a third, mesolimbic DA system, acts to integrate both the conditioned olfactory cue and its rewarding sexual outcome. Three common regions, the piriform cortex, mPOA, and VTA, are activated in male and female rats by conditioned olfactory stimuli. Opioid actions in the VTA potentiate mesolimbic DA activation, whereas opioid actions in the mPOA inhibit sexual arousal and desire. Neurotransmitter systems or their receptors in red are excitatory for sexual motivation whereas those in blue are inhibitory. Black pathways signify major inputs and outputs of the system. Note that opioids can be excitatory in the VTA, inhibitory in the mPOA, or either in the VMH (depending on the receptor type). A similar system is activated in humans.[63] Abbreviations: ACC, anterior cingulate cortex; ArcN, arcuate nucleus of the hypothalamus; CB1, cannabinoid Type 1 receptor; CPu, caudate-putamen (striatum); DA, dopamine; δ, delta opioid receptors; GnRH, gonadotropin releasing hormone; LS, lateral septum; MeApd, posterior-dorsal nucleus of the medial amygdala; mPOA, medial preoptic area; MSH, melanocyte stimulating hormone; μ, mu opioid receptors; NAc, nucleus accumbens; NE, norepinephrine OT, oxytocin; PirCtx, piriform cortex; PVN, paraventricular nucleus of the hypothalamus; Tu, olfactory tubercle; VMH, ventromedial nucleus of the hypothalamus; VP, ventral pallidum; VTA, ventral tegmental area; 5-HT, serotonin. *Source: Adapted from Pfaus et al.*[683]

stimuli" to activate cortical, limbic, and hypothalamic circuits involved in the facilitation of sexual arousal and desire and/or in the suppression of inhibitory systems (Figure 50.28). Some of those circuits involve selective processing of the sexual stimulation that generates the reward state (UCS), the olfactory stimulus (CS), and a system that integrates the CS and UCS so that animals can focus their attention and engage appropriate forward-directed locomotion toward the CS when it is present.[38,46] What becomes apparent is that systems for sexual reward and incentive responding overlap with systems proposed for sexual (and maternal) bonding (e.g., Ref. 670). These involve the interaction of at least three neurochemical systems, including mesolimbic DA, hypothalamic OT, and opioids that inhibit hypothalamic structures like the mPOA, but sensitize mesolimbic DA systems through a process of disinhibition.[684] This is strikingly similar to Fisher's[685] proposal of three primary emotional systems for mating, reproduction, and parenting, to the mechanism proposed for romantic love in humans,[686] and to the models of human sexual responding proposed by Georgiadis and colleagues.[45,64]

During conditioning, female rats display solicitations indicative of their desire to copulate. If they receive the appropriate stimulation (i.e., distributed CLS during paced copulation) UCS-detector centers become activated (green circle). The posterior-dorsal nucleus of the medial amygdala is sensitive to paced copulation and projects information about it to the mPOA which serves as a main integrator area. The VTA is activated to provide DA to areas important for motivation and decision making (e.g., NAc, ACC) and motor activity (caudate-putamen, ventral pallidum). POMC cells in the ArcN project to the VTA and release opioids that hyperpolarize inhibitory GABAergic neurons (not shown), and therefore increase DA cell firing in mesolimbic and mesocortical terminal regions. The ArcN also projects α-MSH and opioid terminals to the mPOA to facilitate solicitations and reward, respectively. OT terminals from the PVN may release OT into the mPOA to facilitate reward and bonding. Concurrently the Tu (blue circle) and PirCtx sense the CS. PirCtx projects olfactory information to the mPOA and to the NAc to strengthen the incentive value of the CS. Exposure to the conditioned odor alone will activate the common core areas (pink boxes) which trigger motivation and integrate information (yellow circle) about males that bear the CS. Serotonin (5-HT) and endocannabinoid (CB) release and binding in the cortex, limbic structures, and hypothalamus, is associated with an inhibition of the excitatory mechanisms. In animals with a well-developed visual system, like humans, visual CSs likely provide the dominant priming cues, although auditory and olfactory cues should not be overlooked.

CONCLUSION

The study of sexual behavior in animals and humans has come a very long way since the mechanisms of sexual arousal and copulatory responding, and their hormonal and rudimentary neural bases, began to be studied in the 1960s. In particular, the advent of PDE-5 inhibitors for the treatment of erectile dysfunction in men opened up new vistas in the pharmacology of sexual behavior that thrust previous "basic science" studies of the role of different neurotransmitters in sexual behavior into the light of translational clinical science relating to human sexual function and dysfunction. Animal models of a variety of human sexual responses were determined that had predictive validity.[31] It became clear from brain imaging studies in a variety of species using immediate-early gene expression as a measure of activation, and from fMRI or PET studies as measures of activation in humans that a conserved set of neural pathways and neurochemical systems exist in vertebrates to excite and inhibit sexual behavior. Some of those systems are general, and modulate responses to all unconditioned and conditioned excitatory stimuli (e.g., the mesolimbic DA system and its role in incentive motivation) or inhibitory stimuli (e.g., the mPFC and other cortical systems that mediate behavioral inhibition as part of executive function). Other systems are more specific and mediate the autonomic control of genital arousal and the sexual approach and solicitation behaviors that females display as ovulation approaches. Those pathways share functions for parental behavior, feeding, and drug addiction although some, like feeding and sex, may be mutually inhibitory. To what extent do drugs of abuse utilize and usurp the sex and bonding pathways? Understanding the interrelation of these systems, how they are activated by hormones and/or experience, and how experience can override hormonal priming, is a very promising avenue of cross-translational research. Understanding the similarities of bonding to addiction may well prove useful in the treatment of drug abuse or other obsessive-compulsive addictions.

New data always raise new questions but also reframe some very old ones in the literature that have never been resolved. Several of these are outlined below.

What Can Studies of Female Sexual Behavior in Other Species Tell Us About Hormonal Influences on Sexuality in Women?

Much has been learned concerning the neuroendocrine processes and cellular mechanisms by which steroid hormones influence reproductive behaviors in rodents and other animals. Although cellular studies in humans are presently impossible to perform, mechanistic studies in rodents may provide clues about the

neuroendocrine mechanisms by which hormones act and interact in the brain to influence behavior in all species, including humans. A number of basic principles have been derived from work in nonhuman species. For example, these studies demonstrate the importance of considering the timing of hormone treatments, the dosage of hormone, specific hormone used within a particular class of hormones, form of hormone (e.g., long-acting esterified estradiol or nonesterified estradiol), interactions between hormones, the role of steroid receptor coactivators, route of administration, peripheral factors that may influence hormonal response, and the possible mechanisms of action by which hormones and other factors may influence hormone action and subsequently, sexual behaviors.[687]

These major advances in our knowledge belie a continuing gap in our understanding. In particular, scientists are usually forced to measure either behavior, neurochemistry, electrophysiology or neuronal structure, often in a limited time window or with a specific neuroanatomical focus. A major frontier is to understand the causal sequence of these dynamic, network-wide events. Another major frontier is to understand the relative contribution of each of these hormone-induced changes to behavior, given that so many systems are being regulated simultaneously.

At a more granular level of analysis, recent work has identified different cell types in the VMH; however, the wiring diagram for these neurons is unclear. A better understanding of the neurochemistry and connectivity of these neurons would explain in a concrete manner the apparent excitatory and inhibitory controls of lordosis behavior. At present, the functional significance of the diverse effects of estradiol on the dendritic arbors of these cell types is unclear. Detailed knowledge of VMH microcircuitry would help explain how and to what end estradiol exerts these cell-type-specific effects. Furthermore, our present understanding of estradiol-induced changes in VMH dendrite structure is largely divorced from our knowledge of changes in neurochemistry and electrophysiology. A fascinating future direction will be to determine the interplay between changes in synaptic connections and neurotransmission.

What is the Nature of Female Orgasm?

A critical question that continues to generate controversy concerns the existence of a "G-Spot" that when stimulated properly leads to a "deep vaginal orgasm".[688] Still others argue that such an anatomical entity does not exist[689] citing clinical or case studies of women who have never found theirs despite trying. It may well be the case that not all vaginas are constructed the same way, and that internal sensory inputs come in various sizes and shapes. The "G-Spot" may correspond to an internal portion of the clitoris that has differential sensitivity in different women. It is also the case that experience with external CLS as a sole source of sexual stimulation and orgasm may well preclude the exploration of other stimulation points, especially those that might be hard to get to and are never stimulated adequately by a sex partner. fMRI studies of external and internal clitoral/G-Spot stimulation and subjective awareness could help to solve this issue. Indeed, Komisaruk and colleagues have used fMRI to examine brain activation in women self-stimulating to orgasm[690] and it would appear that orgasms can be induced from external clitoral, internal clitoral, and/or cervical stimulation,[691] despite differences in the areas of activation. How these differ in subjective quality could be examined using validated measures such as Orgasm Rating Scale.[692]

A similar problem exists in the animal literature. Although it is clear that VCS and not CLS potentiates estrus termination, it is not known whether paced CLS, VCS, or both, lead to the reward state necessary for the induction of CPP and/or partner preference. Moreover, it has not been established that the penis of the male rat actually makes contact with the cervix during intromission, although the ejaculatory plug of the male forms around the cervix and pubic bone, which would provide intense and continuous VCS when it occurs and until the plug is removed from the vagina. However, the rate and duration of intromissions is higher in paced vs nonpaced conditions.[523] This, and not the presence or absence of ejaculations, is key in the induction of estrus termination and in the neuroendocrine responses (e.g., nightly prolactin surges) that facilitate pregnancy. Erskine[74] has argued that paced copulation results in stronger intromission thrusts, which may well stimulate both the clitoris and cervix.

How Does Awareness of Sexual Incentives Change across the Menstrual Cycle?

More attention needs to be paid to the menstrual cycle and how it affects women's reactions to sexual incentive cues. These could build upon and extend current knowledge about autonomic, emotional, and cognitive changes across the cycle as they relate to the perception of, and reaction to, external sexual stimuli. Those perceptions could then be examined in terms of brain activation following the presentation of stimuli through goggles worn by the subjects. In this vein, cognitive tasks that examine changes in relative attention toward explicitly sexual visual cues presented either above or below conscious awareness could be used in conjunction with physiological measures of sexual arousal to examine how the two are altered across the cycle (see below).

Will There be Drug Treatments for Sexual Arousal, Desire, and Orgasm Disorders?

Disorders of sexual arousal, desire, and orgasm affect a sizable proportion of pre- and post-menopausal women world-wide depending on the criteria used to define the disorder.[693] This can occur as a function of hormone or neurochemical imbalance, genetic "proneness" to inhibition, depression and other mental illness, and the use of oral contraceptives that generate negative steroid feedback.

As noted above, the development of treatments for sexual disorders in women has benefited greatly from preclinical analyses provided by animal studies. Such studies can examine mechanisms at different levels of analysis (e.g., neuropharmacological to molecular) in ways that simply cannot be done in humans. This requires a complete analysis of the behavior of the animal "models", and some degree of predictive validity that what is being observed in the particular animal model corresponds to the sexual process that requires treatment. In turn, this requires a conceptual understanding of the functional endpoint. For example, approach and solicitation in female rats or macaques serves the same purpose as approach, flirtations, and maintenance of eye-gaze in women who have found someone sexually alluring, This is a critical behavioral juncture: in those with desire disorders such behavior simply does not occur. In extreme cases it is not even thought of. What remains in many women is arousal without a proper context and no sexual incentive to blame it on. Others lose the arousal component as well. And of course, without this there is little chance of sexual gratification, leading to sexual interactions that are likely aversive. This may especially be the case if the woman is prone to inhibition (e.g., Ref. 694).

In some women this is remedied easily by psychodynamic therapies, whereas in others it is not. Such women may well have a physiological blunting of response that is due to hormonal or neurochemical systems that either are no longer operating properly to excite arousal and desire (as may occur in many women during and after menopause), or overactive inhibitory systems that have come online for a variety of reasons, some of which may be genetic and due to an increased sensitivity to sadness and depression (e.g., overexpression of the long allele form of the promoter regions of the serotonin transporter gene[695]).

The discovery of potential pharmacological treatments for arousal and desire disorders in women have been largely serendipitous, with the prosexual effects of the MC-4 receptor agonist bremelanotide discovered during clinical trials of the potential tanning drug MT-II[696] and the sexual effects of flibanserin discovered during its trials as a potential antidepressant.[642] The potential of testosterone as a therapy for the treatment of sexual arousal and desire disorders after menopause was first shown in studies where it was added as an adjunct to estradiol.[138] Current treatments with testosterone include a transdermal testosterone patch (Intrinsa) and labial gel (Libigel), both of which produce rather continuous penetration of testosterone into the circulation. Although these have positive efficacy in the treatment of arousal and desire disorders,[697] they do not mimic the normal testosterone rise during ovulation.[698] The efficacy of applying testosterone as a sublingual bolus was advanced by Tuiten et al.[699] and recently shown in clinical trials that applied it in conjunction with a PDE-5 inhibitor or 5-HT1A agonist to treat hypoactive desire induced by either a "bottom-up" lack of genital sensitivity[700] or an abundance of "top down" inhibition over sexual incentive cues.[694] The first effect has been modeled in female rats.[71] The dose of testosterone is extremely small, making it likely that its action is only in the brain. The PDE-5 inhibitor acts in the periphery to relax smooth muscle in the genitals, allowing for more rapid and complete engorgement. Approximately 4h after administration, the brain is "ready" for sex, having undergone genomic changes induced presumably by testosterone that allow both genital arousal and sexual incentive stimuli to be registered and integrated. Essentially this "tricks" the brain into thinking that ovulation has just occurred. The combination is reported to be well tolerated and devoid of untoward side-effects in a number of treatment regimens. A major advantage is that the combination can be taken "on demand" prior to sexual activity.

It is impossible to know at this point whether any of these drugs will be approved for the treatment of hypoactive sexual desire, interest, and/or arousal. However, as work continues to enhance our understanding of the neurochemical systems involved in sexual excitation and inhibition, it is likely that other drugs will be subjected to clinical trials. It has been predicted that combined pharmacological and traditional talk therapies will have better efficacy than either alone.[701] However it is not yet known whether some women, pre- or post-menopausal, will retain their restoration of sexual arousal and desire if the pharmacological treatment is discontinued. All of this could be modeled in rats or other species using appropriate behavioral analyses. It will also become vitally important to understand how testosterone or other androgens are working in the female brain. Although a good starting point is to examine whether they operate on classic intracellular or membrane bound receptors, it may also be the case that they are aromatized into estrogens to induce their actions. This will be important in determining how testosterone sets up the neurochemical substrates that bring sexual incentive cues into conscious awareness in the female brain. Likewise, a greater understanding of the roles played by OT and prolactin in women is critical in helping to elucidate

the mechanisms of orgasm and its aftermath. This may help to differentiate women with orgasm difficulties who have either never experienced them or experienced them fleetingly vs those that have sluggish brain and/or autonomic reactions to sexual stimulation.

How Does Sexual Responding Change with Age?

As the so-called "Baby Boomer" generation is aging and millions of women world-wide are reaching menopause and beyond, more research must be focused on how the aging female brain and body change in response to the hormonally unstable and ultimately hypogonadal condition that menopause engages (see Chapter 37). Women experience changes in cognition and emotional reactions, in addition to bone density and fat deposition as a function of altered metabolism. Sexuality changes during aging as a function of experience and expectation, relationship status, hormonal alterations, and changes in genital sensitivity. Very little is understood about age-related changes in the brains of women in regards to sexuality, and little has been studied in this regard in animals. Aging brings about cardiovascular and metabolic changes associated with conditions such as diabetes and hypertension, which can blunt sexual arousal and desire in both men and women.[35] The treatments for those conditions also have sexual "side effects" that blunt sexual arousal and possibly desire and orgasm,[35] so treatments that restore sexual function must be able to operate independently of those mechanisms and not exacerbate them.

Can We Infer Mechanisms in Females from Similar Mechanisms in Males?

There is no question that substantial sex differences exist in morphology and brain neurochemical function[702] that underlie differences between females and males in response to sexual stimuli,[703] motivation,[704] control of pituitary hormone systems in rodents,[705] pain,[706] and mental health.[707] Some of these mechanisms have their origin in the initial sexual differentiation that occurs in the neonatal brain,[708,709] whereas others do not.[710,711] In particular, McCarthy et al.[711] note that a common strategy in the experimental approach taken by many neuroendocrinologists is "after I understand the phenomenon in males, I'll check whether it's there in females". This approach has also been taken in understanding the hemodynamic of sexual arousal in men and women, often with the assumption that anything that induces penile erection in men should also work the same way for the induction of labial, clitoral, and vaginal erection in women. Certainly PDE-5 inhibitors do increase vaginal vasocongestion but many women are unaware of that, leading to the phenomenon of "discordance" between subjective reports of sexual arousal and physiological measures of such arousal.[151] Such discordance between subjective and physiological sexual arousal is not observed in men unless they have consumed a threshold dose of alcohol that blunts erection but disinhibits subjective arousal and desire.[13,712] There may be several reasons for this. Sex differences likely exist in the cellular response to cyclic vs continuous gonadal hormone output; in the reaction to context; and in individual responses to preexisting stressors. Individual differences also exist in terms of experiences with, and attitudes toward, different types of erotic stimuli that are presented to provoke a genital response. These sex and individual differences conspire to create differences in conscious sexual response which is reflected in brain activation (e.g., laterality in amygdala responsivity[703]). Although it is difficult to find sex differences in the human sexual response cycle, other than the ability of some women to have multiple orgasms relative to men's inability to do so, there are obvious sex differences in the behavior of female and male rodents. Those differences are typically "reverse-engineered" back to differences in differentiation of brain and body, and to specific differences in brain area, for example, the sexually dimorphic nucleus of the preoptic area. Indeed, lesions of the POA in male rats disrupt erection, ejaculation, and mounting behavior, whereas lesions in female rats disrupt solicitations and pacing, and may affect the hemodynamics of clitoral engorgement or the ability of genital stimulation (clitoral and/or cervical) to induce reward. At one level, those functions and the behaviors they subserve seem very different. At another, however, they both involve the responses to genital stimulation that bring about direct sexual contact: solicitations and pacing in females and mounting in males. In fact, in the human brain activation literature, there is an overwhelming preopoderance of sex *similarities* in regional responses to erotic visual stimuli.[45,713] Thus, in rats, the same regions may respond similarly to sexual stimulation but induce output that appears different at a behavioral level. In humans, the behavioral differences may well have disappeared, or been impinged upon by a greater executive cortical/cognitive control over sexual behavior that is mediated more by context and social learning. However, rats clearly have the capacity to make both Pavlovian (stimulus–stimulus) and operant (response-reinforcer) associations between sexual stimuli and sexual responses that can span first- and second-order conditioning, even to the point of conditioning of sexual fetishes for rodent tethering jackets.[28,714]

Context is also an important component of sexual behavior in both female and male rats.[50,70,668] It may well be the case that sex similarities exist in rats, as they do in humans, especially at "higher" emotional and cognitive processing levels that include cortical processing. It

is the case that human neuropsychology and brain imaging studies on incentive sexual responses have focused on cortical and limbic structures, usually with a mention that the hypothalamus is or is not activated, whereas animal studies have focused largely on hypothalamic and some limbic activation of immediate-early gene products, or microdialysis/voltammetric analyses of neurotransmitter turnover. Rarely do neuroendocrinologists study the cerebral cortex, nor do neuropsychologists delve into the hypothalamus, and this confounds our understanding. In fact, analysis of both animal and human brain activation to sexual stimulation reveals nearly identical cortical responses,[64] suggesting that even "higher processes" have been conserved, and that the sexual brain is not simply reducible to hypothalamic processing.

How Does Environmental Context Modulate Hormonal Action on Female Sexual Behavior?

A number of examples were given earlier of nonsteroid hormone factors that can influence steroid receptors, and subsequently behavioral response. In addition, we discussed the idea that stimuli from the environment (mating stimulation) can activate steroid receptors indirectly by ligand-independent activation. This mechanism provides a means by which an array of environmental influences could alter sexual response. Do these mechanisms come into play in the real world of a wild rat? Do attempted mounts by conspecifics or other environmental factors alter the timing of the commencement of sexual behaviors? And might particular contexts or types of stimulation activate steroid receptors in women thus influencing sexual response? It is also important to consider whether incentive cues associated with sexual reward alter steroid ligand–receptor interactions and whether such changes, if any, can compensate for the loss of hormonal priming that occurs in hypogonadal individuals.

Final Remarks

Our understanding of the neurobiology of female sexual function is fast reaching a level of depth and sophistication that rivals that of male sexual function. Animal models of human female sexual function and dysfunction have been proposed that emphasize reward-related learning and cognitive assessment of context. Within these new paradigms, the role of ovarian hormones, gene expression, neurochemical mediation, and the impact of brain lesions (both surgical and accidental) are beginning to be assessed. The sexual brain integrates sensory and hormonal inputs to the hypothalamus to activate incentive motivation and emotional responses in limbic structures. These are under cortical control to inhibit unnecessary or competing responses, or indeed to inhibit sexual responding altogether in inappropriate contexts or situations. The combined actions of these systems optimize female sexual responding, so that what is most rewarding is also likely to be the most reproductively efficient. That these systems are essentially conserved in all mammals—and perhaps all vertebrates—is a testament to our continued survival on this planet, survival that depends critically on the ability of females to approach, solicit, pace, and engage in rewarding sexual activities under their control.

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References

1. O'Dowd MJ, Philipp EE. *The history of obstetrics and gynaecology*. New York: Informa Health Care; 2000.
2. Pfaus JG. Revisiting the concept of sexual motivation. *Annu Rev Sex Res* 1999;10:120–56.
3. Thompson DW. The works of Aristotle translated into English. In: Smith JA, Ross WD, editors. *Historia animalium*;vol. IV. Oxford: Clarendon Press; 1910.
4. Néret G. *Erotica universalis*. Frankfurt: Taschen; 1994.
5. Gabor M. *The pin-up*. New York: Universe Books; 1973.
6. Fowler OS. *Sexual science*. Philadelphia: National Publishing Co.; 1870.
7. Darwin C. *The descent of man*. New York: D. Appleton and Company; 1871.
8. Maines RP. *The technology of orgasm: "Hysteria," the vibrator, and women's sexual satisfaction*. Baltimore: Johns Hopkins University Press; 1999.
9. Short RV. The discovery of the ovaries. In: 2nd ed. Zuckerman S, Weir BJ, editors. *The ovary*;vol. 1. London: Academic Press; 1977. pp. 1–39.
10. Ford CS, Beach FA. *Patterns of sexual behavior*. New York: Harper and Roe; 1951.
11. Pfaus JG, Gorzalka BB. Opioids and sexual behavior. *Neurosci Biobehav Rev* 1987;11:1–34.
12. Abel EL. A review of alcohol's effects on sex and reproduction. *Drug Alcohol Depend* 1980;5:321–32.
13. Wilson GT. Alcohol and human sexual behavior. *Behav Res Ther* 1977;15:239–52.
14. Beach FA, Etkin W, Rasquin P. Importance of progesterone to induction of sexual receptivity in spayed female rats. *Proc Soc Exp Biol Med* 1942;51:369–71.

15. Larsson K, Heimer L. Mating behaviour of male rats after lesions in the preoptic area. *Nature* 1964;202:413–4.
16. Kennedy GC. Hypothalamic control of the endocrine and behavioural changes associated with oestrus in the rat. *J Physiol* 1964;172:383–92.
17. Kennedy GC, Mitra J. Hypothalamic control of energy balance and the reproductive cycle in the rat. *J Physiol* 1963;166:395–407.
18. Pfaus JG, Heeb MM. Implications of immediate-early gene induction in the brain following sexual stimulation of female and male rodents. *Brain Res Bull* 1997;44:397–407.
19. Kinsey AAC, Pomeroy WB, Martin CCE. *Sexual behavior in the human male*. Indiana University Press; 1948.
20. Kinsey AC, Pomeroy WB, Martin CE, Gebhard PH. *Sexual behavior in the human female*. Indiana University Press; 1953.
21. Masters WH, Johnson VE. *Human sexual response*. Boston: Little; 1966.
22. Freud S. Three essays on the theory of sexuality. *S E* 1905;7.
23. Hite S. *The Hite report: a nationwide study on female sexuality*. New York: Macmillan; 1976.
24. Hite S. *The Hite report on male sexuality*. New York: Knopf; 1981.
25. Tiefer L. Omissions, biases, and nondisclosed conflicts of interest: is there a hidden agenda in the NAMS position statement? *MedGenMed* 2005;7:59.
26. Tiefer L. Female sexual dysfunction: a case study of disease mongering and activist resistance. *PLoS Med* 2006;3:e178.
27. Beach FA. The snark was a boojum. *Am Psychol* 1950;5:115–24.
28. Pfaus JG, Kippin TE, Coria-Avila GA, et al. Who, what, where, when (and maybe even why)? How the experience of sexual reward connects sexual desire, preference, and performance. *Arch Sex Behav* 2012;41:31–62.
29. Agmo A, Ellingsen E. Relevance of non-human animal studies to the understanding of human sexuality. *Scand J Psychol* 2003;44:293–301.
30. Pfaus JG, Giuliano F, Gelez H. Bremelanotide: an overview of preclinical CNS effects on female sexual function. *J Sex Med* 2007;4(Suppl. 4):269–79.
31. Pfaus JG, Kippin TE, Coria-Avila GA. What can animal models tell us about human sexual response? *Annu Rev Sex Res* 2003;14:1–63.
32. Pfaff DW. *Estrogens and brain function: neural analysis of a hormone-controlled mammalian reproductive behavior*. New York: Springer Verlag; 1980.
33. Pfaus JG, Scepkowski LA. The biologic basis for libido. *Curr Sex Health Rep* 2005;2:95–100.
34. Pavlov IP. *Conditioned reflexes*. New York: Dover Publications; 1927.
35. Pfaus JG. Pathways of sexual desire. *J Sex Med* 2009;6:1506–33.
36. Bush G. Dorsal anterior cingulate cortex: a role in reward-based decision making. *Proc Natl Acad Sci USA* 2001;99:523–8.
37. Carter CS, Braver TS, Barch DM, Botvinick MM, Noll D, Cohen JD. Anterior cingulate cortex, error detection, and the online monitoring of performance. *Science* 1998;280:747–9.
38. Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev* 1993;18:247–91.
39. Beach FA. Sexual attractivity, proceptivity, and receptivity in female mammals. *Horm Behav* 1976;7:105–38.
40. Craig W. Appetites and aversions as constituents of instincts. *Biol Bull* 1918.
41. Woodworth RS. *Dynamic psychology*. New York: Columbia University Press; 1918.
42. Mackintosh NJ. *The psychology of animal learning*. London: Academic Press; 1974.
43. Toates F. An integrative theoretical framework for understanding sexual motivation, arousal, and behavior. *J Sex Res* 2009;46:168–93.
44. Kaplan HS. *The new sex therapy*. New York: Brunel/Mazel; 1974.
45. Georgiadis JR, Kringelbach ML. The human sexual response cycle: brain imaging evidence linking sex to other pleasures. *Prog Neurobiol* 2012;98:49–81.
46. Berridge KC, Robinson TE, Aldridge JW. Dissecting components of reward: 'liking', 'wanting', and learning. *Curr Opin Pharmacol* 2009;9:65–73.
47. Basson R. Women's sexual dysfunction: revised and expanded definitions. *CMAJ* 2005;172:1327–33.
48. Wallen K. The evolution of female sexual desire. In: Abramson PR, Pinkerton SD, editors. *Sexual nature, sexual culture*. Chicago: University of Chicago Press; 1995. pp. 57–79.
49. Erskine MS. Solicitation behavior in the estrous female rat: a review. *Horm Behav* 1989;23:473–502.
50. McClintock M. Group mating in the domestic rat as a context for sexual selection: consequences for the analysis of sexual behavior and neuroendocrine responses. *Adv Study Behav* 1984;14:2–50.
51. Adler N. Effects of the male's copulatory behavior on successful pregnancy of the female rat. *J Comp Physiol Psychol* 1969;69:613–22.
52. Coopersmith C, Candurra C, Erskine MS. Effects of paced mating and intromissive stimulation on feminine sexual behavior and estrus termination in the cycling rat. *J Comp Psychol* 1996;110:176–86.
53. Kornberg E, Erskine MS. Effects of differential mating stimulation on the onset of prolactin surges in pseudopregnant rats. *Psychoneuroendocrinology* 1994;19:357–71.
54. Slob AK, Ernste M, Werff ten Bosch JJ. Menstrual cycle phase and sexual arousability in women. *Arch Sex Behav* 1991;20:567–77.
55. Stanislaw H, Rice FJ. Correlation between sexual desire and menstrual cycle characteristics. *Arch Sex Behav* 1988;17:499–508.
56. Singer I, Singer J. Periodicity of sexual desire in relation to time of ovulation in women. *J Biosoc Sci* 1972;4:471–81.
57. Wallen K, Winston LA, Caventa S, Davis-Dasilva M, Collins DC. Periovulatory changes in female sexual behavior and patterns of ovarian steroid secretion in group-living rhesus monkeys. *Horm Behav* 1984;18:431–50.
58. Gizewski ER, Krause E, Karama S, Baars A, Senf W, Forsting M. There are differences in cerebral activation between females in distinct menstrual phases during viewing of erotic stimuli: a fMRI study. *Exp Brain Res* 2006;174:101–8.
59. Krug R, Plihal W, Fehm HL, Born J. Selective influence of the menstrual cycle on perception of stimuli with reproductive significance: an event-related potential study. *Psychophysiology* 2000;37:111–22.
60. Mass R, Hölldorfer M, Moll B, Bauer R, Wolf K. Why we haven't died out yet: changes in women's mimic reactions to visual erotic stimuli during their menstrual cycles. *Horm Behav* 2009;55:267–71.
61. Rupp HA, James TW, Ketterson ED, Sengelaub DR, Janssen E, Heiman JR. Neural activation in the orbitofrontal cortex in response to male faces increases during the follicular phase. *Horm Behav* 2009;56:66–72.
62. Pfaff DW. *Drive: neurobiological and molecular mechanisms of sexual motivation*. Massachusetts: MIT Press; 1999.
63. Hull E, Dominguez JM, Muschamp JW. *Neurochemistry of male sexual behavior*. Springer Verlag; 2007.
64. Georgiadis JR, Kringelbach ML, Pfaus JG. Sex for fun: a synthesis of human and animal neurobiology. *Nat Rev Urol* 2012;9:486–98.
65. Boling JL, Blandau RJ. The estrogen-progesterone induction of mating responses in the spayed female rat. *Endocrinology* 1939;25:359–64.
66. Giraldi A, Marson L, Nappi R, et al. Physiology of female sexual function: animal models. *J Sex Med* 2004;1:237–53.
67. Giuliano F, Pfaus JG, Srilatha B, et al. Experimental models for the study of female and male sexual function. *J Sex Med* 2010;7:2970–95.

68. McMurray G, Casey JH, Naylor AM. Animal models in urological disease and sexual dysfunction. *Br J Pharmacol* 2006;147(Suppl. 2): S62–79.
69. Pfaus JG, Frank A. Beach award. Homologies of animal and human sexual behaviors. *Horm Behav* 1996;30:187–200.
70. Pfaus JG, Smith WJ, Coopersmith CB. Appetitive and consummatory sexual behaviors of female rats in bilevel chambers: I. A correlational and factor analysis and the effects of ovarian hormones. *Horm Behav* 1999;35:224–40.
71. Snoeren EMS, Chan JSW, de Jong TR, Waldinger MD, Olivier B, Oosting RS. A new female rat animal model for hypoactive sexual desire disorder; behavioral and pharmacological evidence. *J Sex Med* 2010;8:44–56.
72. Beach FA, Jordan L. Sexual exhaustion and recovery in the male rat. *Q J Exp Psychol* 1956;8:121–33.
73. Larsson K. *Conditioning and sexual behavior in the male albino rat*. Oxford: Almqvist & Wiksell; 1956.
74. Erskine MS. Effects of paced coital stimulation on estrus duration in intact cycling rats and ovariectomized and ovariectomized-adrenalectomized hormone-primed rats. *Behav Neurosci* 1985;99:151–61.
75. Pfaus JG, Smith WJ, Byrne N, Stephens G. Appetitive and consummatory sexual behaviors of female rats in bilevel chambers II. Patterns of estrus termination following vaginocervical stimulation. *Horm Behav* 2000;37:96–107.
76. Coria-Avila GA, Pfaus JG. Neuronal activation by stimuli that predict sexual reward in female rats. *Neuroscience* 2007;148:623–32.
77. Coria-Avila GA, Jones SL, Solomon CE, Gavrila AM, Jordan GJ, Pfaus JG. Conditioned partner preference in female rats for strain of male. *Physiol Behav* 2006;88:529–37.
78. Coria-Avila GA, Ouimet AJ, Pacheco P, Manzo J, Pfaus JG. Olfactory conditioned partner preference in the female rat. *Behav Neurosci* 2005;119:716–25.
79. Paredes RG. Hormones and sexual reward. In: Litwack G, editor. *Vitamins & hormones*. Oxford: Academic Press; 2010. pp. 241–62.
80. Mendelson SD, Gorzalka BB. Cholecystokinin-octapeptide produces inhibition of lordosis in the female rat. *Pharmacol Biochem Behav* 1984;21:755–9.
81. Paredes RG, Vazquez B. What do female rats like about sex? Paced mating. *Behav Brain Res* 1999;105:117–27.
82. Coria-Avila GA, Gavrila AM, Boulard B, Charron N, Stanley G, Pfaus JG. Neurochemical basis of conditioned partner preference in the female rat: II. Disruption by flupenthixol. *Behav Neurosci* 2008;122:396–406.
83. Coria-Avila GA, Solomon CE, Vargas EB, et al. Neurochemical basis of conditioned partner preference in the female rat: I. Disruption by naloxone. *Behav Neurosci* 2008;122:385–95.
84. Jones SL, Farrell S, Gardner Gregory J, Pfaus JG. Sensitization of sexual behavior in ovariectomized rats by chronic estradiol treatment. *Horm Behav* 2013;64:8–18.
85. Pfaus JG, Mendelson SD, Phillips AG. A correlational and factor analysis of anticipatory and consummatory measures of sexual behavior in the male rat. *Psychoneuroendocrinology* 1990;15:329–40.
86. van Furth WR, van Ree JM. Sexual motivation: involvement of endogenous opioids in the ventral tegmental area. *Brain Res* 1996;729:20–8.
87. Estep DQ, Lanier DL, Dewsbury DA. Copulatory behavior and nest building behavior of wild house mice (*Mus musculus*). *Anim Learn Behav* 1975;3:329–36.
88. Farmer MA, Leja A, Foxen-Craft E, et al. Pain reduces sexual motivation in female, but not male, mice. *J Neurosci*, in press.
89. Harper LV. The transition from filial to reproductive function of "Coitus-Related" responses in young guinea pigs. *Dev Psychobiol* 1972;5:21–34.
90. Pfaus JG, Pinel JPJ. Alcohol inhibits and disinhibits sexual behavior in the male rat. *Psychobiology* 1989;17:195–201.
91. Raible LH, Gorzalka BB. Receptivity in Mongolian gerbils: dose and temporal parameters of ovarian hormone administration. *Lab Anim* 1986;20:109–13.
92. Lisk RD, Reuter LA, Raub JA. Effects of grouping on sexual receptivity in female hamsters. *J Exp Zool* 1974;189:1–6.
93. Petrulis A. Neural mechanisms of individual and sexual recognition in Syrian hamsters (*Mesocricetus auratus*). *Behav Brain Res* 2009;200:260–7.
94. Meisel RL, Mullins AJ. Sexual experience in female rodents: cellular mechanisms and functional consequences. *Brain Res* 2006;1126:56–65.
95. Young LJ, Wang Z, Insel TR. Neuroendocrine bases of monogamy. *Trends Neurosci* 1998;21:71–5.
96. Aragona BJ, Liu Y, Yu YJ, et al. Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. *Nat Neurosci* 2006;9:133–9.
97. Kawano K. Aggressive behavior of the domesticated house musk shrew (*Suncus murinus*) in inter-male, inter-female and heterosexed interactions. *J Ethol* 1992;10:119–31.
98. Rissman EF. Social variables influence female sexual behavior in the musk shrew (*Suncus murinus*). *J Comp Psychol* 1987;101:3–6.
99. Veney SL, Rissman EF. Steroid implants in the medial preoptic area or ventromedial nucleus of the hypothalamus activate female sexual behaviour in the musk shrew. *J Neuroendocrinol* 2000;12:1124–32.
100. Schiml PA, Rissman EF. Cortisol facilitates induction of sexual behavior in the female musk shrew (*Suncus murinus*). *Behav Neurosci* 1999;113:166–75.
101. Bakker J, Baum MJ. Neuroendocrine regulation of GnRH release in induced ovulators. *Front Neuroendocrinol* 2000;21:220–62.
102. Melo AI, Gonzalez-Mariscal G. Communication by olfactory signals in rabbits: its role in reproduction. *Vitam Horm* 2010;83: 351–71.
103. Zehr JL, Maestripieri D, Wallen K. Estradiol increases female sexual initiation independent of male responsiveness in rhesus monkeys. *Horm Behav* 1998;33:95–103.
104. Zumpe D, Michael RP. Redirected aggression and gonadal hormones in captive Rhesus monkeys (*Macaca mulatta*). *Anim Behav* 1970;18:11–9.
105. Zumpe D, Michael RP. The clutching reaction and orgasm in the female rhesus monkey (*Macaca mulatta*). *J Endocrinol* 1968;40:117–23.
106. Beach FA. Factors involved in the control of mounting behavior by female mammals. In: Diamond E, editor. *Perspectives in reproduction and sexual behavior*. Bloomington: Indiana University Press; 1968. pp. 83–131.
107. Michael RP, Bonsall RW. Peri-ovulatory synchronisation of behaviour in male and female rhesus monkeys. *Nature* 1977;265:463–5.
108. Goldfoot DA, Westerborg-van Loon H, Groeneveld W, Slob AK. Behavioral and physiological evidence of sexual climax in the female stump-tailed macaque (*Macaca arctoides*). *Science* 1980;208:1477–9.
109. Vasey PL. Female choice and inter-sexual competition for female sexual partners in Japanese macaques. *Behaviour* 1998;135:579–97.
110. Vasey PL. Sexual partner preference in female Japanese macaques. *Arch Sex Behav* 2002;31:51–62.
111. Vasey PL. Sex differences in sexual partner acquisition, retention, and harassment during female homosexual consortships in Japanese macaques. *Am J Primatol* 2004;64:397–409.
112. Vasey PL, Foroud A, Duckworth N, Kovacovsky SD. Male–female and female–female mounting in Japanese macaques: a comparative study of posture and movement. *Arch Sex Behav* 2006;35:116–28.

113. Furuichi T. Female contributions to the peaceful nature of bonobo society. *Evol Anthropol* 2011;20:131–42.
114. Hohmann G, Fruth B. Use and function of genital contacts among female bonobos. *Anim Behav* 2000;60:107–20.
115. Adams DB, Gold AR, Burt AD. Rise in female-initiated sexual activity at ovulation and its suppression by oral contraceptives. *N Engl J Med* 1978;299:1145–50.
116. Dennerstein L, Gotts G, Brown JB, Morse CA, Farley TM, Pinol A. The relationship between the menstrual cycle and female sexual interest in women with PMS complaints and volunteers. *Psychoneuroendocrinology* 1994;19:293–304.
117. Harvey SM. Female sexual behavior: fluctuations during the menstrual cycle. *J Psychosom Res* 1987;31:101–10.
118. Van Goozen SH, Wiegant VM, Endert E, Helmond FA, Van de Poll NE. Psychoendocrinological assessment of the menstrual cycle: the relationship between hormones, sexuality, and mood. *Arch Sex Behav* 1997;26:359–82.
119. Graham CA, Sherwin BB. The relationship between mood and sexuality in women using an oral contraceptive as a treatment for premenstrual symptoms. *Psychoneuroendocrinology* 1993;18:273–81.
120. Warner P, Bancroft J. Mood, sexuality, oral contraceptives and the menstrual cycle. *J Psychosom Res* 1988;32:417–27.
121. Bancroft J. Sexual effects of androgens in women: some theoretical considerations. *Fertil Steril* 2002;77:55–9.
122. Mathur RS, Landgrebe SC, Moody LO, Semmens JP, Williamson HO. The effect of estrogen treatment on plasma concentrations of steroid hormones, gonadotropins, prolactin and sex hormone-binding globulin in post-menopausal women. *Maturitas* 1985;7:129–33.
123. Rothman MS, Carlson NE, Xu M, et al. Reexamination of testosterone, dihydrotestosterone, estradiol and estrone levels across the menstrual cycle and in postmenopausal women measured by liquid chromatography-tandem mass spectrometry. *Steroids* 2011;76:177–82.
124. Zimmerman Y, Eijkemans MJC, Coelingh Bennink HJT, Blankenstein MA, Fauser BCJM. The effect of combined oral contraception on testosterone levels in healthy women: a systematic review and meta-analysis. *Hum Reprod Update* 2013. http://dx.doi.org/10.1093/humupd/dmt038.
125. Schwenkhagen A, Studd J. Role of testosterone in the treatment of hypoactive sexual desire disorder. *Maturitas* 2009;63:152–9.
126. Davis SR, Braunstein GD. Efficacy and safety of testosterone in the management of hypoactive sexual desire disorder in postmenopausal women. *J Sex Med* 2012;9:1134–48.
127. Shifren JL, Braunstein GD, Simon JA, et al. Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. *N Engl J Med* 2000;343:682–8.
128. Leiblum SR, Koochaki PE, Rodenberg CA, Barton IP, Rosen RC. Hypoactive sexual desire disorder in postmenopausal women: US results from the Women's International Study of Health and Sexuality (WISHeS). *Menopause* 2006;13:46–56.
129. Dennerstein L, Koochaki P, Barton I, Graziottin A. Hypoactive sexual desire disorder in menopausal women: a survey of western European women. *J Sex Med* 2006;3:212–22.
130. Giuliano F, Rampin O, Allard J. Neurophysiology and pharmacology of female genital sexual response. *J Sex Marital Ther* 2002;28:S101–21.
131. Braunstein GD. Androgen insufficiency in women. *Growth Horm IGF Res* 2006;16:109–17.
132. Masters WH, Johnson VE, Kolodny RC. *Masters and Johnson on sex and human loving*. Little Brown and Company; 1986.
133. Burger HG, Hailes J, Menelaus M, Nelson J, Hudson B, Balazs N. The management of persistent menopausal symptoms with oestradiol-testosterone implants: clinical, lipid and hormonal results. *Maturitas* 1984;6:351–8.
134. van Netten JJ, Georgiadis JR, Nieuwenburg A, Kortekaas R. 8–13 Hz fluctuations in rectal pressure are an objective marker of clitorally-induced orgasm in women. *Arch Sex Behav* 2008;37:279–85.
135. Burger H, Hailes J, Nelson J, Menelaus M. Effect of combined implants of oestradiol and testosterone on libido in postmenopausal women. *Br Med J (Clin Res Ed)* 1987;294:936–7.
136. Nachtigall L, Casson P, Lucas J, Schofield V, Melson C, Simon JA. Safety and tolerability of testosterone patch therapy for up to 4 years in surgically menopausal women receiving oral or transdermal oestrogen. *Gynecol Endocrinol* 2011;27:39–48.
137. Panay N, Al-Azzawi F, Bouchard C, et al. Testosterone treatment of HSDD in naturally menopausal women: the ADORE study. *Climactric* 2010;13:121–31.
138. Sherwin BB, Gelfand MM, Brender W. Androgen enhances sexual motivation in females: a prospective, crossover study of sex steroid administration in the surgical menopause. *Psychosom Med* 1985;47:339–51.
139. Sherwin BB. Randomized clinical trials of combined estrogen-androgen preparations: effects on sexual functioning. *Fertil Steril* 2002;77(Suppl. 4):S49–54.
140. Burton RF, Trans. *The kama sutra of Vatsyayana*. New York: Doctors Books; 1886. Available from: http://www.sacred-texts.com/sex/kama/.
141. Costa RM, Miller GF, Brody S. Women who prefer longer penises are more likely to have vaginal orgasms (but not clitoral orgasms): implications for an evolutionary theory of vaginal orgasm. *J Sex Med* 2012;9:3079–88.
142. Brody S, Klapilova K, Krejčová L. More frequent vaginal orgasm is associated with experiencing greater excitement from deep vaginal stimulation. *J Sex Med* 2013;10:1730–6.
143. Buisson O, Jannini EA. Pilot echographic study of the differences in clitoral involvement following clitoral or vaginal sexual stimulation. *J Sex Med* 2013;10:2734–40.
144. Komisaruk BR, Beyer-Flores C, Whipple B. *The science of orgasm*. Baltimore: John Hopkins University Press; 2006.
145. Leff JJ, Israel M. The relationship between mode of female masturbation and achievement of orgasm in coitus. *Arch Sex Behav* 1983;12:227–36.
146. Levin RJ, Wagner G. Orgasm in women in the laboratory? Quantitative studies on duration, intensity, latency, and vaginal blood flow. *Arch Sex Behav* 1985;14:439–49.
147. Cechetto DF, Shoemaker JK. Functional neuroanatomy of autonomic regulation. *Neuroimage* 2009;47:795–803.
148. Iversen S, Iversen L, Saper CB. The autonomic nervous system and the hypothalamus. In: Kandel E, Schwartz JH, Jessell TM, editors. *Principles of neural science*; 2000.
149. Selye H. Confusion and controversy in the stress field. *J Human Stress* 1975;1:37–44.
150. Myers LS, Dixen J, Morrissette D, Carmichael M, Davidson JM. Effects of estrogen, androgen, and progestin on sexual psychophysiology and behavior in postmenopausal women. *J Clin Endocrinol Metab* 1990;70:1124–31.
151. Chivers ML, Rosen RC. Phosphodiesterase type 5 inhibitors and female sexual response: faulty protocols or paradigms? *J Sex Med* 2010;7:858–72.
152. Shafik A, Sibai El O, Shafik AA. Vaginal response to clitoral stimulation: identification of the clitorovaginal reflex. *J Reprod Med* 2008;53:111–6.
153. Peters LC, Kristal MB, Komisaruk BR. Sensory innervation of the external and internal genitalia of the female rat. *Brain Res* 1987;408:199–204.
154. Pacheco P, Martinez-Gomez M, Whipple B, Beyer C. Somatomotor components of the pelvic and pudendal nerves of the female rat. *Brain Res* 1989;490:85–94.

155. Giuliano F, Allard J, Compagnie S, Alexandre L, Droupy S, Bernabe J. Vaginal physiological changes in a model of sexual arousal in anesthetized rats. *Am J Physiol Regul Integr Comp Physiol* 2001;281:R140–9.
156. Cruz Y, Zempoalteca R, Angelica Lucio R, Pacheco P, Hudson R, Martínez-Gómez M. Pattern of sensory innervation of the perineal skin in the female rat. *Brain Res* 2004;1024:97–103.
157. Hall K. The clitoris of the rat after ovariectomy and the injection of sex hormones. *J Pathol Bacteriol* 1938;14:19–26.
158. Munarriz R, Kim SW, Kim NN, Traish A, Goldstein I. A review of the physiology and pharmacology of peripheral (vaginal and clitoral) female genital arousal in the animal model. *J Urol* 2003;170:S40–4.
159. Pessina MA, Hoyt RF, Goldstein I, Traish AM. Differential regulation of the expression of estrogen, progesterone, and androgen receptors by sex steroid hormones in the vagina: immunohistochemical studies. *J Sex Med* 2006;3:804–14.
160. Parada M, Chamas L, Censi S, Coria-Avila G, Pfaus JG. Clitoral stimulation induces conditioned place preference and Fos activation in the rat. *Horm Behav* 2010;57:112–8.
161. Parada M, Abdul-Ahad F, Censi S, Sparks L, Pfaus JG. Context alters the ability of clitoral stimulation to induce a sexually-conditioned partner preference in the rat. *Horm Behav* 2011; 59:520–7.
162. Parada M, Vargas EB, Kyres M, Burnside K, Pfaus JG. The role of ovarian hormones in sexual reward states of the female rat. *Horm Behav* 2012;62:442–7.
163. Parada M, Jafari N, Pfaus JG. Sexual experience blocks the ability of clitoral stimulation to induce a conditioned place preference in the rat. *Physiol Behav* 2013;119:97–102.
164. Pfaff DW, Montgomery M, Lewis C. Somatosensory determinants of lordosis in female rats: behavioral definition of the estrogen effect. *J Comp Physiol Psychol* 1977;91:134–45.
165. Buisson O, Foldes P, Paniel B-J. Sonography of the clitoris. *J Sex Med* 2008;5:413–7.
166. Foldes P, Buisson O. The clitoral complex: a dynamic sonographic study. *J Sex Med* 2009;6:1223–31.
167. Ladas AK, Whipple B, Perry JD. *The G spot and other recent discoveries about human sexuality*. New York: Holt, Rinehart, and Winston; 1982.
168. Pfaus JG, Kleopoulos SP, Mobbs CV, Gibbs RB, Pfaff DW. Sexual stimulation activates c-fos within estrogen-concentrating regions of the female rat forebrain. *Brain Res* 1993;624:253–67.
169. Pfaus JG, Marcangione C, Smith WJ, Manitt C, Abillamaa H. Differential induction of Fos in the female rat brain following different amounts of vaginocervical stimulation: modulation by steroid hormones. *Brain Res* 1996;741:314–30.
170. Marson L. Central nervous system control of sexual function in males and females. In: Carson CC, Kirby RS, Goldstein I, Wyllie MG, editors. *Textbook of erectile dysfunction*. New York: Informa HealthCare; 2009. pp. 73–88.
171. Marson L, McKenna KE. CNS cell groups involved in the control of the ischiocavernosus and bulbospongiosus muscles: a transneuronal tracing study using pseudorabies virus. *J Comp Neurol* 1996;374:161–79.
172. McKenna KE. Central nervous system pathways involved in the control of penile erection. *Annu Rev Sex Res* 1999;10:157–83.
173. Adler NT, Davis PG, Komisaruk BR. Variation in the size and sensitivity of a genital sensory field in relation to the estrous cycle in rats. *Horm Behav* 1977;9:334–44.
174. Cai RS, Alexander MS, Marson L. Activation of somatosensory afferents elicit changes in vaginal blood flow and the urethrogenital reflex via autonomic efferents. *J Urol* 2008;180:1167–72.
175. Wiedey J, Alexander MS, Marson L. Spinal neurons activated in response to pudendal or pelvic nerve stimulation in female rats. *Brain Res* 2008;1197:106–14.
176. Komisaruk BR, Adler NT, Hutchison J. Genital sensory field: enlargement by estrogen treatment in female rats. *Science* 1972;178:1295–8.
177. Cutler WB, Zacker M, McCoy N, Genovese-Stone E, Friedman E. Sexual response in women. *Obstet Gynecol* 2000;95:S19.
178. Shafik A, Shafik IA, Sibai El O. Vaginal and uterine pressure response to semen deposition into the vagina and uterus: human study. *Clin Exp Obstet Gynecol* 2006;33:107–9.
179. Blaustein JD, Farrell S, Ghavami G, Laroche J, Mohan G. Non-intromissive mating stimuli are sufficient to enhance sexual behaviors in ovariectomized female rats. *Horm Behav* 2009;55: 404–11.
180. Bennett AL, Blasberg ME, Blaustein JD. Sensory cues mediating mating-induced potentiation of sexual receptivity in female rats. *Horm Behav* 2001;40:77–83.
181. Rajendren G, Dudley CA, Moss RL. Role of the vomeronasal organ in the male-induced enhancement of sexual receptivity in female rats. *Neuroendocrinology* 1990;52:368–72.
182. Gorzalka BB, Moe IV. Adrenal role in proceptivity and receptivity induced by two modes of estradiol treatment. *Physiol Behav* 1994;55:29–34.
183. González-Flores O, Beyer C, Lima-Hernández FJ, et al. Facilitation of estrous behavior by vaginal cervical stimulation in female rats involves alpha1-adrenergic receptor activation of the nitric oxide pathway. *Behav Brain Res* 2007;176:237–43.
184. Sansone GR, Gerdes CA, Steinman JL, et al. Vaginocervical stimulation releases oxytocin within the spinal cord in rats. *Neuroendocrinology* 2002;75:306–15.
185. Sachs BD, Glater GB, O'Hanlon JK. Morphology of the erect glans penis in rats under various gonadal hormone conditions. *Anat Rec* 1984;210:45–52.
186. Taylor GT, Komitowski D, Weiss J. Light and scanning electron microscopic study of testosterone-restored penile papillae in castrated rats. *Anat Rec* 1983;205:277–86.
187. Catelli JM, Sved AF, Komisaruk BR. Vaginocervical probing elevates blood pressure and induces analgesia by separate mechanisms. *Physiol Behav* 1987;41:609–12.
188. Crowley WR, Jacobs R, Volpe J. Analgesic effect of vaginal stimulation in rats: modulation by graded stimulus intensity and hormones. *Physiol Behav* 1976;16:483–8.
189. Whipple B, Komisaruk BR. Elevation of pain threshold by vaginal stimulation in women. *Pain* 1985;21:357–67.
190. Rodriguez-Sierra JF, Crowley WR, Komisaruk BR. Vaginal stimulation in rats induces prolonged lordosis responsiveness and sexual receptivity. *J Comp Physiol Psychol* 1975;89:79–85.
191. Cameron N, Erskine MS. c-FOS expression in the forebrain after mating in the female rat is altered by adrenalectomy. *Neuroendocrinology* 2003;77:305–13.
192. Moss RL, Dudley CA, Schwartz NB. Coitus-induced release of luteinizing hormone in the proestrous rat: fantasy or fact? *Endocrinology* 1977;100:394–7.
193. Gunnet JW, Freeman ME. The mating-induced release of prolactin: a unique neuroendocrine response. *Endocr Rev* 1983;4: 44–61.
194. Erskine MS, Lehmann ML, Cameron NM, Polston EK. Coregulation of female sexual behavior and pregnancy induction: an exploratory synthesis. *Behav Brain Res* 2004;153:295–315.
195. Pfaus JG, Manitt C, Coopersmith CB. Effects of pelvic, pudendal, or hypogastric nerve cuts on Fos induction in the rat brain following vaginocervical stimulation. *Physiol Behav* 2006;89:627–36.
196. Marson L. Autonomic regulation of sexual function. In: Llewellyn-Smith IJ, Verberne AJM, editors. *Central regulation of autonomic functions*. 2nd ed. Oxford, England: Oxford University Press; 2011. pp. 366–418.

197. Komisaruk BR, Whipple B, Crawford A, Liu W-C, Kalnin A, Mosier K. Brain activation during vaginocervical self-stimulation and orgasm in women with complete spinal cord injury: fMRI evidence of mediation by the vagus nerves. *Brain Res* 2004;1024: 77–88.
198. Halata Z, Munger BL. The neuroanatomical basis for the protopathic sensibility of the human glans penis. *Brain Res* 1986;371:205–30.
199. Johnson RD, Halata Z. Topography and ultrastructure of sensory nerve endings in the glans penis of the rat. *J Comp Neurol* 1991;312:299–310.
200. Paick JS, Lee SW. The neural mechanism of apomorphine-induced erection: an experimental study by comparison with electrostimulation-induced erection in the rat model. *J Urol* 1994;152:2125–8.
201. Sipski M, Alexander C, Gómez-Marín O, Spalding J. The effects of spinal cord injury on psychogenic sexual arousal in males. *J Urol* 2007;177:247–51.
202. Lee JW, Erskine MS. Vaginocervical stimulation suppresses the expression of c-fos induced by mating in thoracic, lumbar and sacral segments of the female rat. *Neuroscience* 1996;74:237–49.
203. Fedirchuk B, Song L, Downie JW, Shefchyk SJ. Spinal distribution of extracellular field potentials generated by electrical stimulation of pudendal and perineal afferents in the cat. *Exp Brain Res* 1992;89:517–20.
204. Honda CN. Visceral and somatic afferent convergence onto neurons near the central canal in the sacral spinal cord of the cat. *J Neurophysiol* 1985;53:1059–78.
205. Komisaruk BR, Wise N, Frangos E, Liu W-C, Allen K, Brody S. Women's clitoris, vagina, and cervix mapped on the sensory cortex: fMRI evidence. *J Sex Med* 2011;8:2822–30.
206. Penfield W, Rasmussen T. *The cerebral cortex of man; a clinical study of localization of function*; 1950.
207. Marson L. Central nervous system neurons identified after injection of pseudorabies virus into the rat clitoris. *Neurosci Lett* 1995;190:41–4.
208. Marson L, Carson 3rd CC. Central nervous system innervation of the penis, prostate, and perineal muscles: a transneuronal tracing study. *Mol Urol* 1999;3:43–50.
209. Marson L, Foley KA. Identification of neural pathways involved in genital reflexes in the female: a combined anterograde and retrograde tracing study. *Neuroscience* 2004;127:723–36.
210. Papka RE, Williams S, Miller KE, Copelin T, Puri P. CNS location of uterine-related neurons revealed by trans-synaptic tracing with pseudorabies virus and their relation to estrogen receptor-immunoreactive neurons. *Neuroscience* 1998;84:935–52.
211. Flanagan-Cato LM, Calizo LH, Griffin GD, Lee BJ, Whisner SY. Sexual behaviour induces the expression of activity-regulated cytoskeletal protein and modifies neuronal morphology in the female rat ventromedial hypothalamus. *J Neuroendocrinol* 2006;18:857–64.
212. Bradley KC, Mullins AJ, Meisel RL, Watts VJ. Sexual experience alters D1 receptor-mediated cyclic AMP production in the nucleus accumbens of female Syrian hamsters. *Synapse* 2004;53:20–7.
213. Bradley KC, Meisel RL. Sexual behavior induction of c-Fos in the nucleus accumbens and amphetamine-stimulated locomotor activity are sensitized by previous sexual experience in female Syrian hamsters. *J Neurosci* 2001;21:2123–30.
214. Levin RJ. Sexual arousal—its physiological roles in human reproduction. *Annu Rev Sex Res* 2005;16:154–89.
215. Levin R, Meston C. Nipple/Breast stimulation and sexual arousal in young men and women. *J Sex Med* 2006;3:450–4.
216. Izumi H. Reflex parasympathetic vasodilatation in facial skin. *Gen Pharmacol* 1995;26:237–44.
217. Whetzel TP, Mathes SJ. Arterial anatomy of the face: an analysis of vascular territories and perforating cutaneous vessels. *Plast Reconstr Surg* 1992;89:591–603.
218. Chivers ML, Bailey JM. A sex difference in features that elicit genital response. *Biol Psychol* 2005;70:115–20.
219. Laan E, Everaerd W, van Bellen G, Hanewald G. Women's sexual and emotional responses to male- and female-produced erotica. *Arch Sex Behav* 1994;23:153–69.
220. White NR, Colona LC, Barfield RJ. Sensory cues that elicit ultrasonic vocalizations in female rats (*Rattus norvegicus*). *Behav Neural Biol* 1991;55:154–65.
221. Afonso VM, Lehmann H, Tse M, Woehrling A, Pfaus JG. Estrogen and the neural mediation of female–male mounting in the rat. *Behav Neurosci* 2009;123:369–81.
222. Krug R, Pietrowsky R, Fehm HL, Born J. Selective influence of menstrual cycle on perception of stimuli with reproductive significance. *Psychosom Med* 1994;56:410–7.
223. Wehrum S, Klucken T, Kagerer S, et al. Gender commonalities and differences in the neural processing of visual sexual stimuli. *J Sex Med* 2013;10:1328–42.
224. Barfield MA, Lisk RD. Relative contributions of ovarian and adrenal progesterone to the timing of heat in the 4-day cyclic rat. *Endocrinology* 1974;94:571–5.
225. Collins VJ, Boling JL, Dempsey EW, Young WC. Quantitative studies of experimentally induced sexual receptivity in the spayed guinea-pig. *Endocrinology* 1938;23:188–96.
226. Powers JB. Hormonal control of sexual receptivity during the estrous cycle of the rat. *Physiol Behav* 1970;5:831–5.
227. Dempsey EW, Hertz R. The experimental induction of oestrus (sexual receptivity) in the normal and ovariectomized guinea pig. *Am J Physiol* 1936;116:201–9.
228. Joslyn WD, Wallen K, Goy RW. Cyclic changes in sexual response to exogenous progesterone in female guinea pigs. *Physiol Behav* December 1971;7:915–7.
229. Davidson JM, Rodgers CH, Smith ER, Bloch GJB. Stimulation of female sex behavior in adrenalectomized rats with estrogen alone. *Endocrinology* 1968;82:193–5.
230. Crowley WR, Nock BL, Feder HH. Facilitation of lordosis behavior by clonidine in female guinea pigs. *Pharmacol Biochem Behav* 1978;8:207–9.
231. Carter CS, Landauer MR, Tierney BM, Jones T. Regulation of female sexual behavior in the golden hamster: behavioral effects of mating and ovarian hormones. *J Comp Physiol Psychol* 1976; 90:839–50.
232. Mani SK, Blaustein JD, O'Malley BW. Progesterone receptor function from a behavioral perspective. *Horm Behav* 1997;31:244–55.
233. Tennent BJ, Smith ER, Davidson JM. The effects of estrogen and progesterone on female rat proceptive behavior. *Horm Behav* 1980;14:65–75.
234. Edwards DA. Induction of estrus in female mice: estrogen-progesterone interactions. *Horm Behav* 1970;1:299–304.
235. Jones SL, Ismail N, King L, Pfaus JG. The effects of chronic administration of testosterone propionate with or without estradiol on the sexual behavior and plasma steroid levels of aged female rats. *Endocrinology* 2012;153:5928–39.
236. Blaustein JD, Wade GN. Sequential inhibition of sexual behavior by progesterone in female rats: comparison with a synthetic antiestrogen. *J Comp Physiol Psychol* 1977;91:752–60.
237. Zucker I. Facilitatory and inhibitory effects of progesterone on sexual responses of spayed guinea pigs. *J Comp Physiol Psychol* 1966;62:376–81.
238. Edwards D, Whalen R, Nadler R. Induction of estrus: estrogen-progesterone interactions. *Physiol Behav* 1968;3:29–33.
239. Sodersten P, Eneroth P. Evidence that progesterone does not inhibit the induction of sexual receptivity by oestradiol-17 in the rat. *J Endocrinol* 1981;89:63–9.
240. Feder HH, Marrone BL. Progesterone: its role in the central nervous system as a facilitator and inhibitor of sexual behavior and gonadotropin release. *Ann N Y Acad Sci* 1977;286:331–54.

241. Green R, Luttge WG, Whalen RE. Induction of receptivity in ovariectomized female rats by a single intravenous injection of estradiol-17. *Physiol Behav* 1970;5:137–41.
242. Glaser JH, Rubin BS, Barfield RJ. Onset of the receptive and proceptive components of feminine sexual behavior in rats following the intravenous administration of progesterone. *Horm Behav* 1983;17:18–27.
243. Kubli-Garfias C, Whalen RE. Induction of lordosis behavior in female rats by intravenous administration of progestins. *Horm Behav* 1977;9:380–6.
244. McGinnis MY, Parsons B, Rainbow TC, Krey LC, McEwen BS. Temporal relationship between cell nuclear progestin receptor levels and sexual receptivity following intravenous progesterone administration. *Brain Res* 1981;218:365–71.
245. Meyerson B. Latency between intravenous injection of progestins and the appearance of estrous behavior in estrogen-treated ovariectomized rats. *Horm Behav* 1972;3:1–9.
246. Lisk RD. A comparison of the effectiveness of intravenous, as opposed to subcutaneous, injection of progesterone for the induction of estrous behavior in the rat. *Can J Biochem Physiol* 1960;38:1381–3.
247. Mani SK, Oyola MG. Progesterone signaling mechanisms in brain and behavior. *Front Endocrinol* 2012;3:7.
248. Munchrath LA, Hofmann HA. Distribution of sex steroid hormone receptors in the brain of an African cichlid fish, Astatotilapia burtoni. *J Comp Neurol* 2010;518:3302–26.
249. Young LJ, Nag PK, Crews D. Species differences in behavioral and neural sensitivity to estrogen in whiptail lizards: correlation with hormone receptor messenger ribonucleic acid expression. *Neuroendocrinology* 1995;61:680–6.
250. Dellovade TL, Blaustein JD, Rissman EF. Neural distribution of estrogen receptor immunoreactive cells in the female musk shrew. *Brain Res* 1992;595:189–94.
251. Lehman MN, Ebling FJ, Moenter SM, Karsch FJ. Distribution of estrogen receptor-immunoreactive cells in the sheep brain. *Endocrinology* 1993;133:876–86.
252. Osterlund MK, Gustafsson JA, Keller E, Hurd YL. Estrogen receptor beta (ERbeta) messenger ribonucleic acid (mRNA) expression within the human forebrain: distinct distribution pattern to ERalpha mRNA. *J Clin Endocrinol Metab* 2000;85:3840–6.
253. Donahue JE, Stopa EG, Chorsky RL, et al. Cells containing immunoreactive estrogen receptor-alpha in the human basal forebrain. *Brain Res* 2000;856:142–51.
254. Gundlah C, Kohama SG, Mirkes SJ, Garyfallou VT, Urbanski HF, Bethea CL. Distribution of estrogen receptor beta (ERbeta) mRNA in hypothalamus, midbrain and temporal lobe of spayed macaque: continued expression with hormone replacement. *Brain Res Mol Brain Res* 2000;76:191–204.
255. Bethea CL, Brown NA, Kohama SG. Steroid regulation of estrogen and progestin receptor messenger ribonucleic acid in monkey hypothalamus and pituitary. *Endocrinology* 1996;137:4372–83.
256. Michael RP. Estrogen-sensitive neurons and sexual behavior in female cats. *Science* 1962;136:322–3.
257. Michael RP. Oestrogens in the central nervous system. *Br Med Bull* 1965;21:87–90.
258. Pfaff DW. Uptake of 3H-estradiol by the female rat brain. An autoradiographic study. *Endocrinology* 1968;82:1149–55.
259. Stumpf WE. Estradiol-concentrating neurons: topography in the hypothalamus by dry-mount autoradiography. *Science* 1968;162:1001–3.
260. Merchenthaler I, Lane MV, Numan S, Dellovade TL. Distribution of estrogen receptor alpha and beta in the mouse central nervous system: in vivo autoradiographic and immunocytochemical analyses. *J Comp Neurol* 2004;473:270–91.
261. Zigmond RE, McEwen BS. Selective retention of oestradiol by cell nuclei in specific brain regions of the ovariectomized rat. *J Neurochem* 1970;17:889–99.
262. Rainbow TC, Parsons B, MacLusky NJ, McEwen BS. Estradiol receptor levels in rat hypothalamic and limbic nuclei. *J Neurosci* 1982;2:1439–45.
263. Cintra A. On the cellular localization and distribution of estrogen receptors in the rat tel- and diencephalon using monoclonal antibodies to human estrogen receptor. *Neurochem Int* 1986;8:587–95.
264. Sar M, Parikh I. Immunohistochemical localization of estrogen receptor in rat brain, pituitary and uterus with monoclonal antibodies. *J Steroid Biochem* 1986;24:497–503.
265. Blaustein JD, Turcotte JC. Estrogen receptor-immunostaining of neuronal cytoplasmic processes as well as cell nuclei in guinea pig brain. *Brain Res* 1989;495:75–82.
266. Shughrue PJ, Merchenthaler I. Distribution of estrogen receptor beta immunoreactivity in the rat central nervous system. *J Comp Neurol* 2001;436:64–81.
267. Blaustein JD, Lehman MN, Turcotte JC, Greene G. Estrogen receptors in dendrites and axon terminals in the guinea pig hypothalamus. *Endocrinology* 1992;131:281–90.
268. Milner TA, McEwen BS, Hayashi S, Li CJ, Reagan LP, Alves SE. Ultrastructural evidence that hippocampal alpha estrogen receptors are located at extranuclear sites. *J Comp Neurol* 2000;429:355–71.
269. Simerly RB, Chang C, Muramatsu M, Swanson LW. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. *J Comp Neurol* 1990;294:76–95.
270. Shughrue PJ, Lane MV, Merchenthaler I. Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. *J Comp Neurol* 1997;388:507–25.
271. Tetel MJ, Getzinger MJ, Blaustein JD. Fos expression in the rat brain following vaginal-cervical stimulation by mating and manual probing. *J Neuroendocrinol* 1993;5:397–404.
272. Musatov S, Chen W, Pfaff DW, Kaplitt MG, Ogawa S. RNAi-mediated silencing of estrogen receptor {alpha} in the ventromedial nucleus of hypothalamus abolishes female sexual behaviors. *Proc Natl Acad Sci USA* 2006;103:10456–60.
273. Roy EJ, Wade GN. Binding of [3-H]estradiol by brain cell nuclei and female rat sexual behavior: inhibition by antiestrogens. *Brain Res* 1977;126:73–87.
274. Etgen AM. Antiestrogens: effects of tamoxifen, nafoxidine, and CI-628 on sexual behavior, cytoplasmic receptors, and nuclear binding of estrogen. *Horm Behav* 1979;13:97–112.
275. Mazzucco CA, Walker HA, Pawluski JL, Lieblich SE, Galea LAM. ERα, but not ERβ, mediates the expression of sexual behavior in the female rat. *Behav Brain Res* 2008;191:111–7.
276. Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS, Smithies O. Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *Proc Natl Acad Sci USA* 1993;90:1162–6.
277. Krege JH, Hodgin JB, Couse JF, et al. Generation and reproductive phenotypes of mice lacking estrogen receptor beta. *Proc Natl Acad Sci USA* 1998;95:15677–82.
278. Couse JF, Korach KS. Estrogen receptor null mice: what have we learned and where will they lead us? *Endocr Rev* 1999;20:358–417.
279. Ogawa S, Eng V, Taylor J, Lubahn DB, Korach KS, Pfaff DW. Roles of estrogen receptor-alpha gene expression in reproduction-related behaviors in female mice. *Endocrinology* 1998;139:5070–81.
280. Rissman EF, Early AH, Taylor JA, Korach KS, Lubahn DB. Estrogen receptors are essential for female sexual receptivity. *Endocrinology* 1997;138:507–10.
281. Kudwa AE, Rissman EF. Double oestrogen receptor alpha and beta knockout mice reveal differences in neural oestrogen-mediated progestin receptor induction and female sexual behaviour. *J Neuroendocrinol* 2003;15:978–83.

282. Ogawa S, Chan J, Chester AE, Gustafsson JA, Korach KS, Pfaff DW. Survival of reproductive behaviors in estrogen receptor beta gene-deficient (betaERKO) male and female mice. *Proc Natl Acad Sci USA* 1999;96:12887–92.
283. Musatov S, Chen W, Pfaff DW, et al. Silencing of estrogen receptor alpha in the ventromedial nucleus of hypothalamus leads to metabolic syndrome. *Proc Natl Acad Sci USA* 2007;104:2501–6.
284. Antal MC, Krust A, Chambon P, Mark M. Sterility and absence of histopathological defects in nonreproductive organs of a mouse ERbeta-null mutant. *Proc Natl Acad Sci USA* 2008;105:2433–8.
285. Antal MC, Petit-Demoulière B, Meziane H, Chambon P, Krust A. Estrogen dependent activation function of ERβ is essential for the sexual behavior of mouse females. *Proc Natl Acad Sci USA* 2012;109:19822–7.
286. Lauber AH, Mobbs CV, Muramatsu M, Pfaff DW. Estrogen receptor messenger RNA expression in rat hypothalamus as a function of genetic sex and estrogen dose. *Endocrinology* 1991;129:3180–6.
287. Simerly RB, Young BJ. Regulation of estrogen receptor messenger ribonucleic acid in rat hypothalamus by sex steroid hormones. *Mol Endocrinol* 1991;5:424–32.
288. DonCarlos LL, Malik K, Morrell JI. Region-specific effects of ovarian hormones on estrogen receptor immunoreactivity. *Neuroreport* 1995;6:2054–8.
289. Gréco B, Allegretto EA, Tetel MJ, Blaustein JD. Coexpression of ER beta with ER alpha and progestin receptor proteins in the female rat forebrain: effects of estradiol treatment. *Endocrinology* 2001;142:5172–81.
290. Meredith JM, Auger CJ, Blaustein JD. Down-regulation of estrogen receptor immunoreactivity by 17 beta-estradiol in the guinea pig forebrain. *J Neuroendocrinol* 1994;6:639–48.
291. Shughrue PJ, Bushnell CD, Dorsa DM. Estrogen receptor messenger ribonucleic acid in female rat brain during the estrous cycle: a comparison with ovariectomized females and intact males. *Endocrinology* 1992;131:381–8.
292. Patisaul HB, Whitten PL, Young LJ. Regulation of estrogen receptor beta mRNA in the brain: opposite effects of 17β-estradiol and the phytoestrogen, coumestrol. *Brain Res Mol Brain Res* 1999;67:165–71.
293. Österlund M, Kuiper GG, Gustafsson JA, Hurd YL. Differential distribution and regulation of estrogen receptor-alpha and -beta mRNA within the female rat brain. *Brain Res Mol Brain Res* 1998;54:175–80.
294. Suzuki S, Handa RJ. Regulation of estrogen receptor-beta expression in the female rat hypothalamus: differential effects of dexamethasone and estradiol. *Endocrinology* 2004;145:3658–70.
295. Attardi B. Facilitation and inhibition of the estrogen-induced luteinizing hormone surge in the rat by progesterone: effects on cytoplasmic and nuclear estrogen receptors in the hypothalamus-preoptic area, pituitary, and uterus. *Endocrinology* 1981;108:1487–96.
296. Blaustein J, Brown TJ. Progesterone decreases the concentration of hypothalamic and anterior pituitary estrogen receptors in ovariectomized rats. *Brain Res* 1984;304:225–36.
297. Brown TJ, MacLusky NJ. Progesterone modulation of estrogen receptors in microdissected regions of the rat hypothalamus. *Mol Cell Neurosci* 1994;5:283–90.
298. Smanik EJ, Young HK, Muldoon TG, Mahesh VB. Analysis of the effect of progesterone in vivo on estrogen receptor distribution in the rat anterior pituitary and hypothalamus. *Endocrinology* 1983;113:15–22.
299. Burrows LJ, Basha M, Goldstein AT. The effects of hormonal contraceptives on female sexuality: a review. *J Sex Med* 2012;9:2213–23.
300. Warnock JK, Clayton A, Croft H, Segraves R, Biggs FC. Comparison of androgens in women with hypoactive sexual desire disorder: those on combined oral contraceptives (COCs) vs. those not on COCs. *J Sex Med* 2006;3:878–82.
301. Clark AS, Roy EJ. Behavioral and cellular responses to pulses of low doses of estradiol-17β. *Physiol Behav* 1983;30:561–5.
302. Sodersten P, Eneroth P, Hansen S. Induction of sexual receptivity in ovariectomized rats by pulse administration of oestradiol-17B. *J Endocrinol* 1981;89:55–62.
303. Parsons B, McEwen BS, Pfaff DW. A discontinuous schedule of estradiol treatment is sufficient to activate progesterone-facilitated feminine sexual behavior and to increase cytosol receptors for progestins in the hypothalamus of the rat. *Endocrinology* 1982;110:613–9.
304. Parsons B, McEwen BS. Sequential inhibition of sexual receptivity by progesterone is prevented by a protein synthesis inhibitor and is not causally related to decreased levels of hypothalamic progestin receptors in the female rat. *J Neurosci* 1981;1:527–31.
305. Roy EJ, Lynn DM, Clark AS. Inhibition of sexual receptivity by anesthesia during estrogen priming. *Brain Res* 1985;337:163–6.
306. Blaustein JD, Dudley SD, Gray JM, Roy EJ, Wade GN. Long-term retention of estradiol by brain cell nuclei and female rat sexual behavior. *Brain Res* 1979;173:355–9.
307. Albert DJ, Jonik RH, Gorzalka BB, Newlove T, Webb B, Walsh ML. Serum estradiol concentration required to maintain body weight, attractivity, proceptivity, and receptivity in the ovariectomized female rat. *Physiol Behav* 1991;49:225–31.
308. Babcock A, Bloch G, Micevych P. Injections of cholecystokinin into the ventromedial hypothalamic nucleus inhibit lordosis behavior in the rat. *Physiol Behav* 1988;43:195–9.
309. Beach FA, Orndoff RK. Variation in the responsiveness of female rats to ovarian hormones as a function of preceding hormonal deprivation. *Horm Behav* 1974;5:201–5.
310. Blaustein JD, Finkbohner R, Delville Y. Estrogen-induced and estrogen-facilitated female rat sexual behavior is not mediated by progestin receptors. *Neuroendocrinology* 1987;45:152–9.
311. Gerall AA, Dunlap JL, Hendricks SE. Effect of ovarian secretions on female behavioral potentiality in the rat. *J Comp Physiol Psychol* 1973;82:449–65.
312. Kow LM, Pfaff DW. Induction of lordosis in female rats: two modes of estrogen action and the effect of adrenalectomy. *Horm Behav* 1975;6:259–76.
313. Parsons B, MacLusky NJ, Krieger MS, McEwen BS, Pfaff DW. The effects of long-term estrogen exposure on the induction of sexual behavior and measurements of brain estrogen and progestin receptors in the female rat. *Horm Behav* 1979;13:301–13.
314. Whalen RE, Nakayama K. Induction of oestrous behaviour: facilitation by repeated hormone treatments. *J Endocrinol* 1965;33:525–6.
315. Lonard DM, O'Malley BW. Expanding functional diversity of the coactivators. *Trends Biochem Sci* 2005;30:126–32.
316. Gelez H, Greggain-Mohr J, Pfaus JG, Allers KA, Giuliano F. Flibanserin treatment increases appetitive sexual motivation in the female rat. *J Sex Med* 2013;10:1231–9.
317. Tetel MJ, Acharya KD. Nuclear receptor coactivators: regulators of steroid action in brain and behavior. *J Neuroendocrinol* 2013. http://dx.doi.org/10.1111/jne.12065.
318. O'Malley BW. Coregulators: from whence came these 'master genes'. *Mol Endocrinol* 2007;21:1009–13.
319. Molenda HA, Griffin AL, Auger AP, McCarthy MM, Tetel MJ. Nuclear receptor coactivators modulate hormone-dependent gene expression in brain and female reproductive behavior in rats. *Endocrinology* 2002;143:436–44.
320. Molenda-Figueira HA, Williams CA, Griffin AL, Rutledge EM, Blaustein JD, Tetel MJ. Nuclear receptor coactivators function in estrogen receptor- and progestin receptor-dependent aspects of sexual behavior in female rats. *Horm Behav* 2006;50:383–92.

321. Apostolakis EM, Ramamurphy M, Zhou D, Oñate S, O'Malley BW. Acute disruption of select steroid receptor coactivators prevents reproductive behavior in rats and unmasks genetic adaptation in knockout mice. *Mol Endocrinol* 2002;16:1511–23.
322. Glidewell-Kenney C, Hurley LA, Pfaff L, Weiss J, Levine JE, Jameson JL. Nonclassical estrogen receptor alpha signaling mediates negative feedback in the female mouse reproductive axis. *Proc Natl Acad Sci USA* 2007;104:8173–7.
323. McDevitt MA, Glidewell-Kenney C, Weiss J, Chambon P, Jameson JL, Levine JE. Estrogen response element-independent estrogen receptor (ER)- signaling does not rescue sexual behavior but restores normal testosterone secretion in male er knockout mice. *Endocrinology* 2007;148:5288–94.
324. Micevych PE, Dewing P. Membrane-initiated estradiol signaling regulating sexual receptivity. *Front Endocrinol* 2011;2:26.
325. Pedram A, Razandi M, Sainson RCA, Kim JK, Hughes CC, Levin ER. A conserved mechanism for steroid receptor translocation to the plasma membrane. *J Biol Chem* 2007;282:22278–88.
326. Dewing P, Boulware MI, Sinchak K, Christensen A, Mermelstein PG, Micevych P. Membrane estrogen receptor-alpha interactions with metabotropic glutamate receptor 1a modulate female sexual receptivity in rats. *J Neurosci* 2007;27:9294–300.
327. Christensen A, Micevych P. A novel membrane estrogen receptor activated by STX induces female sexual receptivity through an interaction with mGluR1a. *Neuroendocrinology* 2013;97:363–8.
328. Kow L-M, Pfaff DW. The membrane actions of estrogens can potentiate their lordosis behavior-facilitating genomic actions. *Proc Natl Acad Sci USA* 2004;101:12354–7.
329. Stevis PE, Deecher DC, Suhadolnik L, Mallis LM, Frail DE. Differential effects of estradiol and estradiol-BSA conjugates. *Endocrinology* 1999;140:5455–8.
330. Temple JL, Wray S. Bovine serum albumin-estrogen compounds differentially alter gonadotropin-releasing hormone-1 neuronal activity. *Endocrinology* 2005;146:558–63.
331. Razandi M, Pedram A, Greene GL, Levin ER. Cell membrane and nuclear estrogen receptors (ERs) originate from a single transcript: studies of ERalpha and ERbeta expressed in Chinese hamster ovary cells. *Mol Endocrinol* 1999;13:307–19.
332. Levin ER. Integration of the extranuclear and nuclear actions of estrogen. *Mol Endocrinol* 2005;19:1951–9.
333. Wade CB, Robinson S, Shapiro RA, Dorsa DM. Estrogen receptor (ER)alpha and ERbeta exhibit unique pharmacologic properties when coupled to activation of the mitogen-activated protein kinase pathway. *Endocrinology* 2001;142:2336–42.
334. Blaustein JD. Cytoplasmic estrogen receptors in rat brain: immunocytochemical evidence using three antibodies with distinct epitopes. *Endocrinology* 1992;131:1336–42.
335. Sar M, Stumpf WE. Neurons of the hypothalamus concentrate [3H]progesterone or its metabolites. *Science* 1973;182:1266–8.
336. Warembourg M. Radioautographic study of the brain and pituitary after [3H] progesterone injection into estrogen-primed ovariectomized guinea pigs. *Neurosci Lett* 1978;7:1–5.
337. Blaustein JD, King JC, Toft DO, Turcotte J. Immunocytochemical localization of estrogen-induced progestin receptors in guinea pig brain. *Brain Res* 1988;474:1–15.
338. Hagihara K, Hirata S, Osada T, Hirai M, Kato J. Distribution of cells containing progesterone receptor mRNA in the female rat di- and telencephalon: an in situ hybridization study. *Brain Res Mol Brain Res* 1992;14:239–49.
339. Kastrup Y, Hallbeck M, Amandusson A, Hirata S, Hermanson O, Blomqvist A. Progesterone receptor expression in the brainstem of the female rat. *Neurosci Lett* 1999;275:85–8.
340. Godwin J, Crews D. Hormonal regulation of progesterone receptor mRNA expression in the hypothalamus of whiptail lizards: regional and species differences. *J Neurobiol* 1999; 39:287–93.
341. Diotel N, Servili A, Gueguen M-M, et al. Nuclear progesterone receptors are up-regulated by estrogens in neurons and radial glial progenitors in the brain of zebrafish. *PLoS One* 2011;6:e28375.
342. Intlekofer KA, Petersen SL. Distribution of mRNAs encoding classical progestin receptor, progesterone membrane components 1 and 2, serpine mRNA binding protein 1, and progestin and ADIPOQ receptor family members 7 and 8 in rat forebrain. *Neuroscience* 2011;172:55–65.
343. Intlekofer KA, Petersen SL. 17β-estradiol and progesterone regulate multiple progestin signaling molecules in the anteroventral periventricular nucleus, ventromedial nucleus and sexually dimorphic nucleus of the preoptic area in female rats. *Neuroscience* 2011;176:86–92.
344. Delville Y, Blaustein JD. A site for estradiol priming of progesterone-facilitated sexual receptivity in the ventrolateral hypothalamus of female guinea pigs. *Brain Res* 1991;559:191–9.
345. Blaustein JD, Turcotte JC. Estradiol-induced progestin receptor immunoreactivity is found only in estrogen receptor-immunoreactive cells in guinea pig brain. *Neuroendocrinology* 1989;49:454–61.
346. Kastner P, Krust A, Turcotte B, et al. Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *EMBO J* 1990;9:1603–14.
347. Conneely OM, Kettelberger DM, Tsai MJ, Schrader WT, O'Malley BW. The chicken progesterone receptor A and B isoforms are products of an alternate translation initiation event. *J Biol Chem* 1989;264:14062–4.
348. Kato J, Hirata S, Nozawa A, Yamada-Mouri N. Gene expression of progesterone receptor isoforms in the rat brain. *Horm Behav* 1994;28:454–63.
349. Camacho-Arroyo I, Guerra-Araiza C, Cerbón MA. Progesterone receptor isoforms are differentially regulated by sex steroids in the rat forebrain. *Neuroreport* 1998;9:3993–6.
350. Guerra-Araiza C, Cerbón MA, Morimoto S, Camacho-Arroyo I. Progesterone receptor isoforms expression pattern in the rat brain during the estrous cycle. *Life Sci* 2000;66:1743–52.
351. Guerra-Araiza C, Villamar-Cruz O, González-Arenas A, Chavira R, Camacho-Arroyo I. Changes in progesterone receptor isoforms content in the rat brain during the oestrous cycle and after oestradiol and progesterone treatments. *J Neuroendocrinol* 2003;15:984–90.
352. Scott R, Wu Peng X, Pfaff DW. Regulation and expression of progesterone receptor mRNA isoforms A and B in the male and female rat hypothalamus and pituitary following oestrogen treatment. *J Neuroendocrinol* 2002;14:175–83.
353. Mendoza-Garcés L, Camacho-Arroyo I, Cerbón MA. Effects of mating on progesterone receptor isoforms in rat hypothalamus. *Neuroreport* 2010;21:513–6.
354. Gómez-Camarillo MA, Beyer C, Lucio RA, et al. Differential effects of progesterone and genital stimulation on sequential inhibition of estrous behavior and progesterone receptor expression in the rat brain. *Brain Res Bull* 2011;85:201–6.
355. Blaustein JD, Brown TJ. Mechanisms of estrogen-progestin interactions on lordosis in female guinea pigs. In: Balthazart J, Pröve E, Gilles R, editors. *Hormones and behaviour in higher vertebrates*. Berlin: Springer-Verlag; 1983. pp. 18–31.
356. Blaustein JD, Olster DH. Gonadal steroid hormone receptors and social behaviors. In: Balthazart J, editor. *Molecular and cellular basis of social behavior in vertebrates*. Berlin: Springer-Verlag; 1989. pp. 31–104.
357. Blaustein JD, Turcotte JC. Down-regulation of progestin receptors in guinea pig brain: new findings using an immunocytochemical technique. *J Neurobiol* 1990;21:675–85.
358. Blaustein JD, Feder HH. Cytoplasmic progestin receptors in female guinea pig brain and their relationship to refractoriness in expression of female sexual behavior. *Brain Res* 1979;177:489–98.

359. Parsons B, Maclusky N, Krey L, Pfaff DW, Mcewen B. The temporal relationship between estrogen-inducible progestin receptors in the female rat brain and the time course of estrogen activation of mating behavior. *Endocrinology* 1980;107:774.
360. Young WC. *Psychobiology of sexual behavior in the guinea pig*. New York: Academic Press; 1969.
361. Blandau RJ, Boling JL, Young WC. The length of heat in the albino rat as determined by the copulatory response. *Anat Rec* 1941;79:453–63.
362. Blaustein JD, Feder HH. Nuclear progestin receptors in guinea pig brain measured by an in vitro exchange assay after hormonal treatments that affect lordosis. *Endocrinology* 1980;106:1061–9.
363. Rainbow TC, McGinnis MY, Krey LC, McEwen BS. Nuclear progestin receptors in rat brain and pituitary. *Neuroendocrinology* 1982;34:426–32.
364. Blaustein JD. Alteration of sensitivity to progesterone facilitation of lordosis in guinea pigs by modulation of hypothalamic progestin receptors. *Brain Res* 1982;243:287–300.
365. Brown TJ, Blaustein JD. Loss of hypothalamic nuclear-bound progestin receptors: factors involved and the relationship to heat termination in female guinea pigs. *Brain Res* 1985;358:180–90.
366. Brown TJ, Blaustein JD. Inhibition of sexual behavior in female guinea pigs by a progestin receptor antagonist. *Brain Res* 1984;301:343–9.
367. Richmond G, Clemens LG. Cholinergic mediation of feminine sexual receptivity: demonstration of progesterone independence using a progestin receptor antagonist. *Brain Res* 1986;373:159–63.
368. Etgen AM, Barfield RJ. Antagonism of female sexual behavior with intracerebral implants of antiprogestin RU 38486: correlation with binding to neural progestin receptors. *Endocrinology* 1986;119:1610–7.
369. Mani SK, Blaustein JD, Allen JM, Law SW, O'Malley BW, Clark JH. Inhibition of rat sexual behavior by antisense oligonucleotides to the progesterone receptor. *Endocrinology* 1994;135:1409–14.
370. Ogawa S, Olazábal UE, Parhar IS, Pfaff DW. Effects of intrahypothalamic administration of antisense DNA for progesterone receptor mRNA on reproductive behavior and progesterone receptor immunoreactivity in female rat. *J Neurosci* 1994;14:1766–74.
371. Pollio G, Xue P, Zanisi M, Nicolin A, Maggi A. Antisense oligonucleotide blocks progesterone-induced lordosis behavior in ovariectomized rats. *Brain Res Mol Brain Res* 1993;19:135–9.
372. Lydon JP, DeMayo FJ, Funk CR, et al. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes Dev* 1995;9:2266–78.
373. Mani SK, Allen JM, Lydon JP, et al. Dopamine requires the unoccupied progesterone receptor to induce sexual behavior in mice. *Mol Endocrinol* 1996;10:1728–37.
374. Mani SK, Reyna AM, Chen JZ, Mulac-Jericevic B, Conneely OM. Differential response of progesterone receptor isoforms in hormone-dependent and -independent facilitation of female sexual receptivity. *Mol Endocrinol* 2006;20:1322–32.
375. Harris HA, Katzenellenbogen JA. Characterization of the biological roles of the estrogen receptors, ERα and ERβ, in estrogen target tissues in vivo through the use of an ERα-selective ligand. *Endocrinology* 2002.
376. Moffatt CA, Rissman EF, Shupnik MA, Blaustein JD. Induction of progestin receptors by estradiol in the forebrain of estrogen receptor-alpha gene-disrupted mice. *J Neurosci* 1998;18:9556–63.
377. Sá SI, Pereira PA, Malikov V, Dulce Madeira M. Role of estrogen receptor α and β in the induction of progesterone receptors in hypothalamic ventromedial neurons. *Neuroscience* 2013;238:159–67.
378. Sá SI, Pereira PA, Malikov V, Ferreira I, Madeira MD. The role of plasma membrane estrogen receptors in mediating the estrogen induction of progesterone receptors in hypothalamic ventromedial neurons. *J Comp Neurol* 2013. http://dx.doi.org/10.1002/cne.23396.
379. Debold JF, Frye CA. Genomic and non-genomic actions of progesterone in the control of female hamster sexual behavior. *Horm Behav* 1994;28:445–53.
380. Mani SK, Blaustein JD. Neural progestin receptors and female sexual behavior. *Neuroendocrinology* 2012;96:152–61.
381. Krebs CJ, Jarvis ED, Chan J, Lydon JP, Ogawa S, Pfaff DW. A membrane-associated progesterone-binding protein, 25-Dx, is regulated by progesterone in brain regions involved in female reproductive behaviors. *Proc Natl Acad Sci USA* 2000;97:12816–21.
382. Sleiter N, Pang Y, Park C, et al. Progesterone receptor A (PRA) and PRB-independent effects of progesterone on gonadotropin-releasing hormone release. *Endocrinology* 2009;150:3833–44.
383. Liu B, Arbogast LA. Gene expression profiles of intracellular and membrane progesterone receptor isoforms in the mediobasal hypothalamus during pro-oestrus. *J Neuroendocrinol* 2009;21:993–1000.
384. Zuloaga DG, Yahn SL, Pang Y, et al. Distribution and estrogen regulation of membrane progesterone receptor-β in the female rat brain. *Endocrinology* 2012;153:4432–43.
385. Zhu Y, Rice CD, Pang Y, Pace M, Thomas P. Cloning, expression, and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. *Proc Natl Acad Sci USA* 2003;100:2231–6.
386. Blaustein JD. Modulation of sex steroid receptors by neurotransmitters: relevant techniques. *Neuroprotocols* 1992;1:42–51.
387. Blaustein JD, Brown TJ, Swearengen ES. Dopamine-beta-hydroxylase inhibitors modulate the concentration of functional estrogen receptors in female rat hypothalamus and pituitary gland. *Neuroendocrinology* 1986;43:150–8.
388. Blaustein JD. The alpha 1-noradrenergic antagonist prazosin decreases the concentration of estrogen receptors in female rat hypothalamus. *Brain Res* 1987;404:39–50.
389. Clark AS, Nock B, Feder HH, Roy EJ. Alpha 1-noradrenergic receptor blockade decreases nuclear estrogen receptor binding in guinea pig hypothalamus and preoptic area. *Brain Res* 1985;330:197–9.
390. Nock B, Blaustein JD, Feder HH. Changes in noradrenergic transmission alter the concentration of cytoplasmic progestin receptors in hypothalamus. *Brain Res* 1981;207:371–96.
391. Thornton JE, Nock B, McEwen BS, Feder HH. Noradrenergic modulation of hypothalamic progestin receptors in female guinea pigs is specific to the ventromedial nucleus. *Brain Res* 1986;377:155–9.
392. Nock B, Feder HH. Alpha 1-noradrenergic regulation of hypothalamic progestin receptors and guinea pig lordosis behavior. *Brain Res* 1984;310:77–85.
393. Montemayor ME, Clark AS, Lynn DM, Roy EJ. Modulation by norepinephrine of neural responses to estradiol. *Neuroendocrinology* 1990;52:473–80.
394. Blaustein JD, Turcotte J. Small apomorphine-induced increase in the concentration of cytosol estrogen receptors in female rat hypothalamus and pituitary. *Brain Res Bull* 1987;18:585–90.
395. Gietzen DW, Hope WG, Woolley DE. Dopaminergic agonists increase [3H]estradiol binding in hypothalamus of female rats, but not of males. *Life Sci* 1983;33:2221–8.
396. Heritage AS, Grant LD, Stumpf WE. 3H estradiol in catecholamine neurons of rat brain stem: combined localization by autoradiography and formaldehyde-induced fluorescence. *J Comp Neurol* 1977;176:607–30.
397. Heritage AS, Stumpf WE, Sar M, Grant LD. Brainstem catecholamine neurons are target sites for sex steroid hormones. *Science* 1980;207:1377–9.

398. Blaustein JD, Turcotte JC. A small population of tyrosine hydroxylase-immunoreactive neurons in the guinea-pig arcuate nucleus contains progestin receptor-immunoreactivity. *J Neuroendocrinol* 1989;1:333–8.
399. Brown TJ, MacLusky NJ, Leranth C, Shanabrough M, Naftolin F. Progestin receptor-containing cells in guinea pig hypothalamus: afferent connections, morphological characteristics, and neurotransmitter content. *Mol Cell Neurosci* 1990;1:58–77.
400. Tetel MJ, Blaustein JD. Immunocytochemical evidence for noradrenergic regulation of estrogen receptor concentrations in the guinea pig hypothalamus. *Brain Res* 1991;565:321–9.
401. Lauber AH, Whalen RE. Muscarinic cholinergic modulation of hypothalamic estrogen binding sites. *Brain Res* 1988;443:21–6.
402. Chen TJ, Chang HC, Hsu C, Peng MT. Effects of anterior roof deafferentation on lordosis behavior and estrogen receptors in various brain regions of female rats. *Physiol Behav* 1992;52:7–11.
403. McGinnis MY, Lumia AR, McEwen BS. Increased estrogen receptor binding in amygdala correlates with facilitation of feminine sexual behavior induced by olfactory bulbectomy. *Brain Res* 1985;334:19–25.
404. Carter CS, Getz LL. *Social and hormonal determinants of reproductive patterns in the prairie vole*. Berlin: Springer Berlin Heidelberg; 1985.
405. Cohen-Parsons M, Roy EJ. Social stimuli augment estrogen receptor binding in preoptic area of female prairie voles. *Brain Res* 1989;476:363–6.
406. Champagne FA, Weaver ICG, Diorio J, Sharma S, Meaney MJ. Natural variations in maternal care are associated with estrogen receptor alpha expression and estrogen sensitivity in the medial preoptic area. *Endocrinology* 2003;144:4720–4.
407. Champagne FA, Weaver ICG, Diorio J, Dymov S, Szyf M, Meaney MJ. Maternal care associated with methylation of the estrogen receptor-alpha1b promoter and estrogen receptor-alpha expression in the medial preoptic area of female offspring. *Endocrinology* 2006;147:2909–15.
408. Perry AN, Paramadilok A, Cushing BS. Neonatal oxytocin alters subsequent estrogen receptor alpha protein expression and estrogen sensitivity in the female rat. *Behav Brain Res* 2009;205:154–61.
409. Kramer KM, Yoshida S, Papademetriou E, Cushing BS. The organizational effects of oxytocin on the central expression of estrogen receptor alpha and oxytocin in adulthood. *BMC Neurosci* 2007;8:71.
410. Blaustein JD. Minireview: neuronal steroid hormone receptors: they're not just for hormones anymore. *Endocrinology* 2004;145.
411. Cenni B, Picard D. Ligand-independent activation of steroid receptors: new roles for old players. *Trends Endocrinol Metab* 1999;10:41–6.
412. Power RF, Mani SK, Codina J, Conneely OM, O'Malley BW. Dopaminergic and ligand-independent activation of steroid hormone receptors. *Science* 1991;254:1636–9.
413. Mani SK, Allen JM, Clark JH, Blaustein JD, O'Malley BW. Convergent pathways for steroid hormone- and neurotransmitter-induced rat sexual behavior. *Science* 1994;265:1246–9.
414. Mani SK, Fienberg AA, O'Callaghan JP, et al. Requirement for DARPP-32 in progesterone-facilitated sexual receptivity in female rats and mice. *Science* 2000;287:1053–6.
415. Mani S, Portillo W. Activation of progestin receptors in female reproductive behavior: interactions with neurotransmitters. *Front Neuroendocrinol* 2010;31:157–71.
416. Foreman MM, Moss RL. Effects of subcutaneous injection and intrahypothalamic infusion of releasing hormones upon lordotic response to repetitive coital stimulation. *Horm Behav* 1977;8:219–34.
417. Rajendren G, Moss RL. The role of the medial nucleus of amygdala in the mating-induced enhancement of lordosis in female rats: the interaction with luteinizing hormone-releasing hormone neuronal system. *Brain Res* 1993;617:81–6.
418. Auger A, Moffatt C, Blaustein J. Progesterone-independent activation of rat brain progestin receptors by reproductive stimuli. *Endocrinology* 1997;138:511.
419. Bennett AL, Blasberg ME, Blaustein JD. Mating stimulation required for mating-induced estrous abbreviation in female rats: effects of repeated testing. *Horm Behav* 2002;42:206–11.
420. Meredith JM, Moffatt CA, Auger AP, Snyder GL, Greengard P, Blaustein JD. Mating-related stimulation induces phosphorylation of dopamine- and cyclic AMP-regulated phosphoprotein-32 in progestin receptor-containing areas in the female rat brain. *J Neurosci* 1998;18:10189–95.
421. Quysner A, Blaustein JD. A dopamine antagonist blocks vaginocervical stimulation-induced neuronal responses in the rat forebrain. *Brain Res* 2001;921:173–82.
422. Micevych P, Sinchak K. Synthesis and function of hypothalamic neuroprogesterone in reproduction. *Endocrinology* 2008;149:2739–42.
423. Kohlert JG, Rowe RK, Meisel RL. Intromissive stimulation from the male increases extracellular dopamine release from fluorogold-identified neurons within the midbrain of female hamsters. *Horm Behav* 1997;32:143–54.
424. Matuszewich L, Lorrain DS, Hull EM. Dopamine release in the medial preoptic area of female rats in response to hormonal manipulation and sexual activity. *Behav Neurosci* 2000;114:772–8.
425. Mermelstein PG, Becker JB. Increased extracellular dopamine in the nucleus accumbens and striatum of the female rat during paced copulatory behavior. *Behav Neurosci* 1995;109:354–65.
426. Vathy I, Etgen AM. Hormonal activation of female sexual behavior is accompanied by hypothalamic norepinephrine release. *J Neuroendocrinol* 1989;1:383–8.
427. Auger AP, Moffatt CA, Blaustein JD. Reproductively-relevant stimuli induce Fos-immunoreactivity within progestin receptor-containing neurons in localized regions of female rat forebrain. *J Neuroendocrinol* 1996;8:831–8.
428. Beyer C, Gonzalez-Flores O, González-Mariscal G. Progesterone receptor participates in the stimulatory effect of LHRH, prostaglandin E2, and cyclic AMP on lordosis and proceptive behaviours in rats. *J Neuroendocrinol* 1997;9:609–14.
429. Acosta-Martinez M, González-Flores O, Etgen AM. The role of progestin receptors and the mitogen-activated protein kinase pathway in delta opioid receptor facilitation of female reproductive behaviors. *Horm Behav* 2006;49:458–62.
430. Chu HP, Etgen AM. Ovarian hormone dependence of alpha(1)-adrenoceptor activation of the nitric oxide–cGMP pathway: relevance for hormonal facilitation of lordosis behavior. *J Neurosci* 1999;19:7191–7.
431. Mani SK, Allen JM, Rettori V, McCann SM, O'Malley BW, Clark JH. Nitric oxide mediates sexual behavior in female rats. *Proc Natl Acad Sci USA* 1994;91:6468–72.
432. Gonzalez-Flores O, Etgen AM, Komisaruk BK, et al. Antagonists of the protein kinase A and mitogen-activated protein kinase systems and of the progestin receptor block the ability of vaginocervical/flank-perineal stimulation to induce female rat sexual behaviour. *J Neuroendocrinol* 2008;20:1361–7.
433. Chu HP, Morales JC, Etgen AM. Cyclic GMP may potentiate lordosis behaviour by progesterone receptor activation. *J Neuroendocrinol* 1999;11:107–13.
434. Apostolakis EM, Garai J, Lohmann JE, Clark JH, O'Malley BW. Epidermal growth factor activates reproductive behavior independent of ovarian steroids in female rodents. *Mol Endocrinol* 2000;14:1086–98.
435. Holder MK, Hadjimarkou MM, Zup SL, et al. Methamphetamine facilitates female sexual behavior and enhances neuronal activation in the medial amygdala and ventromedial nucleus of the hypothalamus. *Psychoneuroendocrinology* 2010;35:197–208.

436. Beyer C, González-Mariscal G, Eguíbar JR, Gómora P. Lordosis facilitation in estrogen primed rats by intrabrain injection of pregnanes. *Pharmacol Biochem Behav* 1988;31:919–26.
437. Beyer C, Gonzalez-Flores O. Ring A reduced progestins potently stimulate estrous behavior in rats: paradoxical effect through the progesterone receptor. *Physiol Behav* 1995;58:985–93.
438. Glaser JH, Etgen AM, Barfield RJ. Intrahypothalamic effects of progestin agonists on estrous behavior and progestin receptor binding. *Physiol Behav* 1985;34:871–7.
439. González-Flores O, Ramírez-Orduña JM, Lima-Hernández FJ, García-Juárez M, Beyer C. Differential effect of kinase A and C blockers on lordosis facilitation by progesterone and its metabolites in ovariectomized estrogen-primed rats. *Horm Behav* 2006;49:398–404.
440. Lima-Hernández FJ, Beyer C, Gómora-Arrati P, et al. Src kinase signaling mediates estrous behavior induced by 5β-reduced progestins, GnRH, prostaglandin E2 and vaginocervical stimulation in estrogen-primed rats. *Horm Behav* 2012;62:579–84.
441. Gonzalez-Mariscal G, Gonzalez-Florez O, Beyer C. Intrahypothalamic injection of RU486 antagonizes the lordosis induced by ring A-reduced progestins. *Physiol Behav* 2011;46:435–8.
442. González-Flores O, Beyer C, Gómora-Arrati P, et al. A role for Src kinase in progestin facilitation of estrous behavior in estradiol-primed female rats. *Horm Behav* 2010;58:223–9.
443. Calizo LH, Flanagan-Cato LM. Estrogen-induced dendritic spine elimination on female rat ventromedial hypothalamic neurons that project to the periaqueductal gray. *J Comp Neurol* 2002;447:234–48.
444. Calizo LH, Flanagan-Cato LM. Hormonal-neural integration in the female rat ventromedial hypothalamus: triple labeling for estrogen receptor-alpha, retrograde tract tracing from the periaqueductal gray, and mating-induced Fos expression. *Endocrinology* 2003;144:5430–40.
445. Tetel MJ, Celentano DC, Blaustein JD. Intraneuronal convergence of tactile and hormonal stimuli associated with female reproduction in rats. *J Neuroendocrinol* 1994;6:211–6.
446. Woolley CS, McEwen BS. Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. *J Comp Neurol* 1993;336:293–306.
447. Tang Y, Janssen WGM, Hao J, et al. Estrogen replacement increases spinophilin-immunoreactive spine number in the prefrontal cortex of female rhesus monkeys. *Cereb Cortex* 2004;14:215–23.
448. Meisel RL, Pfaff DW. Brain region specificity in estradiol effects on neuronal ultrastructure in rats. *Mol Cell Endocrinol* 1985;40:159–66.
449. Calizo LH, Flanagan-Cato LM. Estrogen selectively regulates spine density within the dendritic arbor of rat ventromedial hypothalamic neurons. *J Neurosci* 2000;20:1589–96.
450. Griffin GD, Flanagan-Cato LM. Estradiol and progesterone differentially regulate the dendritic arbor of neurons in the hypothalamic ventromedial nucleus of the female rat (*Rattus norvegicus*). *J Comp Neurol* 2008;510:631–40.
451. Griffin GD, Ferri-Kolwicz SL, Reyes BAS, Van Bockstaele EJ, Flanagan-Cato LM. Ovarian hormone-induced reorganization of oxytocin-labeled dendrites and synapses lateral to the hypothalamic ventromedial nucleus in female rats. *J Comp Neurol* 2010;518:4531–45.
452. Frankfurt M, Gould E, Woolley CS, McEwen BS. Gonadal steroids modify dendritic spine density in ventromedial hypothalamic neurons: a Golgi study in the adult rat. *Neuroendocrinology* 1990;51:530–5.
453. Frankfurt M, McEwen BS. Estrogen increases axodendritic synapses in the VMN of rats after ovariectomy. *Neuroreport* 1991;2:380–2.
454. Nishizuka M, Pfaff DW. Intrinsic synapses in the ventromedial nucleus of the hypothalamus: an ultrastructural study. *J Comp Neurol* 1989;286:260–8.
455. Ferri SL, Flanagan-Cato LM. Oxytocin and dendrite remodeling in the hypothalamus. *Horm Behav* 2012;61:251–8.
456. Hennessey AC, Camak L, Gordon F, Edwards DA. Connections between the pontine central gray and the ventromedial hypothalamus are essential for lordosis in female rats. *Behav Neurosci* 1990;104:477–88.
457. Chung SK, Pfaff DW, Cohen RS. Estrogen-induced alterations in synaptic morphology in the midbrain central gray. *Exp Brain Res* 1988;69:522–30.
458. VanderHorst VG, Holstege G. Estrogen induces axonal outgrowth in the nucleus retroambiguus-lumbosacral motoneuronal pathway in the adult female cat. *J Neurosci* 1997;17:1122–36.
459. Meisel RL, Luttrell VR. Estradiol increases the dendritic length of ventromedial hypothalamic neurons in female Syrian hamsters. *Brain Res Bull* 1990;25:165–8.
460. Ferri SL, Rohrbach CJ, Way SE, Curtis KS, Curtis JT, Flanagan-Cato LM. Dendritic arbor of neurons in the hypothalamic ventromedial nucleus in female prairie voles (*Microtus ochrogaster*). *Horm Behav* 2013;63:173–9.
461. Blaustein JD, Gréco B. A progestin antagonist blocks vaginocervical stimulation-induced fos expression in neurones containing progestin receptors in the rostral medial preoptic area. *J Neuroendocrinol* 2002;14:109–15.
462. Flanagan-Cato LM, Lee BJ, Calizo LH. Co-localization of midbrain projections, progestin receptors, and mating-induced fos in the hypothalamic ventromedial nucleus of the female rat. *Horm Behav* 2006;50:52–60.
463. Bennett AL, Gréco B, Blasberg ME, Blaustein JD. Response to male odours in progestin receptor- and oestrogen receptor-containing cells in female rat brain. *J Neuroendocrinol* 2002;14:442–9.
464. Gréco B, Blasberg ME, Kosinski EC, Blaustein JD. Response of ERα-IR and ERβ-IR cells in the forebrain of female rats to mating stimuli. *Horm Behav* 2003;43:444–53.
465. DonCarlos LL, Garcia-Ovejero D, Sarkey S, Garcia-Segura LM, Azcoitia I. Androgen receptor immunoreactivity in forebrain axons and dendrites in the rat. *Endocrinology* 2003;144:3632–8.
466. Tabori NE, Stewart LS, Znamensky V, et al. Ultrastructural evidence that androgen receptors are located at extranuclear sites in the rat hippocampal formation. *Neuroscience* 2005;130:151–63.
467. Pfaff DW. Autoradiographic localization of radioactivity in rat brain after injection of tritiated sex hormones. *Science* 1968;161:1355–6.
468. Sar M, Stumpf WE. Distribution of androgen target cells in rat forebrain and pituitary after [3H]-dihydrotestosterone administration. *J Steroid Biochem* 1977;8:1131–5.
469. Michael RP, Rees HD. Neurons in the brain of fetal rhesus monkeys accumulate 3H-testosterone or its metabolites. *Life Sci* 1986;38:1673–7.
470. McAbee MD, DonCarlos LL. Ontogeny of region-specific sex differences in androgen receptor messenger ribonucleic acid expression in the rat forebrain. *Endocrinology* 1998;139:1738–45.
471. Gréco B, Edwards DA, Zumpe D, Michael RP, Clancy AN. Fos induced by mating or noncontact sociosexual interaction is colocalized with androgen receptors in neurons within the forebrain, midbrain, and lumbosacral spinal cord of male rats. *Horm Behav* 1998;33:125–38.
472. Roselli C, Horton L. Distribution and regulation of aromatase activity in the rat hypothalamus and limbic system. *Endocrinology* 1985;117:2471–7.
473. Abdelgadir SE, Resko JA, Ojeda SR, Lephart ED, McPhaul MJ, Roselli CE. Androgens regulate aromatase cytochrome P450 messenger ribonucleic acid in rat brain. *Endocrinology* 1994;135:395–401.

474. Roselli CE, Ellinwood WE, Resko JA. Regulation of brain aromatase activity in rats. *Endocrinology* 1984;114:192–200.
475. Wagner CK, Morrell JI. Distribution and steroid hormone regulation of aromatase mRNA expression in the forebrain of adult male and female rats: a cellular-level analysis using in situ hybridization. *J Comp Neurol* 1996;370:71–84.
476. Cohen RE, Wade J. Aromatase and 5α-reductase type 2 mRNA in the green anole forebrain: an investigation of the effects of sex, season and testosterone manipulation. *Gen Comp Endocrinol* 2012;176:377–84.
477. Manson JE, Chlebowski RT, Stefanick ML, et al. Menopausal hormone therapy and health outcomes during the intervention and extended poststopping phases of the Women's Health Initiative randomized trials. *JAMA* 2013;310:1353–68.
478. Rosenbaum S, Jones SL, Gardner Gregory J, Pfaus JG. Blocking aromatization facilitates sexual behavior in ovariectomized rats treated with estradiol and testosterone. In: *Poster presented at the Society for Neuroscience*; October 13–18, 2012. New Orleans, LA.
479. Perelman MA. The sexual tipping point: a mind/body model for sexual medicine. *J Sex Med* 2009;6:629–32.
480. Gray JA. *The psychology of fear and stress*. 2nd ed. Cambridge: Cambridge University Press; 1987.
481. Bancroft J, Janssen E. The dual control model of male sexual response: a theoretical approach to centrally mediated erectile dysfunction. *Neurosci Biobehav Rev* 2000;24:571–9.
482. Pfaus JG, Wilkins MF, DiPietro N, et al. Inhibitory and disinhibitory effects of psychomotor stimulants and depressants on the sexual behavior of male and female rats. *Horm Behav* 2010;58:163–76.
483. Fink G, Sumner BE, Rosie R, Grace O, Quinn JP. Estrogen control of central neurotransmission: effect on mood, mental state, and memory. *Cell Mol Neurobiol* 1996;16:325–44.
484. Kow L-M, Mobbs CV, Pfaff DW. Roles of second-messenger systems and neuronal activity in the regulation of lordosis by neurotransmitters, neuropeptides, and estrogen: a review. *Neurosci Biobehav Rev* 1994;18:251–68.
485. Acosta-Martinez M, Etgen AM. Activation of mu-opioid receptors inhibits lordosis behavior in estrogen and progesterone-primed female rats. *Horm Behav* 2002;41:88–100.
486. Petitti N, Karkanias GB, Etgen AM. Estradiol selectively regulates alpha 1B-noradrenergic receptors in the hypothalamus and preoptic area. *J Neurosci* 1992;12:3869–76.
487. Jenkins WJ, Becker JB. Dynamic increases in dopamine during paced copulation in the female rat. *Eur J Neurosci* 2003;18:1997–2001.
488. Pfaus JG, Damsma G, Wenkstern D, Fibiger HC. Sexual activity increases dopamine transmission in the nucleus accumbens and striatum of female rats. *Brain Res* 1995;693:21–30.
489. Luine VN. Serotonin, catecholamines and metabolites in discrete brain areas in relation to lordotic responding on proestrus. *Neuroendocrinology* 1993;57:946–54.
490. Frohmader KS, Bateman KL, Lehman MN. Effects of methamphetamine on sexual performance and compulsive sex behavior in male rats. *Psychopharmacology* 2010;212:93–104.
491. Graham MD, Pfaus JG. Differential regulation of female sexual behaviour by dopamine agonists in the medial preoptic area. *Pharmacol Biochem Behav* 2010;97:284–92.
492. Graham MD, Pfaus JG. Differential effects of dopamine antagonists infused to the medial preoptic area on the sexual behavior of female rats primed with estrogen and progesterone. *Pharmacol Biochem Behav* 2012;102:532–9.
493. Silva LE, Castro M, Amaral FC, Antunes-Rodrigues J, Elias LL. Estradiol-induced hypophagia is associated with the differential mRNA expression of hypothalamic neuropeptides. *Braz J Med Biol Res* 2010;43:759–66.
494. Taylor JA, Goubillon M-L, Broad KD, Robinson JE. Steroid control of gonadotropin-releasing hormone secretion: associated changes in pro-opiomelanocortin and preproenkephalin messenger RNA expression in the ovine hypothalamus. *Biol Reprod* 2007;76:524–31.
495. Menard CS, Dohanich GP. Estrogen dependence of cholinergic systems that regulate lordosis in cycling female rats. *Pharmacol Biochem Behav* 1994;48:417–21.
496. Romano GJ, Harlan RE, Shivers BD, Howells RD, Pfaff DW. Estrogen increases proenkephalin messenger ribonucleic acid levels in the ventromedial hypothalamus of the rat. *Mol Endocrinol* 1988;2:1320–8.
497. Yang T-S, Wang H-L, Chen Y-J, Chang S-P, Yuan C-C. Effect of continuous administration of conjugated estrogen plus medroxyprogesterone acetate (Premelle) in postmenopausal women in Taiwan. *J Chin Med Assoc* 2004;67:336–43.
498. Robbins TW. Shifting and stopping: fronto-striatal substrates, neurochemical modulation and clinical implications. *Philos Trans R Soc Lond B Biol Sci* 2007;362:917–32.
499. Sinchak K, Mills RH, Eckersell CB, Micevych PE. Medial preoptic area delta-opioid receptors inhibit lordosis. *Behav Brain Res* 2004;155:301–6.
500. Afonso VM, Mueller D, Stewart J, Pfaus JG. Amphetamine pretreatment facilitates appetitive sexual behaviors in the female rat. *Psychopharmacology* 2009;205:35–43.
501. Winland C, Haycox C, Bolton JL, et al. Methamphetamine enhances sexual behavior in female rats. *Pharmacol Biochem Behav* 2011;98:575–82.
502. Afonso VM, Bablekis V, Pfaus JG. Sensory mediation of female–male mounting in the rat: II. Role of tactile and conspecific cues. *Physiol Behav* 2006;87:863–9.
503. Afonso VM, Pfaus JG. Hormonal and experiential control of female–male mounting in the female rat. *Horm Behav* 2006;49:30–7.
504. Afonso VM, Woehrling A, Pfaus JG. Sensory mediation of female–male mounting in the rat: I. Role of olfactory cues. *Physiol Behav* 2006;87:857–62.
505. Hoshina Y, Takeo T, Nakano K, Sato T, Sakuma Y. Axon-sparing lesion of the preoptic area enhances receptivity and diminishes proceptivity among components of female rat sexual behavior. *Behav Brain Res* 1994;61:197–204.
506. Afonso VM, Sison M, Lovic V, Fleming AS. Medial prefrontal cortex lesions in the female rat affect sexual and maternal behavior and their sequential organization. *Behav Neurosci* 2007;121:515–26.
507. Kondo Y, Koizumi T, Arai Y, Kakeyama M, Yamanouchi K. Functional relationships between mesencephalic central gray and septum in regulating lordosis in female rats: effect of dual lesions. *Brain Res Bull* 1993;32:635–8.
508. Yamanouchi K, Arai Y. The septum as origin of a lordosis-inhibiting influence in female rats: effect of neural transection. *Physiol Behav* 1990;48:351–5.
509. Grierson JP, James MD, Pearson JR, Wilson CA. The effect of selective D1 and D2 dopaminergic agents on sexual receptivity in the female rat. *Neuropharmacology* 1988;27:181–9.
510. Ismail N, Laroche C, Girard-Bériault F, Menard S, Greggain-Mohr JA, Pfaus JG. Conditioned ejaculatory preference in male rats paired with haloperidol-treated females. *Physiol Behav* 2010;100:116–21.
511. Hansen S, Harthon C, Waslin E, Löfberg L, Svensson K. Mesotelencephalic dopamine system and reproductive behavior in the female rat: effects of ventral tegmental 6-hydroxydopamine lesions on maternal and sexual responsiveness. *Behav Neurosci* 1991;105:588–98.
512. Wilson CA, Thody AJ, Hole DR, Grierson JP, Celis ME. Interaction of estradiol, alpha-melanocyte-stimulating hormone, and dopamine in the regulation of sexual receptivity in the female rat. *Neuroendocrinology* 1991;54:14–22.

513. Paredes RG, Alonso A. Sexual behavior regulated (paced) by the female induces conditioned place preference. *Behav Neurosci* 1997;111:123–8.
514. Cooper PE, Martin JB. Neuroendocrinology and brain peptides. *Ann Neurol* 1980;8:551–7.
515. Khorram O, de Castro JB, McCann SM. The effect of the estrous cycle and estrogen on the release of immunoreactive α-melanocyte-stimulating hormone. *Peptides* 1985;6:503–8.
516. Pfaus JG, Shadiack A, Van Soest T, Tse M, Molinoff P. Selective facilitation of sexual solicitation in the female rat by a melanocortin receptor agonist. *Proc Natl Acad Sci USA* 2004;101:10201–4.
517. Rössler A-S, Pfaus JG, Kia HK, Bernabé J, Alexandre L, Giuliano F. The melanocortin agonist, melanotan II, enhances proceptive sexual behaviors in the female rat. *Pharmacol Biochem Behav* 2006;85:514–21.
518. Greggain-Mohr JA, Antonie R, Pfaus JG. Effect of melanotan-II on sexual solicitations in the female rat: enhancement with subchronic administration. *Pharmacol Biochem Behav*, submitted for publication.
519. Georgescu M, Pfaus JG. Role of glutamate receptors in the ventromedial hypothalamus in the regulation of female rat sexual behaviors: I. Behavioral effects of glutamate and its selective receptor agonists AMPA, NMDA and kainate. *Pharmacol Biochem Behav* 2006;83:322–32.
520. Georgescu M, Pfaus JG. Role of glutamate receptors in the ventromedial hypothalamus in the regulation of female rat sexual behaviors. II. Behavioral effects of selective glutamate receptor antagonists AP-5, CNQX, and DNQX. *Pharmacol Biochem Behav* 2006;83:333–41.
521. Pfaus JG, Pfaff DW. [mu]-,[delta]-, and [kappa]-opioid receptor agonists selectively modulate sexual behaviors in the female rat: differential dependence on progesterone. *Horm Behav* 1992;26:457–73.
522. Konorski J. *Integrative activity of the brain*. Chicago: Chicago University Press; 1967.
523. Erskine MS, Kornberg E, Cherry JA. Paced copulation in rats: effects of intromission frequency and duration on luteal activation and estrus length. *Physiol Behav* 1989;45:33–9.
524. Barnett SA. *The rat: a study in behaviour*. Chicago: Aldine Publishing Company; 1963.
525. Xiao K, Kondo Y, Sakuma Y. Differential regulation of female rat olfactory preference and copulatory pacing by the lateral septum and medial preoptic area. *Neuroendocrinology* 2005;81:56–62.
526. Guarraci FA, Megroz AB, Clark AS. Paced mating behavior in the female rat following lesions of three regions responsive to vaginocervical stimulation. *Brain Res* 2004;999:40–52.
527. Meerts SH, Clark AS. Lesions of the medial preoptic area interfere with the display of a conditioned place preference for vaginocervical stimulation in rats. *Behav Neurosci* 2009;123:752–7.
528. Bracha V, Zbarska S, Parker K, Carrel A, Zenitsky G, Bloedel JR. The cerebellum and eye-blink conditioning: learning versus network performance hypotheses. *Neuroscience* 2009;162:787–96.
529. Thompson RF, Steinmetz JE. The role of the cerebellum in classical conditioning of discrete behavioral responses. *Neuroscience* 2009;162:732–55.
530. Kow L-M, Pfaff DW. Estrogen effects on neuronal responsiveness to electrical and neurotransmitter stimulation: an in vitro study on the ventromedial nucleus of the hypothalamus. *Brain Res* 1985;347:1–10.
531. Grattan DR, Selmanoff M. Sex differences in the activity of gamma-aminobutyric acidergic neurons in the rat hypothalamus. *Brain Res* 1997;775:244–9.
532. Jang IS, Rhee JS, Watanabe T, Akaike N, Akaike N. Histaminergic modulation of GABAergic transmission in rat ventromedial hypothalamic neurones. *J Physiol* 2001;534:791–803.
533. Mirkes SJ, Bethea CL. Oestrogen, progesterone and serotonin converge on GABAergic neurones in the monkey hypothalamus. *J Neuroendocrinol* 2001;13:182–92.
534. Commons KG, Kow L-M, Milner TA, Pfaff DW. In the ventromedial nucleus of the rat hypothalamus, GABA-immunolabeled neurons are abundant and are innervated by both enkephalin- and GABA-immunolabeled axon terminals. *Brain Res* 1999;816:58–67.
535. O'Connor LH, Nock B, McEwen BS. Regional specificity of gamma-aminobutyric acid receptor regulation by estradiol. *Neuroendocrinology* 1988;47:473–81.
536. Schumacher M, Coirini HEC, McEwen BS. Regulation of high-affinity GABAa receptors in specific brain regions by ovarian hormones. *Neuroendocrinology* 1989;50:315–20.
537. Warembourg M, Leroy D. AMPA glutamate receptor subunits in the guinea pig hypothalamus: distribution and colocalization with progesterone receptor. *J Comp Neurol* 2002;453:305–21.
538. Diano S. Gonadal steroids target AMPA glutamate receptor-containing neurons in the rat hypothalamus, septum and amygdala: a morphological and biochemical study. *Endocrinology* 1997;138:778–89.
539. Mccarthy M. Functional significance of steroid modulation of GABAergic neurotransmission: analysis at the behavioral, cellular, and molecular levels. *Horm Behav* 1995;29:131–40.
540. Kow LM, Harlan RE, Shivers BD, Pfaff DW. Inhibition of the lordosis reflex in rats by intrahypothalamic infusion of neural excitatory agents: evidence that the hypothalamus contains separate inhibitory and facilitatory elements. *Brain Res* 1985;341:26–34.
541. McCarthy MM, Curran GH, Feder HH. Excitatory amino acid modulation of lordosis in the rat. *Neurosci Lett* 1991;126:94–7.
542. Eyigor O, Lin W, Jennes L. Identification of neurones in the female rat hypothalamus that express oestrogen receptor-alpha and vesicular glutamate transporter-2. *J Neuroendocrinol* 2004;16:26–31.
543. Luine VN, Wu V, Hoffman CS, Renner KJ. GABAergic regulation of lordosis: influence of gonadal hormones on turnover of GABA and interaction of GABA with 5-HT. *Neuroendocrinology* 1999;69:438–45.
544. Watanabe T, Inoue S, Hiroi H, Orimo A, Muramatsu M. NMDA receptor type 2D gene as target for estrogen receptor in the brain. *Brain Res Mol Brain Res* 1999;63:375–9.
545. McCarthy MM, Masters DB, Fiber JM, et al. GABAergic control of receptivity in the female rat. *Neuroendocrinology* 1991;53:473–9.
546. Luine VN, Grattan DR, Selmanoff M. Gonadal hormones alter hypothalamic GABA and glutamate levels. *Brain Res* 1997;747:165–8.
547. Hudgens ED, Ji L, Carpenter CD, Petersen SL. The GAD2 promoter is a transcriptional target of estrogen receptor (ER) α and ERβ: a unifying hypothesis to explain diverse effects of estradiol. *J Neurosci* 2009;29:8790–7.
548. Hoffman CS, Westin TM, Miner HM, Johnson PL, Summers CH, Renner KJ. GABAergic drugs alter hypothalamic serotonin release and lordosis in estrogen-primed rats. *Brain Res* 2002;946:96–103.
549. McCarthy MM, Malik KF, Feder HH. Increased GABAergic transmission in medial hypothalamus facilitates lordosis but has the opposite effect in preoptic area. *Brain Res* 1990;507:40–4.
550. McCarthy MM, Masters DB, Rimvall K, Schwartz-Giblin S, Pfaff DW. Intracerebral administration of antisense oligodeoxynucleotides to GAD65 and GAD67 mRNAs modulate reproductive behavior in the female rat. *Brain Res* 1994;636:209–20.
551. Booth C, Wayman CP, Jackson VM. An ex vivo multi-electrode approach to evaluate endogenous hormones and receptor subtype pharmacology on evoked and spontaneous neuronal activity within the ventromedial hypothalamus; translation from female receptivity. *J Sex Med* 2010;7:2411–23.
552. Willoughby JO, Blessing WW. Origin of serotonin innervation of the arcuate and ventromedial hypothalamic region. *Brain Res* 1987;418:170–3.

553. Crowley WR, Rodriguez-Sierra JF, Komisaruk BR. Monoaminergic mediation of the antinociceptive effect of vaginal stimulation in rats. *Brain Res* 1977;137:67–84.
554. Etgen AM. Intrahypothalamic implants of noradrenergic antagonists disrupt lordosis behavior in female rats. *Physiol Behav* 1990;48:31–6.
555. Hansen S, Stanfield EJ, Everitt BJ. The role of ventral bundle noradrenergic neurones in sensory components of sexual behaviour and coitus-induced pseudopregnancy. *Nature* 1980;286:152–4.
556. Hansen S, Stanfield EJ, Everitt BJ. The effects of lesions of lateral tegmental noradrenergic neurons on components of sexual behavior and pseudopregnancy in female rats. *Neuroscience* 1981;6:1105–17.
557. Thornton JE, Goy RW, McEwen BS, Feder HH. Alpha 1 noradrenergic antagonism decreases hormonally-induced and hormonally-independent lordosis. *Pharmacol Biochem Behav* 1989;32:421–4.
558. Vathy I, van der Plas J, Vincent PA, Etgen AM. Intracranial dialysis and microinfusion studies suggest that morphine may act in the ventromedial hypothalamus to inhibit female rat sexual behavior. *Horm Behav* 1991;25:354–66.
559. Vincent PA, Etgen AM. Steroid priming promotes oxytocin-induced norepinephrine release in the ventromedial hypothalamus of female rats. *Brain Res* 1993;620:189–94.
560. VanderHorst VGJM, Gustafsson J-A, Ulfhake B. Estrogen receptor-α and -β immunoreactive neurons in the brainstem and spinal cord of male and female mice: relationships to monoaminergic, cholinergic, and spinal projection systems. *J Comp Neurol* 2005;488:152–79.
561. Nomura M, Akama KT, Alves SE, et al. Differential distribution of estrogen receptor (ER)-alpha and ER-beta in the midbrain raphe nuclei and periaqueductal gray in male mouse: predominant role of ER-beta in midbrain serotonergic systems. *Neuroscience* 2005;130:445–56.
562. Etgen AM, Ungar S, Petitti N. Estradiol and progesterone modulation of norepinephrine neurotransmission: implications for the regulation of female reproductive behavior. *J Neuroendocrinol* 1992;4:255–71.
563. Etgen AM, Karkanias GB. Estrogen regulation of noradrenergic signaling in the hypothalamus. *Psychoneuroendocrinology* 1994;19:603–10.
564. Donoso AO, Broitman ST. Effects of a histamine synthesis inhibitor and antihistamines on the sexual behavior of female rats. *Psychopharmacology* 1979;66:251–5.
565. Dohanich GP, Clemens LG. Brain areas implicated in cholinergic regulation of sexual behavior. *Horm Behav* 1981;15:157–67.
566. Rainbow TC, Snyder L, Berck DJ, McEwen BS. Correlation of muscarinic receptor induction in the ventromedial hypothalamic nucleus with the activation of feminine sexual behavior by estradiol. *Neuroendocrinology* 1984;39:476–80.
567. Dupré C, Lovett-Barron M, Pfaff DW, Kow L-M. Histaminergic responses by hypothalamic neurons that regulate lordosis and their modulation by estradiol. *Proc Natl Acad Sci USA* 2010;107:12311–6.
568. Coirini H, McEwen BS. Progestin receptor induction and sexual behavior by estradiol treatment in male and female rats. *J Neuroendocrinol* 1990;2:467–72.
569. McCarthy MM, Kleopoulos SP, Mobbs CV, Pfaff DW. Infusion of antisense oligodeoxynucleotides to the oxytocin receptor in the ventromedial hypothalamus reduces estrogen-induced sexual receptivity and oxytocin receptor binding in the female rat. *Neuroendocrinology* 1994;59:432–40.
570. Schumacher M, Coirini H, Johnson AE, et al. The oxytocin receptor: a target for steroid hormones. *Regul Pept* 1993;45:115–9.
571. Kow LM, Johnson AE, Ogawa S, Pfaff DW. Electrophysiological actions of oxytocin on hypothalamic neurons in vitro: neuropharmacological characterization and effects of ovarian steroids. *Neuroendocrinology* 1991;54:526–35.
572. Inenaga K, Karman H, Yamashita H, Tribollet E, Raggenbass M, Dreifuss JJ. Oxytocin excites neurons located in the ventromedial nucleus of the Guinea-pig hypothalamus. *J Neuroendocrinol* 1991;3:569–73.
573. Schulze HG, Gorzalka BB. Oxytocin effects on lordosis frequency and lordosis duration following infusion into the medial preoptic area and ventromedial hypothalamus of female rats. *Neuropeptides* 1991;18:99–106.
574. Flanagan LM, Pfaus JG, Pfaff DW, McEwen BS. Induction of FOS immunoreactivity in oxytocin neurons after sexual activity in female rats. *Neuroendocrinology* 1993;58:352–8.
575. Kruger THC, Schiffer B, Eikermann M, Haake P, Gizewski E, Schedlowski M. Serial neurochemical measurement of cerebrospinal fluid during the human sexual response cycle. *Eur J Neurosci* 2006;24:3445–52.
576. Dudley CA, Moss RL. Facilitation of lordosis in female rats by CNS-site specific infusions of an LH-RH fragment, Ac-LH-RH-(5-10). *Brain Res* 1988;441:161–7.
577. Gargiulo PA, Muñoz V, Donoso AO. Inhibition by *N*-methyl-D-aspartic acid (NMDA) receptor antagonist of lordosis behavior induced by estrogen followed by progesterone or luteinizing hormone-releasing hormone (LHRH) in the rat. *Physiol Behav* 1992;52:737–9.
578. Harlan RE, Shivers BD, Pfaff DW. Midbrain microinfusions of prolactin increase the estrogen-dependent behavior, lordosis. *Science* 1983;219:1451–3.
579. Moss RL, McCann SM. Induction of mating behavior in rats by luteinizing hormone-releasing factor. *Science* 1973;181:177–9.
580. Merchenthaler I, Göres T, Sétáló G, Petrusz DP, Flerkó B. Gonadotropin-releasing hormone (GnRH) neurons and pathways in the rat brain. *Cell Tissue Res* 1984;237:15–29.
581. Jennes L, Eyigor O, Janovick JA, Conn PM. Brain gonadotropin releasing hormone receptors: localization and regulation. *Recent Prog Horm Res* 1997;52:475–90.
582. Bakowska JC, Morrell JI. Atlas of the neurons that express mRNA for the long form of the prolactin receptor in the forebrain of the female rat. *J Comp Neurol* 1997;386:161–77.
583. Nishizuka M, Shivers BD, Leranth C, Pfaff DW. Ultrastructural characterization of prolactin-like immunoreactivity in rat medial basal hypothalamus. *Neuroendocrinology* 1990;51:249–54.
584. Pfaus JG, Jakob A, Kleopoulos SP, Gibbs RB, Pfaff DW. Sexual stimulation induces Fos immunoreactivity within GnRH neurons of the female rat preoptic area: interaction with steroid hormones. *Neuroendocrinology* 1994;60:283–90.
585. Kruger THC, Leeners B, Naegeli E, et al. Prolactin secretory rhythm in women: immediate and long-term alterations after sexual contact. *Hum Reprod* 2012;27:1139–43.
586. Leeners B, Kruger THC, Brody S, Schmidlin S, Naegeli E, Egli M. The quality of sexual experience in women correlates with post-orgasmic prolactin surges: results from an experimental prototype study. *J Sex Med* 2013;10:1313–9.
587. Ingram SM, Krause RG, Baldino F, Skeen LC, Lewis ME. Neuronal localization of cholecystokinin mRNA in the rat brain by using in situ hybridization histochemistry. *J Comp Neurol* 1989;287:260–72.
588. Fulwiler CE, Saper CB. Cholecystokinin-immunoreactive innervation of the ventromedial hypothalamus in the rat: possible substrate for autonomic regulation of feeding. *Neurosci Lett* 1985;53:289–96.
589. Zaborszky L, Beinfeld MC, Palkovits M, Heimer L. Brainstem projection to the hypothalamic ventromedial nucleus in the rat: a CCK-containing long ascending pathway. *Brain Res* 1984;303:225–31.
590. Inagaki S, Shiotani Y, Yamano M, et al. Distribution, origin, and fine structures of cholecystokinin-8-like immunoreactive terminals in the nucleus ventromedialis hypothalami of the rat. *J Neurosci* 1984;4:1289–99.

591. Schumacher M, Coirini H, McEwen BS, Záborszky L. Binding of [3H]cholecystokinin in the ventromedial hypothalamus modulated by an afferent brainstem projection but not by ovarian steroids. *Brain Res* 1991;564:102–8.
592. Akesson TR, Mantyh PW, Mantyh CR, Matt DW, Micevych PE. Estrous cyclicity of 125I-cholecystokinin octapeptide binding in the ventromedial hypothalamic nucleus. Evidence for downmodulation by estrogen. *Neuroendocrinology* 1987;45:257–62.
593. Acosta-Martinez M, Etgen AM. The role of delta-opioid receptors in estrogen facilitation of lordosis behavior. *Behav Brain Res* 2002;136:93–102.
594. Nicot A, Ogawa S, Berman Y, Carr KD, Pfaff DW. Effects of an intrahypothalamic injection of antisense oligonucleotides for preproenkephalin mRNA in female rats: evidence for opioid involvement in lordosis reflex. *Brain Res* 1997;777:60–8.
595. Romano GJ, Mobbs CV, Howells RD, Pfaff DW. Estrogen regulation of proenkephalin gene expression in the ventromedial hypothalamus of the rat: temporal qualities and synergism with progesterone. *Brain Res Mol Brain Res* 1989;5:51–8.
596. Turcotte JC, Blaustein JD. Convergence of substance P and estrogen receptor immunoreactivity in the midbrain central gray of female guinea pigs. *Neuroendocrinology* 1997;66:28–37.
597. Akesson TR, Micevych PE. Estrogen concentration by substance P-immunoreactive neurons in the medial basal hypothalamus of the female rat. *J Neurosci Res* 1988;19:412–9.
598. Dornan WA, Malsbury CW, Penney RB. Facilitation of lordosis by injection of substance P into the midbrain central gray. *Neuroendocrinology* 1987;45:498–506.
599. Dornan WA, Akesson TR, Micevych PE. A substance P projection from the VMH to the dorsal midbrain central gray: implication for lordosis. *Brain Res Bull* 1990;25:791–6.
600. Barbaresi P. Immunocytochemical localization of substance P receptor in rat periaqueductal gray matter: a light and electron microscopic study. *J Comp Neurol* 1998;398:473–90.
601. Foreman MM, Moss RL. Role of hypothalamic serotonergic receptors in the control of lordosis behavior in the female rat. *Horm Behav* 1978;10:97–106.
602. Luine VN, Frankfurt M, Rainbow TC, Biegon A, Azmitia E. Intrahypothalamic 5,7-dihydroxytryptamine facilitates feminine sexual behavior and decreases [3H]imipramine binding and 5-HT uptake. *Brain Res* 1983;264:344–8.
603. James MD, Hole DR, Wilson CA. Differential involvement of 5-hydroxytryptamine (5HT) in specific hypothalamic areas in the mediation of steroid-induced changes in gonadotrophin release and sexual behaviour in female rats. *Neuroendocrinology* 1989;49:561–9.
604. Maswood N, Sarkar J, Uphouse L. Modest effects of repeated fluoxetine on estrous cyclicity and sexual behavior in Sprague Dawley female rats. *Brain Res* 2008;1245:52–60.
605. Miryala CSJ, Hiegel C, Uphouse L. Sprague-Dawley and Fischer female rats differ in acute effects of fluoxetine on sexual behavior. *J Sex Med* 2013;10:350–61.
606. Sarkar J, Hiegel C, Maswood N, Uphouse L. Daily male exposure attenuates estrous cycle disruption by fluoxetine. *Behav Brain Res* 2008;189:83–91.
607. Guptarak J, Sarkar J, Hiegel C, Uphouse L. Role of 5-HT1A receptors in fluoxetine-induced lordosis inhibition. *Horm Behav* 2010. http://dx.doi.org/10.1016/j.yhbeh.2010.03.003.
608. Sinchak K, Dewing P, Ponce L, et al. Modulation of the arcuate nucleus-medial preoptic nucleus lordosis regulating circuit: a role for GABAB receptors. *Horm Behav* 2013;64:136–43.
609. Mahavongtrakul M, Kanjiya MP, Maciel M, Kanjiya S, Sinchak K. Estradiol dose-dependent regulation of membrane estrogen receptor-α, metabotropic glutamate receptor-1a, and their complexes in the arcuate nucleus of the hypothalamus in female rats. *Endocrinology* 2013;154:3251–60.
610. Micevych P, Sinchak K. Temporal and concentration dependent estradiol effects on neural pathways mediating sexual receptivity. *J Neuroendocrinol* 2013;25:1012–23.
611. Sinchak K, Wagner EJ. Estradiol signaling in the regulation of reproduction and energy balance. *Front Neuroendocrinol* 2012;33:342–63.
612. Sternson SM, Shepherd GMG, Friedman JM. Topographic mapping of VMH→arcuate nucleus microcircuits and their reorganization by fasting. *Nat Neurosci* 2005;8:1356–63.
613. Bannai M, Ichikawa M, Nishihara M, Takahashi M. Effect of injection of antisense oligodeoxynucleotides of GAD isozymes into rat ventromedial hypothalamus on food intake and locomotor activity. *Brain Res* 1998;784:305–15.
614. Berridge KC. 'Liking' and "wanting" food rewards: brain substrates and roles in eating disorders. *Physiol Behav* 2009.
615. Mahler SV, Berridge KC. What and when to 'want'? Amygdala-based focusing of incentive salience upon sugar and sex. *Psychopharmacology* 2012;221:407–26.
616. Takaki A, Aou S, Oomura Y, Okada E, Hori T. Feeding suppression elicited by electrical and chemical stimulations of monkey hypothalamus. *Am J Physiol* 1992;262:R586–94.
617. Lodder J, Zeilmaker GH. Role of pelvic nerves in the postcopulatory abbreviation of behavioral estrus in female rats. *J Comp Physiol Psychol* 1976;90:925–9.
618. Reading DS, Blaustein JD. The relationship between heat abbreviation and neural progestin receptors in female rats. *Physiol Behav* 1984;32:973–81.
619. Goldfoot DA, Goy RW. Abbreviation of behavioral estrus in guinea pigs by coital and vagino-cervical stimulation. *J Comp Physiol Psychol* 1970;72:426–34.
620. Carter CS. Stimuli contributing to the decrement in sexual receptivity of female golden hamsters (*Mesocricetus auratus*). *Anim Behav* 1973;21:827–34.
621. Wallen K, Winston LA. Social complexity and hormonal influences on sexual behavior in rhesus monkeys (*Macaca mulatta*). *Physiol Behav* 1984;32:629–37.
622. Ramos SM, Debold JF. Protein synthesis in the medial preoptic area is important for the mating-induced decrease in estrus duration in hamsters. *Horm Behav* 1999;35:177–85.
623. Blaustein J, Brown T, Reading D. Failure of protein synthesis inhibition to block progesterone desensitization of lordosis in female rats. *Physiol Behav* 1982;29:475–81.
624. Blaustein JD, Brown TJ. Neural progestin receptors: regulation of progesterone-facilitated sexual behaviour in female guinea pigs. In: Gilles R, Balthazart J, editors. *Neurobiology: current comparative approaches*. Berlin: Springer Verlag; 1985. pp. 60–76.
625. Morin LP, Feder HH. Multiple progesterone injections and the duration of estrus in ovariectomized guinea pigs. *Physiol Behav* 1973;11:861–5.
626. Blaustein JD, Tetel MJ, Meredith JM. Neurobiological regulation of hormonal response by progestin and estrogen receptors. In: Micevych PE, Hammer R, editors. *Neurobiological effects of sex steroid hormones*. New York: Cambridge University Press; 1995. pp. 324–49.
627. Goy RW, Phoenix CH, Young WC. Inhibitory action in the corpus luteum on the hormonal induction of estrous behavior in the guinea pig. *Gen Comp Endocrinol* 1966;6:267–75.
628. Nadler RD. A biphasic influence of progesterone on sexual receptivity of spayed female rats. *Physiol Behav* 1970;5:95–7.
629. Moguilewsky M, Raynaud JP. The relevance of hypothalamic and hyphophyseal progestin receptor regulation in the induction and inhibition of sexual behavior in the female rat. *Endocrinology* 1979;105:516–22.
630. Parsons B, McGinnis MY, McEwen BS. Sequential inhibition of progesterone: effects on sexual receptivity and associated changes in brain cytosol progestin binding in the female rat. *Brain Res* 1981;221:149–60.

631. Blaustein JD. Progesterone in high doses may overcome progesterone's desensitization effect on lordosis by translocation of hypothalamic progestin receptors. *Horm Behav* 1982;16:175–90.
632. Joslyn WD, Feder HH. Facilitatory and inhibitory effects of supplementary estradiol benzoate given to ovariectomized, estrogen-primed guinea pigs. *Horm Behav* 1971.
633. Hansen S, Sodersten P. Reversal of progesterone inhibition of sexual behaviour in ovariectomized rats by high doses of progesterone. *J Endocrinol* 1979;80:381–8.
634. Sodersten P, Hansen S. Induction of sexual receptivity by oestradiol benzoate in cyclic female rats: influence of ovarian secretions before injection of oestradiol benzoate. *J Endocrinol* 1979;80:389–95.
635. Powers JB, Moreines J. Progesterone: examination of its postulated inhibitory actions on lordosis during the rat estrous cycle. *Physiol Behav* 1976;17:493–8.
636. Hansen S, Sodersten P. Effects of subcutaneous implants of progesterone on the induction and duration of sexual receptivity in ovariectomized rats. *J Endocrinol* 1978;77:373–9.
637. Georgescu M, Sabongui C, Del Corpo A, Marsan L, Pfaus JG. Vaginocervical stimulation induces Fos in glutamate neurons in the ventromedial hypothalamus: attenuation by estrogen and progesterone. *Horm Behav* 2009;56:450–6.
638. Georgescu M, Afonso VM, Graham MD, Pfaus JG. Glutamate release in the ventromedial hypothalamus of the female rat during copulation: modulation by ovarian hormones. *Horm Behav* 2014.
639. Georgescu M, Cyr D, Pfaus JG. AMPA/kainate receptors in the ventromedial hypothalamus mediate the effects of glutamate on estrus termination in the rat. *Pharmacol Biochem Behav* 2012;102:146–50.
640. Georgescu M, Graham MD, Pfaus JG. GABAergic projections to the ventromedial hypothalamus of the female rat, in preparation.
641. Snoeren EMS, Bovens A, Refsgaard LK, et al. Combination of testosterone and vardenafil increases female sexual functioning in sub-primed rats. *J Sex Med* 2011;8:989–1001.
642. Clayton AH, Dennerstein L, Pyke R, Sand M. Flibanserin: a potential treatment for hypoactive sexual desire disorder in premenopausal women. *Women's Health (Lond Engl)* 2010;6:639–53.
643. Diamond LE, Earle DC, Heiman JR, Rosen RC, Perelman MA, Harning R. An effect on the subjective sexual response in premenopausal women with sexual arousal disorder by bremelanotide (PT-141), a melanocortin receptor agonist. *J Sex Med* 2006;3:628–38.
644. Katz M, DeRogatis LR, Ackerman R, et al. Efficacy of flibanserin in women with hypoactive sexual desire disorder: results from the BEGONIA trial. *J Sex Med* 2013;10:1807–15.
645. Perelman MA. Clinical application of CNS-acting agents in FSD. *J Sex Med* 2007;4:S280–90.
646. van der Made F, Bloemers J, Yassem WE, et al. The influence of testosterone combined with a PDE5-inhibitor on cognitive, affective, and physiological sexual functioning in women suffering from sexual dysfunction. *J Sex Med* 2009;6:777–90.
647. Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Prog Neurobiol* 1998;56:613–72.
648. Carter CS, Witt DM, Thompson EG, Carlstead K. Effects of hormonal, sexual, and social history on mating and pair bonding in prairie voles. *Physiol Behav* 1988;44:691–7.
649. Carter CS, Williams JR, Witt DM, Insel TR. Oxytocin and social bonding. *Ann N Y Acad Sci* 1992;652:204–11.
650. Wang Z, Young LJ, De Vries GJR, Insel TR. Voles and vasopressin: a review of molecular, cellular, and behavioral studies of pair bonding and paternal behaviors. *Prog Brain Res* 1999;199:483–99.
651. Young LJ, Murphy Young AZ, Hammock EAD. Anatomy and neurochemistry of the pair bond. *J Comp Neurol* 2005;493:51–7.
652. Lester G, Gorzalka BB. Effect of novel and familiar mating partners on the duration of sexual receptivity in the female hamster. *Behav Neural Biol* 1988;49:398–405.
653. Broekman M, de Bruin M, Smeenk J, Slob AK, van der Schoot P. Partner preference behavior of estrous female rats affected by castration of tethered male incentives. *Horm Behav* 1988;22:324–37.
654. Oldenburger WP, Everitt BJ, de Jonge FH. Conditioned place preference induced by sexual interaction in female rats. *Horm Behav* 1992;26:214–28.
655. Jenkins WJ, Becker JB. Female rats develop conditioned place preferences for sex at their preferred interval. *Horm Behav* 2003;43:503–7.
656. Matthews TJ, Grigore M, Tang L, Doat M, Kow LM, Pfaff DW. Sexual reinforcement in the female rat. *J Exp Anal Behav* 1997;68:399–410.
657. Meerts SH, Clark AS. Artificial vaginocervical stimulation induces a conditioned place preference in female rats. *Horm Behav* 2009;55:128–32.
658. Cibrian-Llanderal T, Tecamachaltzi-Silvaran M, Rio RT-D, Pfaus JG, Manzo J, Coria-Avila GA. Clitoral stimulation modulates appetitive sexual behavior and facilitates reproduction in rats. *Physiol Behav* 2010;100:148–53.
659. González-Flores O, Camacho FJ, Domínguez-Salazar E, Ramírez-Orduna JM, Beyer C, Paredes RG. Progestins and place preference conditioning after paced mating. *Horm Behav* 2004;46:151–7.
660. Corona R, Camacho FJ, García-Horsman P, Guerrero A, Ogando A, Paredes RG. Different doses of estradiol benzoate induce conditioned place preference after paced mating. *Horm Behav* 2011;60:264–8.
661. Normandin JJ, Murphy AZ. Excitotoxic lesions of the nucleus paragigantocellularis facilitate male sexual behavior but attenuate female sexual behavior in rats. *Neuroscience* 2011;175:212–23.
662. Paredes RG, Martínez I. Naloxone blocks place preference conditioning after paced mating in female rats. *Behav Neurosci* 2001;115:1363–7.
663. García-Horsman SP, Agmo A, Paredes RG. Infusions of naloxone into the medial preoptic area, ventromedial nucleus of the hypothalamus, and amygdala block conditioned place preference induced by paced mating behavior. *Horm Behav* 2008;54:709–16.
664. Corona R, Larriva-Sahd J, Paredes RG. Paced-mating increases the number of adult new born cells in the internal cellular (granular) layer of the accessory olfactory bulb. *PLoS One* 2011;6:e19380.
665. Brennan P, Kaba H, Keverne EB. Olfactory recognition: a simple memory system. *Science* 1990;250:1223–6.
666. Brennan PA, Keverne EB. Neural mechanisms of mammalian olfactory learning. *Prog Neurobiol* 1997;51:457–81.
667. Bales KL, Carter CS. Sex differences and developmental effects of oxytocin on aggression and social behavior in prairie voles (*Microtus ochrogaster*). *Horm Behav* 2003;44:178–84.
668. Ismail N, Gelez H, Lachapelle I, Pfaus JG. Pacing conditions contribute to the conditioned ejaculatory preference for a familiar female in the male rat. *Physiol Behav* 2009;96:201–8.
669. Holley A, Shalev S, Bellevue S, Pfaus JG. Conditioned mate-guarding behavior in the female rat. *Physiol Behav* 2014, in press.
670. Young LJ, Wang Z. The neurobiology of pair bonding. *Nat Neurosci* 2004;7:1048–54.
671. Wang ZX, Liu Y, Young LJ, Insel TR. Hypothalamic vasopressin gene expression increases in both males and females postpartum in a biparental rodent. *J Neuroendocrinol* 2000;12:111–20.
672. Harris JA. The acquisition of conditioned responding. *J Exp Psychol Anim Behav Process* 2011;37:151–64.
673. Rescorla RA, Wagner AR. A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement. In: Black AH, Prokasy WF, editors. *Classical conditioning II: current research and theory*. New York: Appleton Century Crofts; 1972. pp. 64–99.

674. Flagel SB, Robinson TE, Clark JJ, et al. An animal model of genetic vulnerability to behavioral disinhibition and responsiveness to reward-related cues: implications for addiction. *Neuropsychopharmacology* 2010;35:388–400.
675. Blackburn JR, Pfaus JG, Phillips AG. Dopamine functions in appetitive and defensive behaviours. *Prog Neurobiol* 1992;39:247–79.
676. Succu S, Sanna F, Melis T, Boi A, Argiolas A, Melis MR. Stimulation of dopamine receptors in the paraventricular nucleus of the hypothalamus of male rats induces penile erection and increases extra-cellular dopamine in the nucleus accumbens: involvement of central oxytocin. *Neuropharmacology* 2007;52:1034–43.
677. Vuong C, Van Uum SH, O'Dell LE, Lutfy K, Friedman TC. The effects of opioids and opioid analogs on animal and human endocrine systems. *Endocr Rev* 2010;31:98–132.
678. Letourneau EJ, O'Donohue W. Classical conditioning of female sexual arousal. *Arch Sex Behav* 1997.
679. Both S, Laan E, Spiering M, Nilsson T, Oomens S, Everaerd W. Appetitive and aversive classical conditioning of female sexual response. *J Sex Med* 2008;5:1386–401.
680. Both S, Brauer M, Laan E. Classical conditioning of sexual response in women: a replication study. *J Sex Med* 2011;8:3116–31.
681. Both S, Spiering M, Laan E, Belcome S, van den Heuvel B, Everaerd W. Unconscious classical conditioning of sexual arousal: evidence for the conditioning of female genital arousal to subliminally presented sexual stimuli. *J Sex Med* 2008;5:100–9.
682. Hoffmann H, Janssen E, Turner SL. Classical conditioning of sexual arousal in women and men: effects of varying awareness and biological relevance of the conditioned stimulus. *Arch Sex Behav* 2004;33:43–53.
683. Pfaus JG, Ismail N, Coria-Avila GA. In: Koob GF, Le Moal M, Thompson RF, editors. *Sexual motivation*. Oxford: Academic Press; 2010. pp. 201–9.
684. Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Brain Res Rev* 1991;16:223–44.
685. Fisher H. Lust, attraction, attachment: biology and evolution of the three primary emotion systems for mating, reproduction, and parenting. *J Sex Educ Ther* 2000;25:96–104.
686. Aron A, Fisher H, Mashek DJ, Strong G, Li H, Brown LL. Reward, motivation, and emotion systems associated with early-stage intense romantic love. *J Neurophysiol* 2005;94:327–37.
687. Blaustein JD. Neuroendocrine regulation of feminine sexual behavior: lessons from rodent models and thoughts about humans. *Annu Rev Psychol* 2008;59:93–118.
688. Brody S, Houde S, Hess U. Greater tactile sensitivity and less use of immature psychological defense mechanisms predict women's penile–vaginal intercourse orgasm. *J Sex Med* 2010;7:3057–65.
689. Puppo V, Gruenwald I. Does the G-spot exist? A review of the current literature. *Int Urogynecol J* 2012;23:1665–9.
690. Komisaruk BR, Whipple B. Functional MRI of the brain during orgasm in women. *Annu Rev Sex Res* 2005;16:62–86.
691. Jannini EA, Rubio-Casillas A, Whipple B, Buisson O, Komisaruk BR, Brody S. Female orgasm(s): one, two, several. *J Sex Med* 2012;9:956–65.
692. Mah K, Binik YM. Do all orgasms feel alike? Evaluating a two-dimensional model of the orgasm experience across gender and sexual context. *J Sex Res* 2002;39:104–13.
693. Clayton AH. Epidemiology and neurobiology of female sexual dysfunction. *J Sex Med* 2007;4:260–8.
694. van Rooij K, Poels S, Bloemers J, et al. Toward personalized sexual medicine (part 3): testosterone combined with a serotonin1A receptor agonist increases sexual satisfaction in women with HSDD and FSAD, and dysfunctional activation of sexual inhibitory mechanisms. *J Sex Med* 2013;10:824–37.
695. Hinkelmann K, Dragoi L, Gompf J, Muhtz C. Decreased recognition of negative affect after selective serotonin reuptake inhibition is dependent on genotype. *Psychiatry Res* 2010;177:354–7.
696. Dorr RT, Lines R, Levine N, Brooks C, Xiang L. Evaluation of Melanotan-II, a superpotent cyclic melanotropic peptide in a pilot phase-I clinical study. *Life Sci* 1996.
697. Krapf JM, Simon JA. The role of testosterone in the management of hypoactive sexual desire disorder in postmenopausal women. *Maturitas* 2009;63:213–9.
698. Schreiner-Engel P, Schiavi RC, Smith H, White D. Sexual arousability and the menstrual cycle. *Psychosom Med* 1981;43:199–214.
699. Tuiten A, Van Honk J, Verbaten R, Laan E, Everaerd W. Can sublingual testosterone increase subjective and physiological measures of laboratory-induced sexual arousal? *Arch Gen Psychiatry* 2002;59:465–6.
700. Poels S, Bloemers J, van Rooij K, et al. Toward personalized sexual medicine (part 2): testosterone combined with a PDE5 inhibitor increases sexual satisfaction in women with HSDD and FSAD, and a low sensitive system for sexual cues. *J Sex Med* 2013;10:810–23.
701. Perelman MA. A new combination treatment for premature ejaculation: a sex therapist's perspective. *J Sex Med* 2006;3:1004–12.
702. Abel J, Rissman EF. Location, location, location: genetic regulation of neural sex differences. *Rev Endocr Metab Disord* 2012;13:151–61.
703. Hamann S, Herman RA, Nolan CL, Wallen K. Men and women differ in amygdala response to visual sexual stimuli. *Nat Neurosci* 2004.
704. Becker JB. Sexual differentiation of motivation: a novel mechanism? *Horm Behav* 2009;55:646–54.
705. Semaan SJ, Kauffman AS. Sexual differentiation and development of forebrain reproductive circuits. *Curr Opin Neurobiol* 2010;20:424–31.
706. Vierck CJ, Acosta-Rua AJ, Rossi HL, Neubert JK. Sex differences in thermal pain sensitivity and sympathetic reactivity for two strains of rat. *J Pain* 2008;9:739–49.
707. Mendrek A, Stip E. Sexual dimorphism in schizophrenia: is there a need for gender-based protocols? *Expert Rev Neurother* 2011;11:951–9.
708. Auger AP, Auger CJ. Epigenetic turn ons and turn offs: chromatin reorganization and brain differentiation. *Endocrinology* 2011;152:349–53.
709. Lenz KM, McCarthy MM. Organized for sex – steroid hormones and the developing hypothalamus. *Eur J Neurosci* 2010;32:2096–104.
710. Arnold AP. The end of gonad-centric sex determination in mammals. *Trends Genet* 2012;28:55–61.
711. McCarthy MM, Arnold AP, Ball GF, Blaustein JD, de Vries GJ. Sex differences in the brain: the not so inconvenient truth. *J Neurosci* 2012;32:2241–7.
712. Miller N, Gold M. The human sexual response and alcohol and drugs. *J Subst Abuse Treat* 1988;5:171–7.
713. Cacioppo S, Bianchi-Demicheli F, Frum C, Pfaus JG, Lewis JW. The common neural bases between sexual desire and love: a multilevel kernel density fMRI analysis. *J Sex Med* 2012;9:1048–54.
714. Pfaus JG, Erickson KA, Talianakis S. Somatosensory conditioning of sexual arousal and copulatory behavior in the male rat: a model of fetish development. *Physiol Behav* 2013;120:114–23.