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Neural mechanisms of sexual motivation and performance in females

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Introduction

Although females and males of most mammalian species engage in mutual and complementary patterns of sexual activity, it is the females who have the final say in allowing successful sexual interaction. However, this does not imply that female sexual behavior exists along a single dimension of sexual receptivity. Far from being passive (or somewhat active) recipients of a male's sexual advances, females in the wild control virtually all aspects of sexual interaction, including the initiation and temporal patterning of copulation. This occurs by means of a complex integration of *appetitive* behaviors, used to attract and solicit sexual contact, *pacing* behaviors, used to control the rate of copulatory contact, and *defensive* behaviors, used either to pace copulatory contact if the female cannot do so otherwise, or to terminate the sexual interaction [1,2,3,4]. These behaviors serve to optimize the rate and strength of sexual stimulation that the female receives, which in turn initiates neuroendocrine reflexes associated with fertility and pregnancy [5].

Much of what is known about the neural mechanisms that underlie these behaviors comes from studies that have examined the effects of systemic or intracranial administration of hormones or drugs, or lesions of discrete brain areas [6]. However, recent advances in immunocytochemistry, molecular histology (e.g., *in situ* hybridization), and *in vivo* neurochemical analyses using brain microdialysis or voltammetry, have made it possible to visualize and quantify neurochemical and protein synthetic changes that occur in brain during sexual activity. Detection of the mRNA or protein products of immediate-early genes, such as *c-fos*, has proven exceptionally useful in marking regions of the brain that are activated during sexual behavior. In contrast to metabolic markers like 2-deoxyglucose (2-DG), the protein products of these genes are expressed in the nucleus and thus provide cellular resolution. It is also possible to identify neurochemical subgroups of cells that are activated by sexual stimulation by examining the co-localization of the protein products of these genes with known cytoplasmic proteins. This paper will explore recent findings using these techniques as they relate to earlier work using lesions and pharmacological manipulations. This analysis will be restricted mainly to the sexual behavior of female rodents because it represents the largest data base on which to draw conclusions.

Conceptual Issues

Studies of the neural mechanisms of sexual behavior in females have been concerned largely with the hormonal and neurochemical modulation of sexually-receptive behaviors, such as lordosis [6,7]. However, it is difficult to relate measures of female sexual receptivity to human sexuality, as lordosis has no obvious counterpart in human females. Beach [1] recognized the heuristic value of separating sexual behaviors into their respective appetitive and consummatory phases. This scheme has been further refined by the elegant work of Madlafousek and Hliňák

[3] and McClintock [4]. With the advent of behavioral techniques to assess the incentive motivational components of sexual behavior independently of the ability to copulate, it has become possible to dissect the neuroanatomical and neurochemical basis of sexual motivation and performance in animals in a way that has relevance to the study of human sexuality.

Appetitive and Consummatory Sexual Behaviors

Appetitive behaviors serve to bring animals into contact with incentive stimuli. This class of behaviors can be subdivided into *anticipatory* behaviors, which are often displayed conditionally in anticipation of the arrival of a goal or reward, and subdivided further into *preparatory* behaviors, which are required for animals to gain access to a goal, or which prepare animals to interact with a goal. Although some of these behaviors are displayed prior to the arrival of a sexually receptive partner, others, termed *proceptive* behaviors, are used by female rats during copulation to initiate and terminate bouts of sexual contact by alternating between the appetitive and consummatory components. This gives females a rich behavioral repertoire with which to control copulatory contact in different environments. Examples of preparatory behaviors displayed prior to the arrival of a sexual partner include (a) instrumental behavior of female rats maintained by sexual reinforcement [8]; (b) crossing an electrified grid to obtain a sexually receptive male [9]; (c) partner preference, that is, the preference of a female for a sexually receptive male versus a sexually nonreceptive male [9], and (d) conditioned place preference (produced by allowing paced sexual interaction within a distinctive environment [10]). Examples of anticipatory behavior displayed prior to the arrival of a sexual partner include conditioned level changing in a bilevel chamber [11].

Once the consummatory phase of copulation begins, females engage in proceptive behaviors, such as solicitation, runaway, hopping, and darting, to initiate and pace bouts of copulatory contact that result in lordosis. The manner in which these behaviors are displayed depends to a large extent on the type of testing chamber used to observe copulatory behavior. In the wild [2], and in the large open fields used by McClintock [3], females mate in groups with males. Group mating allows an unambiguous observation of the female's role in the initiation and pacing of copulation. Females orient their forward direction toward a male, then abruptly run away, causing the males to chase them. After a brief chase, the females will stop and assume a pre-lordosis "crouch" or presenting posture, which often becomes a full lordosis when the male palpates the flanks. Some females will hop several times just out of range of the male's mount prior to assuming the crouch. Once the male has mounted, the females may dart a short distance away and then crouch again to accept a second mount by the male. These behaviors are more pronounced if the male achieves vaginal intromission. The pattern of solicitation, runaway, and lordosis define a bout of copulation for the female, and she will pace the number of intromissions she receives prior to ejaculation using the duration or distance of the runaway.

This is not the case in traditional unilevel chambers. The female is typically oriented toward the male much of the time and has no room to run away. She is forced to use defensive behaviors, such as boxing or rearing, to enforce her desired interintromission interval. Often, she receives more intromissions prior to ejaculation than she would in a large open field. Her inability to pace the copulatory contact efficiently allows for only a rudimentary assessment of her sexual motivation (inferred from her display of proceptive and defensive behaviors) and lordosis. It is also difficult to differentiate "true" rejection responses from those defensive behaviors that the female must use to pace the male. One alternative is to test females in chambers with several compartments, some with entry holes small enough to allow her to escape the male. Females can also be tested with males that are tethered to one side of a large

chamber. Both testing situations allow the females to pace more efficiently; however, the former situation requires the use of females small enough to crawl through the entry holes, whereas the latter often results in females that run away and assume pre-lordosis crouches just outside the perimeter of the male's reach.

Another alternative is to use bilevel chambers [11]. These chambers are narrow in width, which maintains an optimal sideways orientation of the animals to the viewer, and consist of two levels connected by a set of ramps on either side. Females pace their copulatory contact with males by running from level to level, which forces the males to chase them. Level changes per mount can be quantified as a measure of pacing, and can be used in conjunction with other measures of proceptivity. These chambers also allow the anticipatory measure of conditioned level changing to be observed prior to the introduction of a sexually receptive male, and thus provide both appetitive and consummatory measures for analysis.

Hormonal Control of Female Sexual Behaviors

The gonadal steroids, estrogen and progesterone, are required for the full expression of sexual behavior in female rodents [7]. In ovariectomized (OVX) rats, estrogen and progesterone regulate receptive and proceptive behaviors in a two-step process, with estrogen promoting lordosis, and progesterone subsequently facilitating lordosis and promoting a range of proceptive and solicitational behaviors. Steroid hormones are believed to regulate these behaviors by binding to intracellular receptors within certain hypothalamic and limbic structures, and inducing protein synthetic changes that lead to long-term changes in neural excitability [12]. In the female rat brain, areas of moderate-to-high ^{3}H-estradiol binding include the olfactory tubercle, lateral septum, suprachiasmatic nucleus, medial preoptic area (mPOA), bed nucleus of the stria terminalis (BNST), anterior hypothalamic area, paraventricular nucleus of the hypothalamus, ventromedial hypothalamus (VMH), arcuate nucleus (ARC), central nucleus of the amygdala, medial amygdala (mAMY), the ventral premammilary nucleus, and the midbrain central grey (MCG) [13]. The application of crystalline estradiol to the ventrolateral region of the VMH in OVX rats promotes lordosis in response to palpation of the flanks [14], and progesterone implants to this region of the VMH are capable of facilitating lordosis in estrogen-primed rats [15]. Blockade of protein synthesis or axonal transport in the VMH during periods of estrogen administration prevents estrogen from promoting lordosis behavior [16]. On the other hand, the facilitatory effect of progesterone on lordosis is relatively rapid, indicating either a direct action on neuronal membranes, ion channels, or an interaction with second messengers [17]. However, the induction of proceptive and solicitational behaviors by progesterone occurs only after several hours, and can be blocked by prior treatment with protein synthesis inhibitors.

Electrophysiological evidence supports the idea that estrogen treatment alters neuronal responsivity to sensory stimulation [7]. Ascending doses of estrogen enlarge the perineal area upon which tactile stimuli will induce lordosis, and similarly increase the number of individual units in the reticular formation that respond to palpation of the flanks. Estrogen also increases the responsiveness ot VMH neurons in slice preparations to stimulation by transmitter substances, such as noradrenaline, which are known to facilitate lordosis following infusion to the VMH [18]. As well, estrogen increases spontaneous excitatory postsynaptic potentials in neurons of the mPOA, VMH, and mAMY [7,19,20]. Thus, steroid hormones are thought to promote receptivity and proceptivity in the female rat by acting on hypothalamic and limbic structures to alter neuronal and behavioral responsiveness to specific sensory stimulation. This

is accomplished by changes in the synthesis and transport of proteins likely to be involved in the neurochemical regulation of these behaviors.

Estrogen treatment also affects partner preference and instrumental behaviors in females. When given a choice between a caged male and a caged female in a runway choice paradigm, females in proestrus or estrus approach the male more often than the female [21]. Treatment of OVX rats with estrogen produces the same effect. Additional treatment with progesterone enhances the preference for a male [22]. In contrast, diestrus females, or OVX, oil-treated females, typically approach the female more often than the male. Treatment of OVX rats with estrogen also promotes operant bar-pressing for a male and increases the amount of aversive stimulation that females will endure to obtain a male [8,9]. Thus, in addition to the regulation of sexual receptivity and proceptivity, estrogen and progesterone promote appetitive behaviors that bring females into contact with males.

Behavioral and Neuroendocrine Consequences of Sexual Stimulation

Whereas estrogen and progesterone can be said to prime neural circuits that mediate sexually receptive and proceptive behaviors, stimulation of the female during copulation also results in short- and long-term behavioral and neuroendocrine changes, nearly all of which are due to vaginocervical stimulation (VCS) produced by multiple penile intromissions by the male. Although it is difficult to isolate the different types of stimulation provided by the male during copulation (including the strength of each intromission), the effects of VCS can be induced and controlled experimentally by direct electrical stimulation of the cervix or by manual insertion of a lubricated glass rod. Studies that have employed these techniques have shown that small amounts of VCS facilitate lordosis, proceptive behaviors, and pacing; moderate amounts facilitate the progestational state required for pregnancy (and pseudopregnancy), and large amounts lead to a faster termination of behavioral estrus.

Copulation with intromission or manual VCS potentiates lordosis elicited in response to palpation of the flanks in estrogen-primed females [23,24]. These effects are prevented by denervation of the genital tract. Manual VCS also potentiates lordosis in OVX rats not treated with estrogen or progesterone [24], and represents the only knbown stimulus other than estrogen to promote lordosis. Paradoxically, lordosis is potentiated by VCS for a longer period of time in OVX, oil-treated rats compared with OVX estrogen-treated rats [24], sugggesting that estrogen may exert an inhibitory influence on the potentiation of lordosis by VCS. The overall period of behavioral estrus, during which an estrogen-primed female remains sexually receptive and proceptive, can be terminated faster in females that receive a large number of intromissions by the male [25]. This is especially true if the intromissions are paced by the female, an action that results in more robust intromissive stimulation by the male [26]. In a variety of species, copulation with intromission or manual VCS also results in decreased locomotion [23], naloxone-reversible analgesia [27], increased sperm transport and fertility [28], and the increased release of pituitary hormones, such as luteinizing hormone (LH), oxytocin, and prolactin [29]. LH is secreted by the anterior pituitary following the action of gonadotropin releasing hormone (GnRH), a neuropeptide synthesized in cell bodies of the anterior POA; thus the LH secretion that occurs following VCS likely results from the increased activity of GnRH-containing neurons. The secretion of oxytocin from the posterior pituitary provokes uterine contractions that aid in the facilitation of sperm transport. The release of prolactin in intact females stimulates ovarian progesterone secretion which prepares the uterus for implantation by the ova. The induction of this progestational state is positively

correlated with the number of paced intromissions received by females during copulation [29], and leads either to the establishment and maintenance of pregnancy, or to pseudopregnancy, a prolonged ceassation of the estrous cycle. Interestingly, in some species such as the ewe, VCS during parturition is critical for the induction of maternal behavior and maternal bonding [30].

It is unclear whether female rats find sexual stimulation rewarding. Although estrogen and progesterone alone facilitate appetitive behaviors, the degree of sexual stimulation during copulation correlates negatively with subsequent appetitive or consummatory sexual behavior. Studies of instrumental responding have shown that fully primed females that receive mounts with intromission are slower to respond subsequently for a male compared with females that receive mounts without intromission [8]. Sexually receptive females who copulate with males that either mount with intromission or without intromission (following the application of lidocaine to the penis) show a preference for the males that mounted without intromission in a subsequent partner preference test [25], although in a similar situation, the speed to reach either male was not affected by prior sexual experience [31]. As noted above, a small number of mounts with intromission or VCS facilitates lordosis, wherease a large number leads to a faster termination of estrous behavior. In certain testing situations, a large number of mounts with intromission or VCS can also lead to an increased display of rejection responses [25]. Thus, it would appear that sexual stimulation, and in particular a large amount of VCS, serves to decrease subsequent appetitive and consummatory sexual motivation.

Neuroanatomical Circuits for Female Sexual Behaviors

The cascade of anticipatory behaviors that lead to proceptive behaviors that result in lordosis can be viewed as an example of the temporal organization of mutually-exclusive behavioral patterns described by Konorski [32]. This is especially true for the organization of consummatory behaviors: lordosis cannot be executed while females are pacing, or vice-versa. Such regulation requires the existence of both facilitatory and inhibitory systems in the brain that control the timing or sequence of these behaviors, and it is likely that different types of sexual stimulation serve to control the onset and offset of these systems. For example, penile intromission by a male rat appears to be a potent sensory signal to the female that turns off lordosis and turns on pacing. In a more general sense, the induction of proceptive and receptive sexual behaviors requires some form of inhibition of the fighting and rejection responses that females will normally display if they are mounted during nonreceptive phases of their estrous cycles, e.g., metestrus and diestrus. An idea considered in 1910 by Steinach [33], and that has appeared from time to time in the literature [e.g., 34], is that hormones may "eroticize" the brain by a process of disinhibition, in which they inhibit the activity of regions that normally suppress sexual behavior. Thus, estrogen may synthesize substances that facilitate female sexual behaviors either by activating excitatory regions or by depressing the activity of inhibitory regions.

Lesion Studies

The effects of electrolytic or excitotoxic lesions to discrete brain regions on female sexual behavior generally support the existence of inhibitory and excitatory systems. Much of this work has been devoted to an understanding of lordosis, although recent studies have begun to examine the effect of lesions on proceptive behaviors. Virtually nothing is known about the neuroanatomical regulation of other appetitive sexual behaviors in the female.

Lesions of the neocortex have been known since the studies of Klüver and Bucy [35] to result in hypersexuality, and this is certainly the case with lordosis [36]. Decorticate estrogen-primed female rats often show an enhanced lordosis reflex, or lordosis in response to inappropriate stimuli, suggesting that the cortex plays an inhibitory role in the expression of lordosis. Similarly, lesions of the lateral septum, POA, or lateral amygdala increase the probability of lordosis [34]. However, such lesions dramatically disrupt the timing of procepetive behaviors. Moreover, the effect of POA lesions appears to depend on the testing situation and the manner in which estrogen is administered. For example, a facilitation of lordosis is almost always observed in situations in which the female cannot pace the copulatory contact. If females are tested in chambers in which they can escape the male, POA lesions do not appear to affect lordosis, although they reduce the total number of intromissions the female accepts during the copulatory interaction [37]. In contrast, POA lesions can suppress lordosis in OVX rats that have had estrogen implants in the VMH [38]. Interestingly, females with POA lesions require less estrogen to induce lordosis [34]. Axon-sparing excitotoxic lesions of the POA inhibit proceptive solicitation in OVX rats primed with estrogen and progesterone [39]. It is thus possible that the POA represents a site of integration between inhibitory and facilitatory systems.

Lesions of the VMH dramatically inhibit lordosis [7], and this site is considered by many to be the prime locus for estrogen's facilitation of lordosis. However, other estrogen-concentrating sites may also be important. Lesions of a number of midbrain and brainstem sites, many of which also concentrate estrogen, such as the MCG, pontine central grey, lateral vestibular nucleus, and medullary reticular formation, also inhibit lordosis [7]. Knife cuts that sever the lateral descending connections between the VMH and pontine central grey also inhibit lordosis [40]. Lesions of other sites, such as the habenula and dorsal hippocampus, seem to regulate the probability of lordosis by reducing the female's responsiveness to hormones [7].

Activation of Brain Regions by Sexual Stimulation

Another way to assess the neural circuitry for female sexual behavior is to examine neural activity electrophysiologically or with metabolic markers such as 2-DG during sexual activity. In estrogen-primed rats, copulation with intromission or manual VCS increases neural excitability and 2-DG uptake within estrogen-concentrating regions of the forebrain, including the mPOA, anterior hypothalamus, and VMH, in addition to brainstem regions such as the dorsal raphé [41,42]. In unanesthetized estrogen-primed rats, increases in neural activity produced by VCS follow different time-courses within different brain structures: some structures, such as the mPOA and lateral hypothalamus, are activated relatively immediately whereas the activation of other structures, such as the anterior hypothalamus and VMH, is relatively delayed [43]. The circuitry involved in the analgesia produced by VCS has been studied in detail by Komisaruk and colleagues [reviewed in 27], and involves the activation of the hypothalamus, MCG, substantia nigra, and brainstem regions such as the raphé nucleus and medullary reticular formation. Neural circuits involved in the induction of pseudopregnancy convey information from the pelvic nerve along direct and indirect pathways. Direct activation of hypothalamic structures likely occurs via the spinohypothalamic pathway [44], whereas the indirect activation involves loops from the ventromedial and lateral parts of the midbrain to the hypothalamus via the dorsal and ventral tegmental tracts [7].

Induction of the immediate-early gene, *c-fos*, has also been used by several groups to identify populations of neurons in different brain regions that are activated by copulation in female rats [45-48]. These studies have found a remarkably consistent pattern of Fos mRNA

and protein induction in the brains of intact or OVX, hormone-primed rats following copulation with intromission or manual VCS. This pattern includes estrogen-concentrating regions of the mPOA, BNST, PVN, ventrolateral VMH, mAMY, in addition to lateral septum, lateral habenula, ventral premammilary nuclei, and midbrain, including the MCG and peripeduncular nuclei. Fos is also induced in regions of the brain that do not readily concentrate estrogen, such as the cortex and striatum. The induction of Fos within many of these regions could be reduced significantly following transection of the pelvic nerve [47], an effect consistent with the interpretation that VCS was the primary stimulus.

Our initial studies on Fos induction [46] were designed to accomplish two goals: first to identify the type(s) of sexual stimulation that induce Fos in these brain regions, and second to use the induction of Fos as a method to examine whether treatment with estrogen and progesterone enhance the cellular response to sensory stimulation. Identical groups of sexually experienced OVX rats received injections of estradiol benzoate 48 hr and progesterone 4 hr, or the oil vehicle, before testing. Females in each hormone group were then either placed into bilevel chambers with sexually vigorous males for 1 hr of copulation, received 50 manual stimulations of the flanks for distributed over 1 hr (to approximate the number of flank clasps that a female normally receives during 1 hr of copulation), received 50 manual VCSs distributed over 1 hr (to approximate the number of penile intromissions that a female normally receives during 1 hr of copulation), or were left in their home cages, to examine the effects of hormone treatment alone. Each flank stimulation and VCS lasted approximately 2 sec, and clusters of 5 stimulations were presented every 6 min. Consistent with previous findings, copulation with intromission in hormone-primed rats induced Fos mRNA and protein within the regions noted above. Manual VCS produced nearly identical effects in these regions, whereas manual flank stimulation produced a much smaller induction of Fos, similar to that observed in oil-treated females that received mounts without intromission during copulation. Hormone treatment alone did not induce Fos, *nor did it enhance the ability of either VCS or flank stimulation to induce Fos*. This was especially obvious in the VMH. Thus, VCS was identified as the prime stimulus for Fos induction during copulation, a finding confirmed by others [48]. However, no evidence was found that hormone treatment altered Fos induction.

The lack of a hormone effect was puzzling, given current interpretations of the ability of hormones, especially estrogen, to augment neuronal sensitivity to stimulation. One possible explanation concerned the use of 50 VCSs, which was designed to approximate the number of penile intromissions received by females during 1 hr of copulation. This amount was clearly higher than the amount necessary to facilitate sexual behavior or to induce pseudopregnancy, and might have produced a ceiling effect on the number of cells activated, thus masking any effect of hormone treatment. This possibility was subsequently examined by comparing the effects of different amounts of VCS [49]. The goal here was to identify the threshold amount of VCS necessary to induce Fos protein within different brain regions (mPOA, BNST, VMH, mAMY, and MCG), and to examine whether hormone treatment altered the thresholds. Identical groups of sexually experienced OVX females were administered estradiol benzoate and progesterone, or the oil vehicle, prior to receiving 0, 1, 5, 10, 20, 30, 40, or 50 VCSs, as presented in the first study. Although the results are still being analyzed, two very important effects have emerged. First, the minimum number of VCSs required to induce Fos varied among the different regions studied, despite the fact that nearly all of these regions receive direct afferent input from the spinohypothalamic tract: A single VCS was capable of inducing Fos within the mAMY, intermediate amounts induced Fos further in the mAMY, mPOA, and BNST, whereas only larger amounts induced Fos within the ventrolateral VMH. Second,

estrogen treatment decreased the threshold for induction of Fos in the mAMY, but *increased* the threshold of induction in the VMH. This latter finding has recently been confirmed using a different procedure of continuous VCS [50]. Given that small amounts of VCS enhance female sexual behavior, moderate amounts induce pseudopregnancy, and large amounts facilitate the termination of behavioral estrus, these results raise the possibility that the effects of VCS may be mediated by different regions of the brain in which Fos has been differentially activated. The ability of hormone treatment to increase the threshold for Fos induction in the VMH also suggested that an inhibitory system exists in this region that may mediate the termination of behavioral estrus. Consistent with this interpretation, infusions of the excitatory amino acid glutamate to the VMH inhibited lordosis behavior [51], an effect that was completely blocked by prior infusions of the local anesthetic procaine.

The picture that emerges from these neuroanatomical analyses suggests strongly that both facilitatory and inhibitory systems exist in the brain to modulate sexual receptivity and proceptivity. Some of these may be negative feedback systems that modulate the activity of several brain regions, whereas others may exist within a single region, e.g., the POA or VMH. Although by no means established, it is likely that similar systems exist to modulate appetitive sexual behaviors in the female.

Neurochemical Correlates of Female Sexual Behavior

Hormones are believed to stimulate sexual behaviors by modulating the activity of different neurotransmitter systems in the brain. The list of drugs and neuropeptides that affect the display of female sexual behavior is enormous, and the reader is directed to several reviews of this work for further information [6,52-54]. An important assumption is that drugs, and in particular receptor agonists, affect behavior in ways that mimic the endogenous activity of the neurotransmitter systems. Conversely, the effects of receptor antagonists, much like lesion effects, are often interpreted as inverse evidence of the role that a particular neurotransmitter system plays in behavior. Although useful conceptually, it is easy to be misled by these assumptions. Drug effects on behavior always beg the question of what the neurotransmitter systems are actually doing, and it is here that the recent application of *in vivo* techniques like brain microdialysis has had an enormous impact. The release of certain neurotransmitters can now be monitored during appetitive and consummatory phases of sexual behavior. It is also possible to detect Fos protein within neurochemically-identified cells following different types of sexual activity or stimulation. This latter technique provides neuroanatomical specificity and can be used to determine the activation of neurochemical systems that cannot be monitored with microdialysis. This section will briefly review the actions of neurotransmitter systems that have been amenable to *in vivo* analyses. Details and specific references are found in [52].

Dopamine

Dopamine plays a critical role in incentive motivation and reward [55,56]. Although its role in appetitive and consummatory aspects of male sexual behavior has been studied extensively, very little comparable work has been done in females. The ability of estrogen to stimulate dopamine (DA) release and to augment DA release and behavior in response to amphetamine is now well-established. Systemic administration of DA agonists can facilitate or inhibit lordosis behavior in OVX rats primed with estrogen and progesterone or estrogen alone. Paradoxically, systemic administration of a range of doses of DA antagonists also facilitates lordosis, although the behavioral signature of the effect is different. Whereas DA agonists

produce a small increase in lordosis quotients, but a large increase in proceptive behaviors, DA antagonists produce a large increase in lordosis quotients but abolish proceptivity. These latter effects have led to the suggestion that DA facilitates the "active" behavioral components of female sexual behavior, that is, proceptivity, but inhibits the "passive" component, that is, lordosis. Alternatively, the effects of DA antagonists could be viewed as decreasing the ability of the female to disengage from lordosis once it is initiated, or to switch between lordosis and proceptive behaviors. Both the inhibitory and facilitatory effects of systemic DA agonists appear to act through D_2 receptors, as D_1 agonists and antagonists do not affect lordosis or proceptivity. However, this may not be the case in discrete brain regions. Few studies have examined the central sites of action of DA in the female rat, and those that have do not provide consistent results. Neurochemical lesions of the mesolimbic DA pathway with 6-OHDA are reported either to facilitate or have no effect on female sexual behaviors [57,58]. The former finding lends support to the idea tha mesolimbic DA release plays an inhibitory role in lordosis, yet recent *in vivo* microdialysis studies reveal that DA release in the nucleus accumbens and dorsal striatum is increased during copulation in female rats and hamsters [59,60]. The VMH may also be a site in which DA 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. Dopamine release is also increased in the VMH during copulation [61]. Interestingly, there may be cross-talk between DA receptors and steroid hormone receptors in the VMH. It has recently been shown that the D_1 agonist SKF-38393 facilitates lordosis in OVX, estrogen-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 progesterone receptor [62]. These data indicate that activating D_1 receptors in the VMH is capable of translocating the progesterone receptor, possibly by altering the phosphorylation of the progesterone receptor or a specific transcription cofactor. Thus, instead of progesterone altering the activity of diencephalic dopamine systems, dopamine in the VMH appears to facilitate lordosis by the indirect activation of progesterone receptors. No studies have yet examined the effect of DA agonists or antagonists in other regions of the brain, or on other forms of appetitive sexual responding.

Noradrenaline

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 noradrenaline transmission. Although systemic treatment with α or β 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 noradrenaline concentrations in the VMH [61]. The role of norepinephrine in other forms of appetitive sexual responding in females has not been studied.

Oxytocin and GnRH

Oxytocin and its receptor are regulated in different brain regions during the estrous cycle, an effect that is likely due to changes in estrogen and progesterone secretion. Numbers of oxytocin cells and levels of oxytocin receptor mRNA are stimulated by estrogen and enhanced by progesterone. In fact, within the VMH, estrogen stimulates oxytocin receptor transcription and progesterone quickly modifies this effect by spreading the functional receptor populations laterally to meet the incoming oxytocin innervation. This suggests that estrogen and progesterone act to synchronize the availability of endogenous oxytocin with its receptor. Lordosis behavior is facilitated dramatically following systemic and icv administration of oxytocin, and oxytocin receptors in the mPOA and VMH appear to facilitate the frequency and duration of lordosis, respectively. Oxytocin infusions to the VMH of OVX, estrogen-primed prarie voles reduced rates of aggression and increased the amount of physical contact that the females made with males, although it also led to a faster termination of estrus. Copulation with intromission induces Fos within oxytocin neurons of the paraventricular hypothalamus, but not the supraoptic nucleus [63], suggesting that endogenous oxytocin systems are activated during copulation and may participate in the facilitation of lordosis following intromission. Interestingly, masturbation to orgasm increases plasma oxytocin levels in human females. Nothing is known about the possible effects of oxytocin on appetitive sexual behaviors.

GnRH also produces a dramatic facilitation of lordosis behavior in OVX, estrogen-primed rats following systemic or venrticular administration. Infusions of GnRH into the anterior POA, ARC, or MCG also facilitate lordosis in estrogen-primed rats, whereas infusions of a GnRH antibody to the MCG reduce lordosis quotients. Copulation with intromission, or manual VCS induces Fos within GnRH neurons of the anterior POA [64], indicating that these neurons are activated during copulation. Interestingly, estrogen enhances the proportion of GnRH cells that express Fos following VCS, possibly through a long-term noradrenergic activation. It is not known whether GnRH affects appetitive sexual behaviors. Taken together, these results suggest that the activation of oxytocin and GnRH neurons may form part of the system that facilitates lordosis following VCS.

New Vistas

The study of female sexual behavior is at a crossroads. Years of research have brought converging operations to bear on the hormonal, neuroanatomical, and neurochemical systems that underlie lordosis behavior and other forms of female receptivity. Facilitatory and inhibitory systems exist in the brain, and their neurochemical and molecular bases are being elucidated. Despite this wealth of knowledge, relatively little is known about the behavioral neurobiology of proceptive behaviors, especially pacing, and even less about anticipatory or preparatory sexual behaviors in females. The focus on lordosis has made it difficult to relate findings in animals, especially female rats, to the sexual behavior of human females, much less to place female sexual behavior into a more general incentive motivational context. Behavioral techniques exist that allow a range of appetitive sexual behaviors to be examined in females. These techniques must be exploited, as they have been in males, to gain a full understanding of the neural basis of sexual motivation in the female. To the extent that homologous appetitive behaviors exist in humans (e.g., psychological measures of sexual "desire" that are distinct from physiological measures of sexual "arousal", as emphasized in the DSM-IV), then accurate predictions made from the sophisticated use of appetitive measures in female rats will come to have important clinical relevance, especially concerning drug effects.

It is also imperative to move into the realm of hypothesis testing, and techniques designed to assess neurochemical activation *in vivo* will become vitally important tools in this endeavor. Brain microdialysis and voltammetry, immunocytochemistry, and *in situ* hybridization histochemistry can all be used to assess neurochemical changes during, or as a result of, particular phases of sexual behavior or different types of sexual stimulation. As more and more substances become amenable to analysis by HPLC at short temporal intervals (e.g., a minute or less), the microdialysis will become all the more useful. Part of the problem in establishing a functional role for different neurochemical systems also stems from a lack of selective tools with which to manipulate them, especially receptor antagonists. Use of antisense technology is likely to be important in this regard, as the synthesis of specific proteins can be targetted.

Finally, although not reviewed in this paper, far more research needs to be conducted into the biological and psychological basis of sexual function and dysfunction in human females. Questions regarding the types of stimuli that elicit subjective sexual desire and vaginal blood flow, how their perception is altered in different sexual dysfunctions, and how hormones, especially androgens, drugs, or clinical interventions affect such processes, need to be addressed. It may also be possible to identify brain regions activated in humans by sexually-arousing stimuli, using positron emission tomography or functional magnetic resonance imaging. The activation of structures in the human brain that are homologous to those activated in the rat by sexual stimulation would have important implications for the evolution of sexual motivation and performance. Sex differences in neural activation may also exist. However, the pattern of Fos induction within the brains of female rats following VCS is nearly identical to that following intromission and ejaculation in male rats (i.e., following penile stimulation) [65], suggesting a high degree of similarity in the neural response of female and male rats to genital stimulation. Clearly, there is much that has been learned from the study of animal sexual behavior, but still more that begs to be discovered.

References

1 Beach FA. Horm Behav 1976; 7: 105-138.
2 Calhoun JB. The ecology and sociology of the Norway rat. Washington DC: US Govt Printing Office, 1962.
3 McClintock MK. Adv Stud Behav 1984; 14: 2-50.
4 Madlafousek J, Hliňák Z. Behav 1978; 63: 129-173.
5 Erskine MS. Horm Behav 1989; 23: 473-502.
6 Pfaff DW, Schwartz-Giblin S. In: Knobil E, Neill J, eds. The physiology of reproduction. New York: Raven, 1988; 1487-1568.
7 Pfaff DW. Estrogens and brain function. New York: Springer, 1980
8 Bermant G. Science 1961; 133: 1771-1773.
9 Meyerson BJ, Lindström LH. Acta Physiol Scand Suppl 1973; 389: 1-80.
10 Oldenburger WP, Everitt BJ, de Jonge FH. Horm Behav 1992; 26: 214-228.
11 Pfaus JG, Mendelson SD, Phillips AG. Psychoneuroendocrinol 1990; 15: 329-340.
12 Pfaff DW, McEwen BS. Science 1983; 219: 808-814.
13 Pfaff DW, Keiner MJ Comp Neurol 1973; 151: 121-158.
14 Rubin BS, Barfield RJ. Endocrinol 1980; 106: 504-509.
15 Rubin BS, Barfield RJ. Neuroendocrinol 1983; 37: 218-224.
16 Meisel RL, Pfaff DW. Brain Res Bull 1984; 12: 187-193.
17 Schumacher M. Trends in Neurosci 1990; 13: 359-362.
18 Kow,L-M, Pfaff DW. Brain Res Bull 1988; 20: 857-861.

19 Moss RL, Dudley CA. In: DeVries GJ et al, eds. Sex differences in the brain. New York, Elsevier, 1984; 3-22.
20 Schiss MC, Joels M, Shinnick-Gallagher P. Brain Res 1988; 440: 380-385.
21 Eliasson M, Meyerson BJ. Physiol Behav 1975; 14: 705-710.
22 Edwards DA, Pfeifle JK. Physiol Behav 1983; 30: 437-443.
23 Komisaruk BR, Diakow C. Endocrinol 1973; 93: 548-557.
24 Rodriguez-Sierra JF, Crowley WR, Komisaruk BR. J Comp Physiol Psychol 1975; 89: 79-85.
25 Hardy DF, DeBold JF. J Comp Physiol Psychol 1972; 78: 400-408.
26 Erskine MS, Kornberg E, Cherry JA. Physiol Behav 1989; 45: 33-39.
27 Komisaruk BR, Steinman JL. Ann NY Acad Sci 1986; 474: 64-75.
28 Adler NT, Toner JP. Ann NY Acad Sci 1986; 474: 21-32.
29 Terkel J. Ann NY Acad Sci 1986; 474: 76-94.
30 Kendrick KM, Levy F, Keverne EB. Physiol Behav 1991; 50: 595-600.
31 Bolles RC, Rapp HM, White GC. J Comp Physiol Psychol 1968; 65: 311-313.
32 Konorski J. Integrative activity of the brain. Chicago, Chicago University Press, 1967.
33 Steinach E. Zentralblatt Physiologie 1910; 24: 551-570.
34 Clemens LG. In: McGill TE et al, eds. Sex and behavior: Status and prospectus. New York, Plenum, 1978; 243-266.
35 Klüver H, Bucy PC. Am J Physiol 1937; 119: 352-353.
36 Beach FA. Psychol Rev 1967; 47: 289-316.
37 Whitney JF. Behav Neurosci 1986; 100: 230-235.
38 Bast, JD, Hunts C, Renner KJ, et al. Brain Res Bull 1987; 18: 153-158.
39 Hoshina Y, Takeo T, Nakano K, et al. Behav Brain Res 1994; 61: 197-204.
40 Henessey AC, Camak L, Gordon F, Edwards DA. Behav Neurosci 1990; 104: 477-488.
41 Allen TO, Adler NT, Greenberg JH, Reivich M. Science 1981; 211: 1070-1072.
42 Blake CA, Sawyer CH. Neuroendocrinol 1972; 10: 358-370.
43 Dafney N, Terkel J. Neuroendocrinol 1990; 51: 459-467.
44 Burstein R, Cliffer KD, Giesler GJ. J Comp Neurol 1990; 291: 329-344.
45 Dudley CA, Rajendren G, Moss RL. Mol Cell Neurosci 1992; 3: 360-369.
46 Pfaus JG, Kleopoulos SP, Mobbs CV, Gibbs RB, Pfaff DW. Brain Res 1993; 624: 253-267.
47 Rowe DW, Erskine MS. Brain Res 621: 25-34.
48 Tetel MJ, Getzinger MJ, Blaustein JD. J Neuroendocrinol 1993; 5: 397-404.
49 Pfaus JG, Marcangione C, Abillamaa H. Soc Neurosci Abst 1994; 20: 591.
50 Tetel MJ, Getzinger MJ, Blaustein JD. Brain Res 1994; 646: 267-272.
51 Kow L-M, Harlan RE, Shivers BD, Pfaff DW. Brain Res 1985; 341: 26-34.
52 Pfaus JG, Everitt BJ. In: Bloom FE, Kupfer DJ, eds. Psychopharmacology: The fourth generation of progress. New York, Raven, 1994; in press.
53 Pfaus JG, Gorzalka BB. Neurosci Biobehav Rev 1987; 11: 1-34.
54 Mendelson SD. Neurosci Biobehav Rev 1992; 16: 309-350.
55 Blackburn JR, Pfaus JG, Phillips AG. Prog Neurobiol 1992; 39: 247-279.
56 Wise RA, Rompré P-P. Ann Rev Psychol 1989; 40: 191-225.
57 Robbins TW, Everitt BJ. Int Rev Neurobiol 1982; 23: 303-365.
58 Hansen S, Harthon C, Wallin E, Löfberg L, Svensson K. Behav Neurosci 1991; 105: 588-598.
59 Meisel RL, Camp DM, Robinson TE. Behav Brain Res 1993; 55: 151-157.
60 Mermelstein PG, Becker JB. Submitted.
61 Vathy I, Etgen AM. J Neuroendocrinol 1989; 1: 383-388.
62 Mani SK, Allen JMC, Clark JH, Blaustein JD, O'Malley BW. Science 1994; 265: 1246-1249.
63 Flanagan LM, Pfaus JG, Pfaff DW, McEwen BS. Neuroendocrinol 1993; 58: 352-358.
64 Pfaus JG, Jakob A, Kleopoulos SP, Gibbs RB, Pfaff DW. Neuroendocrinol 1994; 60: 283-290.
65 Baum MJ, Everitt BJ. Neurosci 1992; 50: 627-646.

Discussion - NEURAL MECHANISMS OF SEXUAL MOTIVATION AND PERFORMANCE IN FEMALES

G. Wagner

You know the old observation that giving testosterone to just-born female rats will impair their cyclicity. Have you tried that as a model?

J.G. Pfaus

No, I have not used that as a model. We know, for example, that females who have not been treated neonatally with androgens but who are subsequently treated with androgens in adulthood will show a preference for females that they have mounted. But we know very little about these neurochemical responses in androgenized females, for example when they are mounting males. It could very well be the case that we can get a male-like neurochemical signature out of them, that required androgenization and sexual differentiation of their brain. That, of course, would be a very interesting study to do because it may very well be that the androgenization is, in fact, sculpting the brain in such a way that these patterns are able to be emitted.

J. Herbert

You might like to raise the issue of the use of c-fos in these studies because it has come up in two papers and as you pointed it out, it may well form one of the newer methods of assessing the psychopharmacology of sexual behaviour. Many of you use c-fos and I think it is important that we should begin to discuss the ground rules of what it is showing us. I think the first thing is that it is not showing us anything to do with ongoing sexual behaviour. I will tell you why I think that, and it is very simple: because of the temporal relationship between sexual behaviour and the expression of c-fos. The increased transcription of mRNA of c-fos takes 15 to 30 minutes; the increased translation of the Fos protein takes up to an hour. So c-fos can not be reflecting ongoing sexual behaviour, but maybe it is a footprint or a marker of preceding sexual behaviour, or even of a general central stimulation.

J.G. Pfaus

I think that you are absolutely right. The thing we need to do, specially when we are looking at fos as a marker, is to realize that while some of the areas in which it is induced may be facilitatory for sexual behaviour, some may in fact be inhibitory. Take the VMN. We have now found that when c-fos is induced there is an oestrogenic effect, but the effect of oestrogens is not to enhance but actually to retard its induction. We find that c-fos is induced within cells that concentrate oestrogen and progesterone, but also within cells that co-localize glutamate. It turns out that if one injects glutamate into the VMN, one inhibits lordosis. The other thing is that we only find it in the VMN after a large number of stimulations. It could very well be the case that what we are marking with this is an inhibitory system within the VMN, which is going to come along hours later to help to terminate oestrus. With c-fos we are marking a signal transduction event, but not necessarily the events that we have watched over the course of the hour.

J. Herbert

Another aspect that intrigues many of us is that the pattern of c-fos induction, with the possible exception of the VMN, is extremely similar in the male and the female, is also similar in maternal behaviour and indeed in paternal behaviour. This intrigues me because clearly not only the behavioural expression but also the incentive situation are very different and yet the c-fos is very similar. Will you comment on that?

J.G. Pfaus

I can only wish that someone will finally publish a PET study or a functional MRI study of human sexual arousal in males and females and see where the similarities and differences are. I really wonder if things are identical or if there are subtle differences. There are clear differences in Fos induction between males and females, but they are subtle. There are clearly differences in Fos induction between maternal or paternal behaviour and copulatory behaviour. The steroid-concentrated regions light up but when you ask the animal a conditioned question you can see, just on that basis, other regions of the brain lighting up. Again, I think we need to apply more sophisticated behavioural analysis to these types of studies and more sophisticated molecular

analysis to get a handle on whether these subtleties are real.

J. Bancroft

At the beginning of your talk, you alluded to the conflict between pacing and being still for lordosis, as if they were incompatible mechanisms. I was wondering whether that is implying a complexity or mechanism in the female which is uniquely female. Is there any male counterpart of that sort of conflict?

J.G. Pfaus

Yes, I think there probably is. After a male rat intromits, instead of staying coupled with the female, he will dismount and show general grooming. I think that the stimulation he is getting from the penis is a signal to the brain to say dismount, just as I think the stimulation that the female rat gets from the intromission is a signal that says "run away, pace him".

J. Bancroft

How species specific is that?

J.G. Pfaus

That is quite species specific. There are other rodents, for example, that stay coupled. If we look at a different male behaviour, say with ejaculation and with dopamine release in the accumbens, there is a precipitated drop in dopamine which then follows through the absolute refractory phase and begins to upswing during the relative refractory phase, and that upswing always precedes the reinitiation of copulation. That would be a case of a system that, after ejaculation, is turning off the active goal-directed behaviour of the male.

B.D. Sachs

I would like to comment on the differences between rats and hamsters. The hamster female has a rather different copulatory behaviour, she shows far less hopping and darting than the female rat and can maintain lordosis for many minutes. I wonder whether there are data that would characterize the physiological difference that

mediates the difference in proceptive behaviour between the two species.

J.G. Pfaus

An interesting point is that before showing that longer period of sexual receptivity, female hamsters are fighting males and fending off. It will be very interesting to see what happens to dopamine release under those circumstances. It may increase in both conditions, we just do not know. It would also be interesting to study to what extent does the behaviour that she shows play a role in enticing the male to make these repetitive copulatory advances towards her.

J. Herbert

An important issue is the relationship between noradrenaline and dopamine and indeed serotonin. To what degree can we begin to assign relative roles for these three amines in sexual behaviour or indeed in any behaviour? For example, several people have showed that dopamine is released in great parts of the brain during sexual behaviour. Is the same thing true for noradrenaline and serotonin? and does it occur under the same circumstances? Can we begin to draw up any picture of the contribution of these three systems to sexual behaviour?

J. Bancroft

Indeed. We have an extraordinary wealth of information about dopamine in particular and ongoing information in recent years about serotonin, but rather little attention has been paid to noradrenaline.

M. Mas

The traditional view in pharmacological manipulations of behaviour is that when one increases dopaminergic transmission there is an increase in motivational aspects of behaviour, sexual or otherwise. Increasing serotonergic activity in general leads to some sort of inhibition of feeding or mating.

J.G. Pfaus

I think that it is absolutely critical to use "in vivo" methods. In the examination

of dopamine antagonists in male sexual behaviour we obviously can knockout appetitive measures at doses that do not touch copulation whatsoever, that do not even touch the initiation of mounting, that is the latency to mount. On that basis one could predict that dopamine is necessary during the appetitive phase but probably not that necessary during the consummatory phase. And yet we see exactly the opposite happen in our dialysis samples: we can see more dopamine over a continuous period elicited in response to copulation than we ever see during the appetitive phase, say when a female is behind a screen. I think that sometimes we can be teased by our drug findings to talk about function in a way that really begs the question what is the system really doing?

J.T. Clark

An important issue here is that dopamine is probably the easiest to measure so people measure it. Does that mean that it is the most important neurotransmitter in sexual function? Maybe the thing that is the hardest to measure is the most important. Perhaps there is some peptide changing within the preoptic area that needs to be studied more effectively. The whole issue of enhancing sexual function using angiotensin antagonists, or somatostatin antagonists, or NPY antagonists when they become available will show us where to look. I think the issue of peptides just has to come to sexology.

B.J. Everitt

Concerning the role of noradrenaline, it should be remembered that there are relatively few noradrenergic neurons in the brain. They are in the brain stem; they have diffuse projections that branch repeatedly so you can have for example a neuron in the locus ceruleus which can innervate diencephalic structures, frontal cortex, spinal cord, cerebellum; the same neuron with its branching axon. So the kind of information processing that is subserved by such a system is unlikely to be one of highly spatially and temporally coded sensory events. We actually know quite a lot about the noradrenergic system and when it is active. Noradrenergic neurons in the brain stem are particulary interested in novel stimuli and unpredictable stimuli. Stressors and generally aversive events activate the noradrenaline system. We can then go to

terminal domains of these systems and ask what the consequence of that activation is, and we can see very clearly that if, for example, one looks at the processing of somatosensory stimuli in the somatosensory cortex, the way the cortex handles those stimuli is improved in efficiency; the stimuli become more visible by changing the relationship between signal and noise in those processing domains. The same activation of the locus ceruleus will have the same effect if the stimuli impinging on the animal are visual or auditory, so this simple change in the noradrenergic system enhances information processing in all of the cortical domains that receive that noradrenaline innervation. Specificity of effect will come depending upon what is impinging on the animal at that particular point in time; so it is an interaction between the specific sensory system that relays through the thalamus to the cortex and the diffuse noradrenergic projection system that in some places will coincide. In a very exciting series of experiments published in the 70's, Aston Jones and Bloom showed that if you measure at the response to an auditory tone in the hippocampus, activation of the noradrenergic system potentiates the decrease in firing, but if you pair that tone with food so that it now becomes a conditioned appetitive stimulus that signals to the animal that the food is available, two things happen: first of all, the response in the hippocampus is now excitation and not inhibition and, secondly, coincident activation of the noradrenaline system potentiates that response as well. So we can see that the very modulatory nature of the effect of this diffuse system can be quite specific in effect. I think one of the real challenges ahead of us is actually when we move out of the structures like the cortex and into domains like the hypothalamus, where the nature of the information processing that goes on there and the impact of the diffuse projection systems to these subcortical sites is actually unknown. In justifying my comment I would like to know what else happens to animals and people when they are being treated with yohimbine and I bet that it is not just changes in sexual responsiveness!