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Review

Reverse engineering the lordosis behavior circuit

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ABSTRACT

Reverse engineering takes the facts we know about a device or a process and reasons backwards to infer the principles underlying the structure–function relations. The goal of this review is to apply this approach to a well-studied hormone-controlled behavior, namely the reproductive stance of female rodents, lordosis. We first provide a brief overview on the considerable amount of progress in the analysis of female reproductive behavior. Then, we propose an analysis of the mechanisms of this behavior from a reverse-engineering perspective with the goal of generating novel hypotheses about the properties of the circuitry elements. In particular, the previously proposed neuronal circuit modules, feedback signals, and genomic mechanisms are considered to make predictions in this manner. The lordosis behavior itself appears to proceed ballistically once initiated, but negative and positive hormonal feedback relations are evident in its endocrine controls. Both rapid membrane-initiated and slow genomic hormone effects contribute to the behavior's control. We propose that the value of the reverse-engineering approach is based on its ability to provide testable, mechanistic hypotheses that do not emerge from either traditional evolutionary or simple reductionistic perspectives, and several are proposed in this review. These novel hypotheses may generalize to brain functions beyond female reproductive behavior. In this way, the reverse-engineering perspective can further develop our conceptual frameworks for behavioral and systems neuroscience.

Contents

Introduction

Developing explanations of mammalian behaviors with the highest level of mechanistic accuracy and conceptual depth has required choosing behavioral topics that are simple and straightforward enough that serious progress toward understanding mechanisms can be made. A useful example among mammalian behaviors is the simple mating behavior typical of female quadrupeds, lordosis behavior (Pfaff, 1999). Several factors have led to rapid progress in explaining mechanisms for steroid hormone dependent sex behaviors. Steroid sex hormones, being small rigid molecules, have lent themselves to receptor binding studies and other analyses using all the tools of biochemical endocrinology and steroid pharmacology. Steroid hormone receptors, being transcription factors, have allowed neurobiologists to use molecular endocrine techniques to advantage. Moreover, the relatively simple inducing stimuli and stereotyped responses that comprise rodent mating behavior have facilitated neurophysiological analysis and reliable data collection. The fact that

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many animals perform complete mating behaviors naturally, without lengthy learning protocols, in the laboratory has also helped. All of these factors have permitted scientists studying hormones and behavior to apply biophysics and molecular biology to the understanding of behavioral mechanisms. Currently, this understanding ranges from our knowledge of the lipophilic pits that constitute the ligand binding domains of steroid receptors, measured in angstroms, to the seasonality of reproduction, detected by animals as the yearly variation in photoperiod. As a result, work on lordosis has been prominent both in neurophysiological and molecular genetic explorations into the mechanisms of complete, natural mammalian behaviors.

Within this field of work, extensive quantitative and mechanistic data have been gathered with respect to the control of lordosis behavior. The present review proposes an engineering perspective to the understanding of this behavior. Reverse engineering is an approach newly applied to systems biology in which the scientist looks at the finished product and forms hypotheses about the functionality of the components and the relations between components. The finished product is usually a process that is being dynamically controlled. For example, the reverse-engineering approach has been proposed as a way to understand biological complexity (Csete and Doyle, 2002), especially the complexities of tissue-specific gene expression (de Magalhaes and Toussaint, 2004; Grigorov and van Bladeren, 2007; Schadt and Yum, 2006). In our case, the final product is the suite of physiological and behavioral changes that occur during the sexually receptive period as they relate to lordosis. This approach has not been systematically applied to mechanisms by which the brain controls behavior. The facts reviewed in this paper have been published before; this review provides a new way of gleaning insights from that knowledge.

A value of the reverse-engineering process is that it forces us to assess neurobehavioral mechanisms according to how those mechanisms may highlight previously unappreciated constraints based on neural circuitry, social behavior, and/or the timing of neurohormonal action. It may be contrasted in some ways with an evolutionary approach, which may speculate about selective pressures, generating hypotheses that are difficult to test. It is also different than simple reductionistic approaches aimed at divining proximate mediators of behavior. Both evolutionary and reductionistic approaches have provided numerous insights into the biological basis of female mating behavior, and our understanding of this neurobehavioral system may be complemented with the reverse-engineering approach. In fact, reverse engineering is only applicable in a rigorous fashion when there is a firmly established body of knowledge from which to generalize about mechanistic functions. This requirement limits the scope of this conceptual review; the lordosis response is a heavily studied behavior with many published findings. Some associated changes in brain function, such as cognition and reward, may be outside the scope of this paper. This is not to say that our knowledge of lordosis is complete; however, it has reached a level sufficient for making functional inferences. Reverse engineering can be thought of as a particularly demanding stage of systems biology analysis. The goal of this review is to surmise, for the first time with a mammalian behavior, and with as much precision and detail as possible, the organizing principles manifest in the lordosis circuitry.

The neurobiological system of lordosis behavior includes many of the common features that are the focus of reverse-engineering analyses. For example, both technological and biological complex systems routinely depend on modularity. Modules are component parts that interface with other modules, and protocols are the rules that manage the relations between the modules. In complex systems, these protocols become layered, involving feed back signaling. Reverse engineering often considers that complex systems face a trade-off between robustness and complexity. These elements of reverse-engineering analysis can be readily translated to current thinking about the lordosis system.

The sections below will consider several aspects of lordosis behavior, namely the hierarchical neural network, behavioral feedback mechanisms, and the time course of neurohormonal action. For each of these aspects of lordosis behavior we will consider how the reverse-engineering perspective may lead to new predictions. The utility of the reverse-engineering approach can be judged by its ability to provide testable, mechanistic hypotheses that have not emerged from a simple reductionistic perspective. In addition, these novel hypotheses may relate to brain function beyond the scope of female sexual behavior.

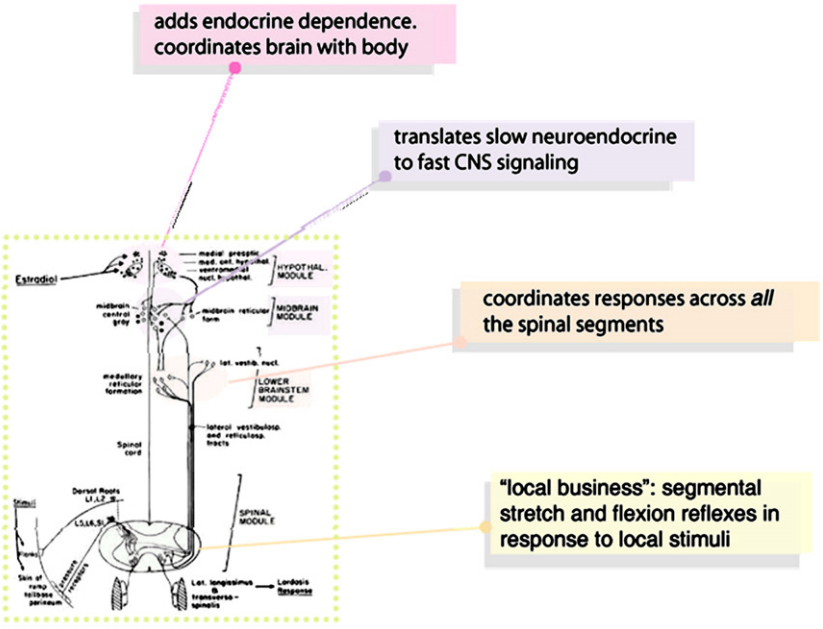

Fig. 1. The neural circuit for producing lordosis behavior. The circuit is modular. Some of the design functions of individual modules are shown. Adapted from (Pfaff, 1980).

Module analysis of the lordosis circuit

Initial investigations of the lordosis response started with the identification of the brain sites mediating estrogen influences and of the sensory modalities for triggering the behavior, leading to the recognition of the neural circuit that mediates the behavior. Neurophysiological and molecular analyses of this circuit proved that specific biochemical reactions in specific nerve cell groups in the mammalian brain govern a specific behavior.

Taking the perspective of evolutionary and comparative biology, a modular organization was conceptualized for this circuitry (Fig. 1; (Pfaff, 1980, 1999)). Modules, in this context, are defined based on neuroanatomical and neurophysiological data revealing a high degree of connectivity within a module compared to a lower degree between modules, especially modules that are not adjacent. With this framework, we have carried out refined electrophysiological, neuropharmacological, neuroanatomical, and molecular investigations. Before we apply a reverse-engineering analysis to the extensive database on the modular control of lordosis behavior, it will be reviewed with an emphasis on the hypothalamic module (Fig. 1).

The lowest module is the *spinal module,* composed of the relevant spinal segments, each of which deals with 'local business': stretch and flexion reflexes in response to local, segmental stimuli within one dermatome. Considering the cellular complexity of the deep dorsal horn of the spinal cord, the central grey and the interneurons providing information to motoneurons, the intricate calculations of spinal motoneurons justify segment-by-segment organization. Nevertheless, integration across spinal segments will be required if the animal is to remain upright and coordinated. This transsegmental level of integration of the motor output, therefore, is the job of the *hindbrain module*, including premotor units in the descending reticular formation.

The *midbrain module* bridges the *hindbrain module* to the *hypothalamic module*. The *hypothalamic module*'s main job is to provide endocrine regulation. Hormonal controls over lordosis behavior keep neural dynamics in tune with reproductive status. As a result, a major function of the hypothalamic module is to ensure that the brain's control over female sexual behavior is in concert with the preparations of the rest of the body for pregnancy. In turn, since endocrine dynamics are precisely regulated by the season of the year in lower mammals, behavioral responses by the female to the male are consequently kept in touch with the environment. Along those lines, another fitness-optimizing requirement met by hypothalamic neurons is to integrate endocrine changes with the animal's nutritional status and with circadian signals. It is important to note that endocrine dynamics are extremely slow; typically, hormonal changes take place over days. Interestingly, VMH neurons that relay to the *midbrain module* comprise a largely discrete subpopulation separate from the estrogen-sensing neurons that express the genes for estrogen receptors (Daniels and Flanagan-Cato, 2000).

In the hypothalamic module, estrogenic hormones work through two gene products, estrogen receptor-alpha and estrogen receptor-beta, to affect numerous genes (Fig. 2). These estrogen-responsive genes have two properties: estrogens elevate their mRNA levels, and their gene products foster female sex behaviors. One line of reasoning follows: (i) Estrogens turn on these genes; (ii) their gene products foster lordosis behavior; (iii) therefore, these obviously comprise an important causal route by which estrogens drive lordosis behavior. These genes are not all similar to each other. They include a transcription factor, the progesterone receptor, neuronal growth-related genes, as well as genes coding for neurotransmitter receptors, neuropeptides and neuropeptide receptors. We have proposed, theoretically, that they can be thought of as governing functional genomic networks that act in parallel and thus help to ensure reliability of the system as noted in the Introduction (Mong and Pfaff, 2004). Such functional networks act in concert for the following parallel processes across several brain regions: greater CNS arousal; remodeling in the

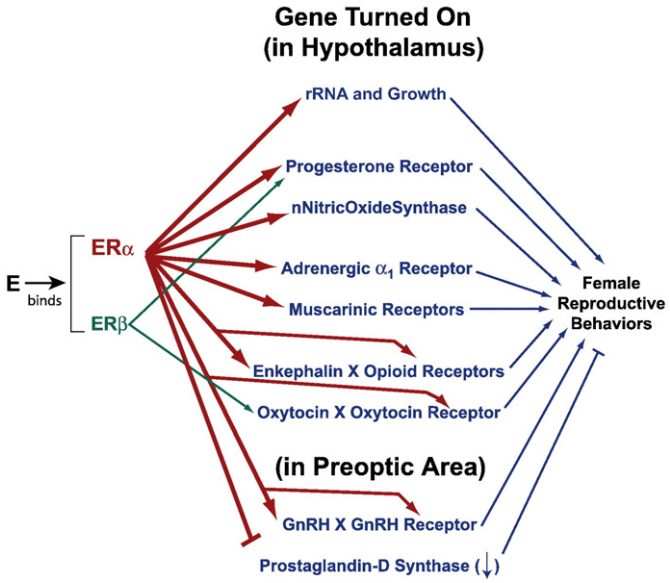

Fig. 2. Genes whose transcript levels are affected by estrogens when expressed in hypothalamic or preoptic neurons. Their products facilitate (except for prostaglandin D synthase) lordosis and related behaviors. Adapted from (Pfaff, 1999).

lordosis circuit; more efficient social recognition; partial analgesia; reduction of anxiety; amplification of other lordosis-producing molecular signals; and synchronization between ovulation and lordosis.

We note that the modules charted in Fig. 1, all those responsible for producing lordosis behavior, match major embryological divisions of the nervous system. The one module, also matching an embryological division, but not pictured in Fig. 1 is the telencephalic module. The forebrain module is not required for *producing* lordosis behavior. Instead, the experimental data indicate that it is useful for *inhibiting* lordosis behavior. While the exact biological interpretation of this fact is not perfectly understood, we hypothesize that its inhibition of a mating behavior would become important under circumstances in which reproduction would not be appropriate. One concrete example of this would be if outputs from the amygdala, signaling fear, indicated that engaging in behaviors leading to reproduction would expose the animal to dangerous environmental situations. Likewise, cognitive processes may inhibit reproduction, such as memories of a dangerous context, involving contextual memory processing.

Reverse-engineering perspective

Several properties of the hierarchical organization of the lordosis circuit have attracted our attention from a reverse-engineering perspective. Here we will describe several testable, novel hypotheses that do not emerge from either traditional evolutionary or simple reductionistic thinking.

1) We hypothesize that an important job of the midbrain module is to mesh extremely slow hypothalamically-mediated endocrine dynamics, measured in hours to days, with the rapid dynamics in the motor control systems of the hindbrain module, measured in milliseconds. This demands integration across time domains. This novel hypothesis predicts that recordings in awake behaving animals would reveal that midbrain neurons are phasically active in correspondence to behavioral elements of reproduction, but hypothalamic neurons, which may show hormone-induced changes in their baseline activity, would not exhibit a firing pattern that correlated with specific behaviors. Alternatively, the role of the midbrain as temporal integrator may be demonstrated by the modulation of midbrain responses to sensory input as a consequence of ongoing hypothalamic activity.

2) We also hypothesize that the midbrain module acts as a switch, permitting lordosis while at the same time suppressing competing responses that otherwise would interfere with the execution of lordosis behavior. One obvious example would be the role of the periaqueductal grey in causing analgesia. The suppression of pain responses would prevent them from competing with the initiation of lordosis. Likewise, the midbrain is also important for defensive reactions and anxiety. We propose that the midbrain suppresses defensive behavior and anxiety towards males while promoting lordosis behavior. To test this hypothesis, one could determine whether hypothalamic treatments that alter lordosis will have reciprocal effects on analgesia and defensive behaviors. We predict that PAG treatments, unlike hypothalamic treatments, may be able to dissociate effects on lordosis, analgesia and defensive behaviors.
3) The permissive signal sent by the VMH to the midbrain is essential for timing the onset and duration of the period during which lordosis may be activated. This period of time is but a portion of the total time during which ovarian steroids are elevated. From an engineering perspective, this precisely timed signal is potentially useful for modulating other behaviors such as exploratory, foraging, feeding and drinking behaviors that might compete with or enhance reproductive behaviors but that do not need to be regulated on a second scale that might be transduced in the midbrain. Thus, we hypothesize that the VMH modulates the circuits regulating these other activities during the entire lordosis responsive period in a manner distinct from any other time. Simply evaluating the amount of time spent performing other behaviors before, during, and after the receptive period would address this hypothesis, especially if the period of receptivity was abbreviated in some cases via any of several methods, for example, progesterone administration or vaginal–cervical stimulation. Experiments in which the VMH is stimulated or inhibited prior to, during, and after the period during which lordosis can be induced, with concomitant evaluation of behaviors in addition to lordosis, would test this hypothesis more rigorously. While the effects of steroids on various behaviors have been studied quite intensively, surprisingly an effect limited to the window of time during receptivity has not been a focus of much attention.
4) We are led by the reverse-engineering perspective to consider the organizing principle leading to the synaptic arrangement of VMH neurons. In particular, we have noted two largely discrete subpopulations of VMH neurons, namely neurons that send axonal projections to the *midbrain module* and estrogen receptor-containing neurons (Calizo and Flanagan-Cato, 2003; Daniels and Flanagan-Cato, 2000). An alternative arrangement could be imagined in which the projection neurons also expressed estrogen receptors. A difference between these two arrangements is that in the observed case, estrogen actions must be exerted on the projection neurons through synaptic activity, whereas in the hypothetical alternative scenario estrogen actions would include genomic actions directly in the midbrain-projecting neurons that would affect the neuron function more globally. Thus, we hypothesize that the observed arrangement segregating estrogen receptor-containing neurons from the midbrain-projecting neurons allows the effects of estrogen to be compartmentalized on the dendritic arbor of the projection neurons. To test this novel hypothesis, it will be necessary to map the synaptic contacts from the estrogen receptor-containing neurons onto the midbrain-projecting neurons. In terms of physiology, we predict that activation of the estrogen receptor-containing neurons will have complex, rather than uniform, interactions with other inputs to the midbrain-projecting neurons.

This arrangement also raises the question of which neuron type, the estrogen receptor-containing neurons versus the midbrain-projecting neurons, serve as the coincidence detectors to integrate the necessary hormonal signals with permissive nutritional, stress and reward signals, thus determining the initiation and the termination of lordosis behavior. Lordosis can only proceed if many conditions are met, including the appropriate hormonal levels, optimal time of day, sufficient energy stores, low stress, and a safe location. If several of these facilitating signals impinged directly on projection neurons, the projection neurons might act as OR gates, with lordosis being permitted whenever one or another of the several environmental, nutritional and social conditions were met. Because lordosis behavior does not occur in the absence of steroid priming, we hypothesize that the steroid-sensitive neurons act as AND gates where all these relevant cues filter through the estrogen receptor-containing neurons positioned upstream of the projection neurons. In this way, a signal may be relayed to the projection neurons, permitting lordosis, only when all of the multiple conditions, including appropriate hormone levels, are met simultaneously. Importantly, this sequential arrangement also may foster reproductive behavior to be expressed as an all-or-none occurrence, rather than as a graded response. To test this novel hypothesis, it will be necessary to determine whether extranuclear afferents, carrying information regarding cues such as circadian phase, metabolic state, and safety cues preferentially innervate the estrogen receptor-containing neurons. Likewise, the major target of estrogen receptor-containing neurons' axons should be the projection neurons.

5) A general engineering question regarding the VMH-midbrain component of this network is whether this pathway is mainly excitatory or disinhibitory. Evidence in favor of disinhibition include the finding that glutamate agonists act in the VMH to inhibit the lordosis response (Georgescu and Pfaus, 2006), although it is not clear how the pharmacological treatments affected the projection neurons versus the local circuitry. In addition, although mating behavior is associated with genomic activation in the VMH (Flanagan et al., 1993; Pfaus et al., 1993), the number of VMH neurons activated by copulation is surprisingly few. This activation may reflect sensory feedback, rather than an activation that drives the behavior. However, lesion studies appear to be at odds with the notion that the VMH normally inhibits lordosis (Pfaff and Sakuma, 1979). We predict that the VMH-midbrain projections may be mixed. Such an arrangement would provide the tightest possible control of this crucial behavior. We also predict that a mixed projection would allow the VMH to exert separate controls over different functional domains in the midbrain. This hypothesis could be tested with a combination of VMH electrical stimulation during recordings in the PAG and neurochemical identification of PAG projecting VMH neurons.
6) The functional genomic network causing structural changes in hypothalamic neurons may not simply widen the lanes of communication through the hypothalamic module, such as by increasing the number of neurons recruited into the circuit. Rather, genomic responses may reconfigure subroutines within the hypothalamic module by dendritic remodeling (Calizo and Flanagan-Cato, 2000; Calizo and Flanagan-Cato, 2002; Frankfurt et al., 1990; Frankfurt and McEwen, 1991) and synaptic changes (Carrer and Aoki, 1982; Chung et al., 1988), as well as changes in the protein synthesis capacity of the neuron (Cohen and Pfaff, 1981). The reorganization of the hypothalamic module for reproduction is likely to include robust veto mechanisms, as in Hypothesis #4, to guarantee robust behavior but only at appropriate times.

Feedback and feedforward systems in lordosis behavior

Once contacted by the male, the female rat's lordosis behavior proceeds in a ballistic fashion, or not at all, depending on the state of the circuit. In less than 50 ms following cutaneous stimuli from the male, the female has begun the vertebral dorsiflexion that constitutes

lordosis behavior (Pfaff and Lewis, 1974). Reflecting on this rapid response, as a simple postural change, lordosis does not require the *negative feedback* guidance that more intricate responses involving visuo-motor coordination, for example, might need. We note that while many motivated behaviors, such as feeding, drinking, salt hunger and temperature control, are essentially homeostatic in nature, mating behaviors need not be so. The performance criterion of reproductive systems is not to hold a critical variable (such as bodyweight or body temperature) constant, but rather to achieve fertilization.

In rats, normal mating behavior includes several episodes of the female displaying lordosis while the male mounts and intromits before he ejaculates. The female actively controls the timing of these intervening intervals to regulate her own neuroendocrine systems that promote pregnancy (Pfaus et al., 1999). In particular, the female alternately avoids then solicits the male's approaches. Unlike the individual lordotic occurrences, the pacing of the receptive responses throughout a mating episode may involve positive and negative feedback cues to optimize the timing of the male's ejaculation (see below). These mechanisms may relate to the control of the female's proceptive approaches to the male, rather than her receptive response specifically. Considering the discussion above, the *midbrain module*, based on its predicted ability to select sets of behaviors, may control the switch between approach and escape motor responses.

Quite separate from the initiation of lordosis behavior, the termination of the time interval during which lordosis can be performed may indeed be subject to negative feedback. The accumulation of mating cues may lead to estrus termination. The signal may include vaginal–cervical stimulation, which in part produces progesterone release (Adler, 1974; Blandau et al., 1941; Pfaus and Heeb, 1997). Recent work has suggested mating-induced changes in spine density in the VMH (Flanagan-Cato et al., 2006), and perhaps mating induced immediate early gene expression is a component of such negative feedback.

Positive feedback systems exhibit the characteristic that slow or small changes can lead to crossing of a threshold and subsequent large and sudden changes in function. Positive feedback relations participate in the endocrine system connected to lordosis behavior, namely in individual neurons and in the hypothalamic/pituitary axis. Thus, for a brief period during estrous and menstrual cycles, high and rising levels of estrogens, supplemented by progesterone cause a sudden higher and faster release of luteinizing hormone releasing hormone (LHRH, also referred to as GnRH) (Levine and Ramirez, 1980) and a surge in the release of luteinizing hormone that in turn causes ovulation. Signals involved in this positive feedback system, including progesterone, synchronize lordosis behavior with ovulation. As this synchronization is critical there may be additional synchronizing processes (see Kinetics below).

In the pre-lordosis proceptive behaviors of female rodents there is ample evidence of *feed-forward* relations that are sex hormone-dependent. For example, if a female rat has high levels of estrogen, supplemented by progesterone, she will, in the presence of a male, exhibit an extremely rapid darting movement of a few feet and then suddenly come skidding to a halt (Fig. 3). The male, if he has high levels of testosterone, will follow the female; under the influence of testosterone he shows a marked preference for her hormone-dependent odors. When she comes skidding to a sudden stop, he contacts her. As charted in Fig. 3B, the female's pre-lordosis behavior displays at least four feed-forward functions: (i.) When the female suddenly halts her forward and downward force, she is braced in the very position that will be needed to support the weight of the male. For example, some stop-action shots of this behavior have shown a 225 g female supporting the entire weight of a 500 g male. (ii.) The muscular tension exhibited by the female itself fosters subsequent lordosis behavior. (iii.) Because the male is approaching the female from the rear, he approaches her in the orientation for proper

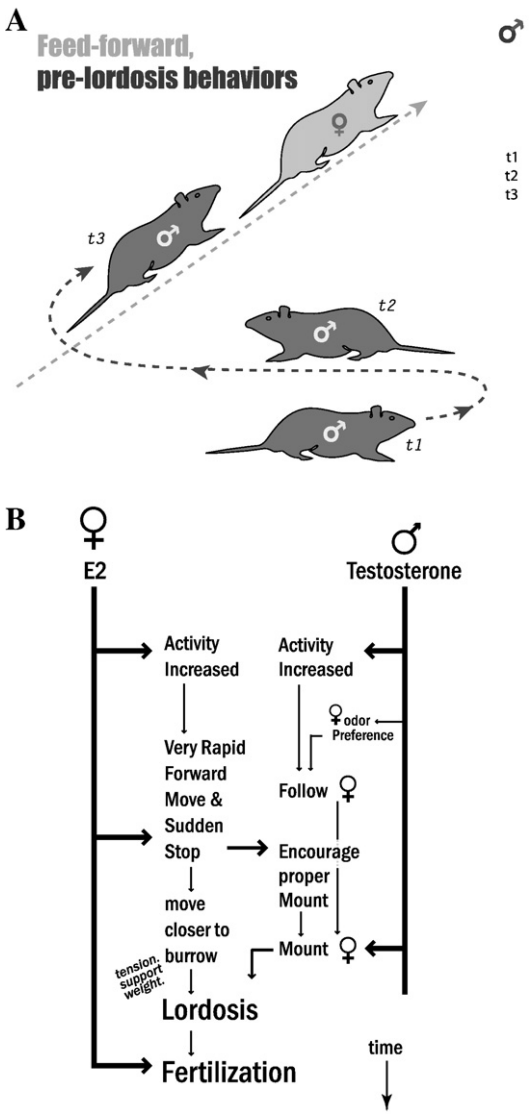

Fig. 3. Panel A. A sketch of rapid prelordotic movements by the female rat that encourage subsequent lordosis and cause the male to mount in the correct position. In this sketch, the female darts rapidly from lower left to upper right. At time 1 (*t*1), the male was going elsewhere but then (*t*2), attracted by the female's darting, he turns toward her. Finally (*t*3), he is following the female so that when she stops suddenly, he will be in the proper position for mounting. Panel B: Feed-forward dynamics in rat mating behavior.

mounting behavior. (iv.) The female's locomotor movements, as described, between mounts have the effect of pacing a series of mounts and intromissions in a manner that has been shown to increase the probability of successful fertilization (Coopersmith and Erskine, 1994).

In the mating behaviors of hamsters, similar feed-forward relations serve to bring reproductively competent males and females closer in space at the correct time (Fig. 4A). If the female has high levels of estrogens, she deposits hormone-dependent scent marks, especially if she smells pheromones from the male. Likewise, the male scent marks with his testosterone-dependent flank gland, especially if he smells odors from the female. As she moves toward her burrow and he proceeds up the pheromone gradient toward her burrow, the positive feed-forward of mutually stimulated ultrasounds come into play. Finally, in the burrow, cutaneous stimulation by the male on the hindquarters of the female causes her to enter a prolonged lordosis posture. Thus, the initial hormone-dependent behaviors of each sex increase the likelihood that the animals will encounter another set of stimuli that promote the next round of behaviors (Fig. 4B), creating a

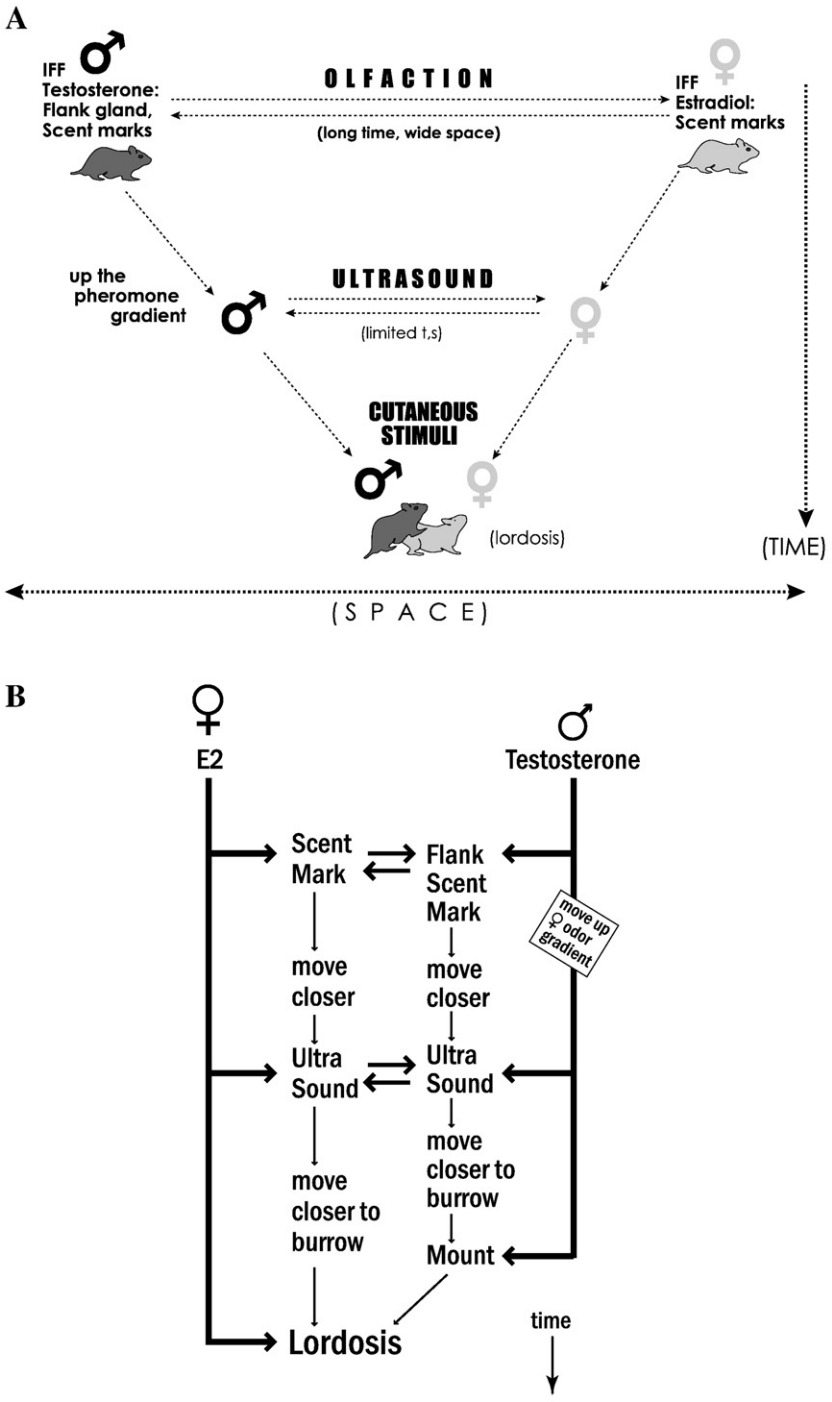

Fig. 4. A. Estrogen-dependent signals in the female hamster and androgen-dependent signals in the male hamster serve to bring the reproductively competent animals together in time and space. B. Feed-forward dynamics in hamster mating behavior.

positive feed-forward series of events that culminate in bringing the animals together to mate in the female's burrow (Floody and Pfaff, 1977; Pfaff and Lewis, 1977).

Reverse-engineering perspective

Several properties of the feedback and feed-forward mechanisms of the lordosis circuit can be considered from a reverse-engineering perspective. Here we will describe several testable, novel hypotheses that do not necessarily emerge from either evolutionary or simple reductionistic thinking.

7) We hypothesize that the activation in the *hypothalamic module*, perhaps in the VMH, during mating mediates the negative feedback leading to estrus termination. In keeping with our Hypothesis #2, this would allow the simultaneous reinstatement of defensive behaviors as lordosis responses wane, through hypothalamic control of the midbrain module. Data consistent with this hypothesis have been reported (Georgescu and Pfaus, 2006; Pfaus et al., 1999).
8) The positive and negative feed-back and feed-forward mechanisms that control the expression of lordosis occur in varying time windows. As a corollary to our Hypothesis #1, we propose that

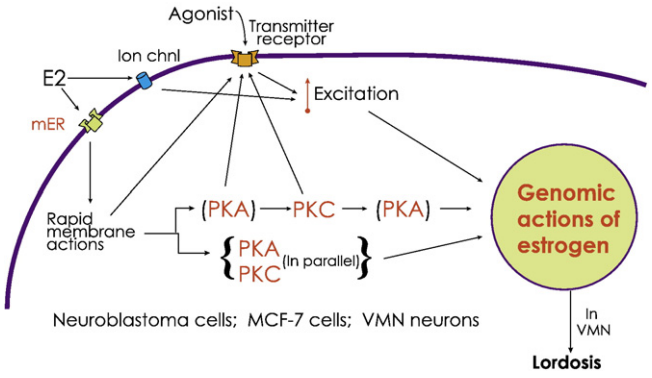

Fig. 5. Both rapid membrane-initiated and slower genomic mechanisms are facilitated by estrogens in such a manner as to facilitate lordosis behavior.

acute feedback processes may be controlled within the midbrain module, where as longer lasting feedback, such as estrus termination, may be processed in the hypothalamic module. Therefore, blocking neural activity, as with a GABA agonist such as muscimol, may disrupt pacing behaviors when administered to the midbrain, but may delay estrus termination when administered to the VMH.

Kinetics of hormone action

A wide range of time constants governs both the molecular mechanisms of sex hormone actions in cells and the controls over lordosis behavior (Vasudevan et al., 2005). In many behavioral experiments, animals are treated with two pulses of estradiol treatment to mimic the chronic exposure that occurs in intact females. In terms of cellular mechanisms, lordosis behavior depends on slow transcriptional steps in the cell nucleus (Fig. 2), preceded by fast, membrane-initiated estrogenic actions (Fig. 5). This evidence has been gathered during transient transfection assays in neuroblastoma cells (Vasudevan et al., 2001), in MCF-7 breast cancer cells (Devidze et al., 2005) and during electrophysiological studies (Kow et al., 2006, 2005) and behavioral analyses (Kow and Pfaff, 2004) of ventromedial hypothalamic cells at the top of the lordosis circuit.

In the transient transfection experiments carried out with neuroblastoma cells, brief pulsatile applications of estrogens were used. A first pulse of estradiol tethered to bovine serum albumin and limited to membrane actions could potentiate the transcriptional response to a second pulse of estradiol allowed to enter the nucleus (Vasudevan et al., 2005; Vasudevan and Pfaff, 2007). In the electrophysiological experiments carried out with slices of ventromedial hypothalamus, estradiol increased the responses to applied neurotransmitters within minutes, far faster than would be required for transcriptional events. Finally, in behavioral experiments carried out with cannulae directed into ventromedial hypothalamus, a pulse of estradiol tethered to BSA potentiated the lordosis behavioral response to a later pulse of estradiol that can enter the nuclei of hypothalamic neurons. These experiments at the molecular, the electrophysiological and behavioral levels lead to the concept of physiologically important interactions between membrane-initiated and slower genomic mechanisms of estrogen action in the context of lordosis.

These data alerted us to the need for systematic quantitative thinking about how two phases of hormone action would be integrated, starting with membrane-initiated hormone effects followed by slower, genomic effects on estrogen receptor-mediated modifications of transcription. Therefore, the following equation was conceived and written in collaboration with Professor Parameswaran Nair, Department of Physics, CCNY:

$$\Delta(t) = F[t, t2, \Delta\mathrm{ERn}, g(t2, t1, \Delta\mathrm{ERm})]$$

where Δt is the change in transcription rate at a defined time after the second phase of hormone administration and ΔERm is the change in output at time $t1$ that is a consequence of estrogen action at the membrane in the first phase. The effect of this change on the system at the later time $t2$ is given by some function g which depends on the signal ΔERm and evolves between the times $t1$ and $t2$. It may be interpreted as the change in the basal state at time $t2$ in response to the signal at time $t1$. The second phase, given by ΔERn, is the change due to the estrogen action in the cell nucleus. The final response of the system is thus given by the function F, which depends on the times involved, the second phase ΔERn and on g. In terms of the relation between the two phases, we know that reversing the order of membrane-initiated and genomic does not work, that is, there is no facilitation of transcription. In future work, we will get data relevant to this equation, for example by systematic variations in the intervals between $t1$ and $t2$; and the interval between $t2$ and t.

Reverse-engineering perspective

Several properties of the kinetics of hormone action within the lordosis circuit can be considered from a reverse-engineering perspective. Several testable, novel hypotheses are proposed.

9) As discussed above, controls over lordosis behavior cover a very large range of time scales, from milliseconds (for the female's response to contact by the male), to minutes (the pacing of mounting and intromission episodes) through hours (for estrus termination) to months (for seasonal control of reproduction). Both rapid membrane-initiated and slow genomic effects of estrogens promote lordosis (Kow and Pfaff, 2004). How these separate neural mechanisms might contribute to the system's performance requires further experimentation to decipher. We hypothesized above that the lordosis behavior circuit is partially comprised of a very slow permissive system in the hypothalamic module, and this may involve the genomic mechanisms involved in estrogenic hormone action, as in Fig. 2. This module is coupled with a more rapid-response module in the midbrain, which may be importantly modulated by membrane-mediated estradiol effects. This hypothesis does not discount possible membrane effects in the hypothalamus, but directs us to test for such effects in the midbrain.
10) Based on the equation proposed above, we predict an interaction between the membrane effects and transcriptional effects of estradiol, providing a new site for the positive modulation of the lordosis circuit. This would be analogous to the positive feed-forward systems discussed above, in which one event (e.g., hop darts) makes a subsequent event (e.g., male–female contact) more probable and thus fertilization more likely. To test this hypothesis, it will be necessary to employ reagents that would selectively prevent the membrane-evoked signaling actions of estradiol and then examine whether the genomic sequelae of estradiol can promote normal sexual behavior.
11) Knowing the shape of the function g encourages one to apply other information regarding the membrane delimited actions of steroids in this neural network. We propose that membrane-initiated actions of estradiol may potentiate genomic actions by either increasing the excitability of VMH neurons, altering second messenger pathways, or both (Kow et al., 2006; Kow and Pfaff, 2004). Considering the interesting observation that estrogen failed to induce lordosis when administered while the rat was anesthetized (Roy et al., 1985), we hypothesize that membrane-initiated actions may potentiate the transcriptional actions by increasing neuronal excitation of VMH. This hypothesis can be tested by administration of the first (to evoke membrane actions) of the two-pulse estrogen application during anesthesia to prevent neuronal excitation. Alternatively, potassium channel

blockers can be used instead of the first estradiol pulse with anesthesia because acutely administered estrogen increased neuronal excitation largely by inhibiting potassium channels (Kow et al., 2006). Under such conditions, one could assess the effects of estradiol on gene expression and behavior.

The membrane-initiated actions of estradiol can also activate a wide variety of signaling systems. Protein kinase A (PKA) and protein kinase C (PKC) are involved in the facilitation of genomic action by membrane-initiated actions (Devidze et al., 2005; Vasudevan et al., 2001). These findings are consistent with the observation that intra-VMH application of PKC activator potentiated estrogen induction of lordosis (Kow et al., 1994). We therefore propose that such membrane-initiated actions facilitate genomic action by activating signaling systems involving PKA and PKC. This hypothesis predicts that both kinds of estrogen action would occur in the same neuron. This can be tested by identifying ER-containing neurons in VMH using virus transfection method (Musatov et al., 2006) and then using the patch clamp technique to determine whether they respond to acutely applied estrogens.

Summary and outlook

This project has focused our attention on some features of the lordosis behavior circuitry as a first attempt at a reverse-engineering approach. Eleven new specific and testable hypotheses have been developed regarding the modular design of the lordosis behavior circuit and how it pertains to feedback systems and the control of the behavior across various time domains.

It was necessary to have picked a behavior simple enough for extensive mechanistic analysis. As a result, we can explain lordosis behavior at several levels of analysis, having used neuroanatomical, neuroendocrine, neurophysiological and genomic tools. Having shown that this area of neurobiology is amenable to systematic experimentation, we have taken a reverse-engineering approach to generate new ideas about brain function. Devising tests for these novel hypotheses will challenge us to refine our knowledge of this biologically critical behavior.

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