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Hormonal and genetic influences on arousal – sexual and otherwise

Donald Pfaff, Jonathan Frohlich and Maria Morgan

Genetic influences on lordosis, a mammalian social behavior, are amenable for study because of the relative simplicity of both stimuli and response. The neural circuit for lordosis involves a supraspinal loop, which is controlled by an estrogen- and progesterone-dependent signal from the medial hypothalamus and results in heightened sexual motivation. In turn, this involves elevated states of arousal, defined by increased sensory alertness, motor activity and emotional reactivity. Mice in which the gene encoding the α form of the estrogen receptor (ERα) has been knocked out show that ERα is crucial for lordosis behavior. Comparing ERα-, ERβ- and double knockouts reveals that different patterns of sexual behaviors in mice require different patterns of ER activity. Understanding how hormonal and genetic effects on deep motivational and arousal processes contribute to their effects on specific sexual and aggressive behaviors pose significant challenges for mouse functional genomics.

A large body of reliable neurobiological results has been enabled by the analysis of hormonal and genetic influences on lordosis, a simple reproductive behavior. Lordosis is the vertebral dorsiflexion performed by female quadrupeds in response to adequate stimuli from a reproductively competent male. Biologically it is important because it permits fertilization and, therefore, reproduction. Strategically it is well chosen for analysis because it depends on the activity of estrogenic hormones facilitated by progestins. Therefore it serves as a virtual expression system for the actions of these steroid hormones and research in this field has been enhanced and accelerated by the tools of steroid chemistry and biochemical endocrinology. Furthermore, the behavior involves simple responses that are triggered by simple stimuli. All are manageable in the laboratory and, crucially, all are relatively easy to study. Because of these advantages the hormonal, neural and genetic determinants of lordosis have been reported in detail [1].

In addition to the spinal circuitry required, there is an obligatory supraspinal loop that brings

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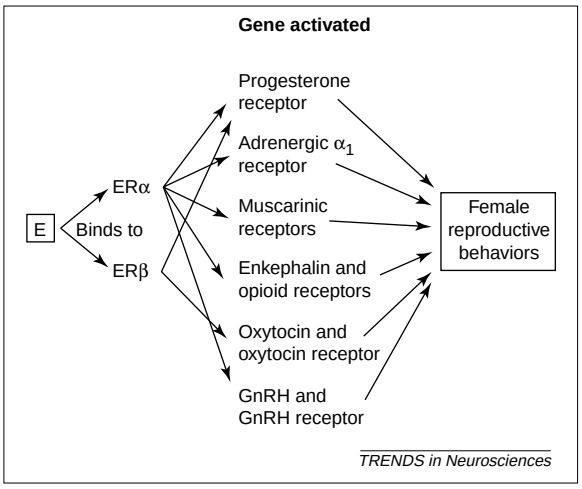

Fig. 1. Ovarian estrogens (E) diffuse from the bloodstream into the brain and through neural tissue by virtue of their lipid solubility. They bind to the classical estrogen receptor (ERα) and to ERβ in specific subsets of neurons. Acting as transcription factors, ligand-activated ERs facilitate transcription of several genes whose products foster female reproductive behaviors in rats and mice. The routes of action of the 'downstream' genes listed are partially understood: the ligand-dependent transcriptional effects of the progesterone receptor increase expression of genes that lead to the amplification of the effects of E; stimulation of adrenergic α-1 receptors and muscarinic receptors by norepinephrine and acetylcholine, respectively, can elevate electrical activity in VMH neurons that have crucial effects on behavior; enkephalin, an opioid peptide, can foster a state of partial analgesia, allowing the female to tolerate stimuli from the male; oxytocin can act as an anxiolytic, permitting sex under conditions of mild stress; gonadotropin releasing hormone (GnRH, also known as LHRH) helps, adaptively, to synchronize mating behavior with ovulation. Note that for neurochemical systems in which E activates transcription of genes for both ligand and the corresponding receptor, there is the possibility of a multiplicative effect on sex behavior. Abbreviation: VMH, ventromedial nucleus of the hypothalamus.

somatosensory stimuli involved in this reproductive behavior to the medullary reticular formation and the midbrain central gray. This circuit, which governs female reproductive behavior, is dependent on the interactions of estrogen and progestins with ER and progesterone receptors within hypothalamic neurons. Analysis of the behavioral functions at the molecular level depends upon the activity of these nuclear hormone receptors as transcription factors. Thus estrogenic effects on hypothalamic neurons that govern female reproductive behavior requires the synthesis of mRNA and protein and the molecular actions of estrogens and progestins in the hypothalamus and basal forebrain guarantee a biologically adaptive synchrony between reproductive behavior and the pituitary–ovarian mechanisms that cause ovulation.

Hormones turning on genes

Which genes are upregulated by estrogens in hypothalamic neurons? Molecular assays over the past 15 years have identified a number of genes whose mRNA is elevated by estradiol administration and which encode proteins that participate in fostering female reproductive behaviors (Fig. 1). How these genes are involved is only partly understood (see legend to Fig. 1). They seem to make separate and individual contributions towards the emergence of a unified mating behavior, although the manner of their orchestration as a function of time remains unknown.

Genes turned on by estrogens include those encoding oxytocin and the oxytocin receptor, and the opioid peptide enkephalin and the delta opioid receptor [1]. Do the ligand/receptor inductions multiply? Although the known biological functions of these proteins and peptides make sense as reproductive-behavior mechanisms, the list of genes cannot be considered complete.

Genes turning on behaviors

Mice in which the genes encoding the two forms of the ER (ERα [2] and ERβ [3]) have been disrupted allow the determination of behaviors controlled by each receptor. The use of gene knockouts to dissect the contributions of individual genes to this hormone dependent circuitry and behavior began with ERα [4], which is required for lordosis behavior in female mice. In fact, female mice that lack ERα function not only behave more like males but are treated as males in social encounters [4]. In contrast, female mice that lack ERβ activity perform lordosis behavior during a larger portion of the estrus cycle than their wild-type littermate controls [5].

While studying the fungus *Neurospora*, Beadle and Tatum discovered mutants with biochemical defects that led to the classical 'one gene one enzyme' hypothesis [6,7], a concept that modern neurobiologists seem to be struggling to step beyond. A wide variety of behavioral and histochemical assays using ERα-, ERβ- and double-knockout mice have addressed the question of which patterns of gene expression are required for which patterns of mammalian behaviors. To date, assays in male and female mice have identified a set of ERα-mediated functions, with some evidence that ERβ stimulation can oppose that of ERα. Furthermore, data from double knockout mice indicate that different patterns of mouse sexual behaviors depend on different patterns of ERα- and ERβ activity [8].

Can this platform of neurobiological information be used as a springboard for studies exploring the motivational and arousal functions that comprise the theoretical underpinnings of reproductive behaviors?

The temporal sequence of events

By necessity, the temporal order of experimental discovery goes from the explanation of concrete, simple mating behaviors, to approach and courtship behaviors in which sexual motivation is expressed, to more generalized arousal functions. However, in females the temporal sequence is: first, the hormone-dependent elevation of arousal; second, the expression of approach and courtship ('proceptive') behaviors; which lead naturally to, third, the mating behaviors themselves.

Donald Pfaff*
Jonathan Frohlich
Maria Morgan
Laboratory of Neurobiology and Behavior, The Rockefeller University, Box 275, 1230 York Avenue, New York, NY10021-6399, USA.
*e-mail: pfaff@rockvax.rockefeller.edu

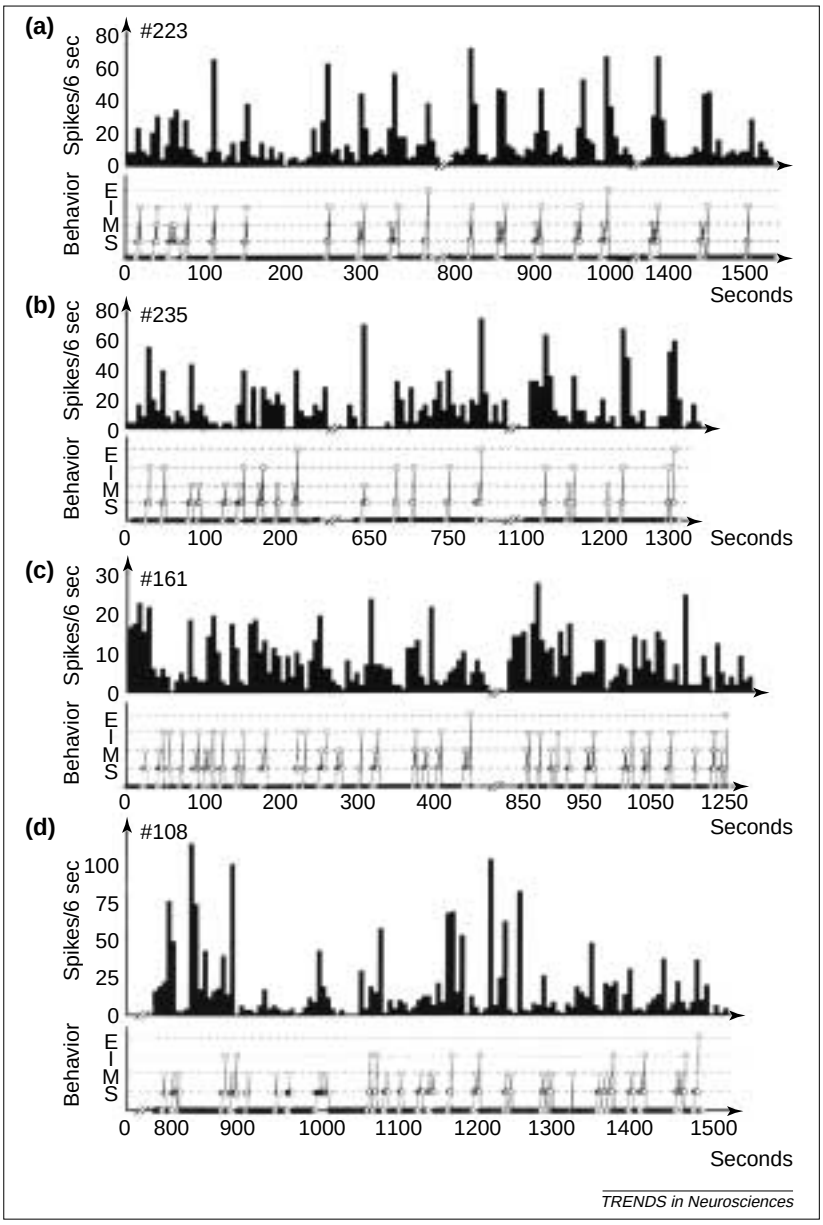

Fig. 2. Temporal relationship between activity of preoptic neurons and sexual behavior in four females (a–d). The electrical activity of 14 of 31 preoptic neurons correlated temporally with bouts of sexual interactions. Presented here are four types of neurons (Types 1–4), whose activity was associated with proceptive behavior (Type 1; S), mounting by the male (Type 2; M), intromission by the male (Type 3; I) and the dismount of the male (Type 4). Abbreviations: E, ejaculation; I, intromission; M, mounting; S, solicitation. Figure reproduced, with permission, from [18].

This review starts by considering the ability of a hormone to increase the expression of a well-defined behavior when all other variables are constant, so proving an underlying hormone-dependent change in motivation. Motivational mechanisms are discussed briefly here. In addition, experiments on motivation indicate a central role for arousal in providing the motivational force that activates behaviors. Therefore new insights on the hormonal and genetic contributions to arousal mechanisms are highlighted. What it does not attempt is a broad review of mammalian gene–behavior relationships. Instead, we hope to deepen consideration of behavioral–functional genomics [9] in mice by building on the especially clear cases identified by analysis of genes encoding nuclear hormone receptors [4,10]. Other areas of progress include the contributions made by regions of the Y chromosome to masculine aggressive behaviors in mice [11,12] and a wide range of affiliative behaviors that are facilitated by transfer of the gene encoding the vasopressin receptor into the basal forebrain of voles [13] (also reviewed in Ref. [9]).

Motivational mechanisms

The existence of motivational states comprising alterations in specific areas of the CNS is supported by decades of experimental analysis of behavior. Prominent in this is the writing of Donald Hebb [14] who incorporated the ascending reticular activating system into his theory of motivation. However, the tools for delving into cell-biological mechanisms are often lacking from early behavioral analyses.

Heightened motivational states, which reflect obvious biological needs, can account for the activation of specific behaviors. Using the experimental tools provided by our knowledge of the hormonal, neural and genetic mechanisms that underlie lordosis, this simple reproductive behavior can be used to elucidate neural concepts deeper than simply the behavior itself. The ability of estrogens to heighten performance of a reproductive behavior elicited by a fixed stimulus in a constant environment, with no changes in age or time of day, indicates an underlying change in biological motivation.

As a result of estrogen treatment amplified by subsequent progesterone treatment, female rodents perform a variety of courtship behaviors accompanied by a vigorous locomotion. The advantages of specific endocrine and genetic tools that have allowed the determination of lordosis-behavior mechanisms can, likewise, be used to further the analysis of sexual motivation. For example, neurons in the preoptic area control the forms of locomotion involved in courtship because local implantation of estradiol significantly increases in running activity [15,16]. To determine the electrophysiological basis of this motivational circuitry, Yasuo Sakuma and colleagues have manipulated neurons of the medial preoptic area to affect locomotion and recorded from this area [17,18]. Impressively, the electrical activity recorded in individual preoptic neurons correlates with locomotive behavior [18] (Fig. 2). These results in rats, as well as similar studies in birds [19], indicate that neuronal alterations related to motivation are not limited to the mechanisms of the simplest sex behaviors themselves.

Brain arousal and behavior

When considering motivational mechanisms, a great deal of attention has been given to explaining the activation of a behavior in an otherwise quiescent animal. Inevitably, experiments along these lines involve observations and manipulations

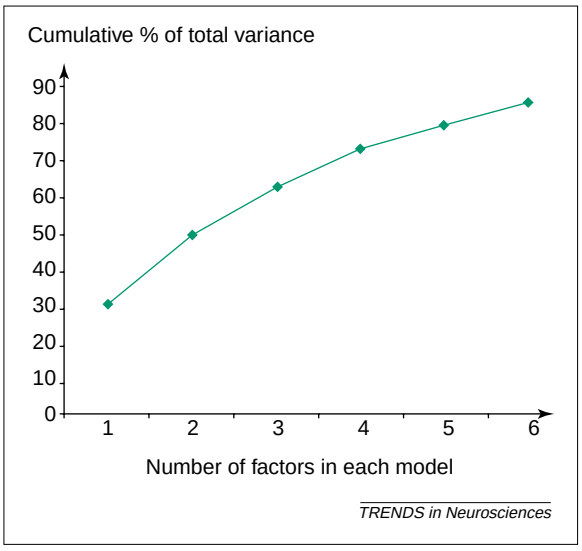

Fig. 3. Sensory alertness, motor activity and emotional reactivity were determined in 48 ovariectomized female mice using a battery of assays. This data was used to form a table of cross–mouse correlations across assays that were then subjected to factor analysis and cluster analysis. Plotted here is the % variance as a function of the number of factors calculated. The 'one-factor' solution shows the % variance accounted for by 'general arousal'. In this, and additional identical experiments, generalized arousal accounted for 29–38% of the behavioral variance. Additional, specific factors raise the total behavior accounted for to 80–90% in this and subsequent experiments. Figure reproduced, with permission, from [21].

of the brainstem ascending reticular systems that underlie arousal.

There are two problems to be overcome when studying arousal. First, from the analysis of human behavior it is obvious that fundamental arousal of the brain is required for any higher cognitive or emotional function to occur. However, in mice, the apparent difficulty in arriving at intuitive definitions of arousal in behavioral studies has caused neurobiologists to discount this type of study. Below, we propose an operational definition that could overcome this difficulty. Second, some experimenters treat arousal as a monolithic function, which totally serves to activate forebrain circuits and motor responses, whereas other researchers argue that arousal does not exist as such, because they view it as subdivided neurochemically, neurophysiologically and functionally. In the following, a thoroughly quantitative approach is taken that is based in neurobiology and avoids extreme categorizations. Initial hormonal and genetic data related to the physiology of arousal is also introduced.

Defining arousal

From both a neuroanatomical and a behavioral perspective, arousal systems form such an important part of mammalian brain activity that a clear definition is required. We consider it best to use an objective, operational definition, in which an animal that is more aroused has: (1) a greater alertness to sensory stimuli; (2) greater motor activity; and (3) greater emotional reactivity.

Measuring arousal

By taking a quantitative, statistical approach to arousal studies it is possible to avoid false controversies, such as the disagreement between those who consider arousal to be a unitary product of ascending reticular activating systems [14] and those who consider the arousal functions so fragmented as to be useless and virtually non-existent [20]. Using the three elements of the operational definition of arousal given above, we compared the responses of 48 mice across a variety of tasks to construct an inter-response correlational matrix. Numerical data from this matrix were used for factor analysis [21]. The percentage of variance accounted for is illustrated in Fig. 3. The value of the 'one-factor solution' on the abscissa represents the percentage of variance accounted for by 'general arousal'. Clearly, in this and similar subsequent experiments, general arousal is an important behavioral feature. However, specific arousal factors are required to account for the remainder of the variance [21].

This mathematical approach to a general arousal function can be compared to the neuroanatomical delineation of the brainstem arousal system. A 'crescent' of neurons along the bottom (ventral) portion of the medullary reticular formation includes large numbers of neurons that respond to multimodal inputs and are perfectly adjusted physiologically to underlie general arousal. Moving forward (anterior) in the brainstem reticular formation, reticular neurons near the midline include many neurons that also respond nonspecifically to auditory and vestibular cues [22,23]. Answering whether the massive brainstem area and large numbers of neurons devoted to these arousal functions qualify as the neurobiological correlate of the generalized arousal behavior mentioned above sets a crucial challenge for future research that deals with the most fundamental basis of cognitive and emotional function.

The dominant feature of arousal systems in the brainstem is that they are not allowed to fail. Because of this it is expected that there is likely to be massive redundancy in the neuroanatomical pathways and neurochemical mechanisms involved, and that these should exert modulatory influences rather than dichotomous on–off responses. The specific and differential effects of hormonal and genetic manipulations referred to below are consistent with these requirements, although it is likely that they impinge on ascending arousal systems at different points of the neuraxis.

The neuroanatomical, neurophysiological and behavioral data quoted above lead to a 'bottom up' approach to the function of brain arousal, as do the hormonal and genetic mechanisms introduced below. This is opposed to 'top-down' approaches. Even the best of the 'top down' ideas invoke *dei ex mechani* such as 'distributed re-entrant networks' [24], which comprise

pseudo-explanations. A direct, neurobiological approach proposes a 'functional pyramid', at the base of which fundamental arousal neurons are necessary, but not sufficient, for awareness, alertness and attention. New experimental protocols are required to test this proposal.

Hormonal influences

That estrogens heighten the arousal state of female rats [25] is evident from their natural behavior. For example, females ready to mate display heightened muscular tension throughout their bodies, rapid alternating movements and rapid locomotor movements. The robust elevations of locomotor activities in rats and mice following estrogen treatment afford additional evidence of augmented arousal [26,27]. Estrogen administration also increases the emotional reactivity of female mice, which is evident not only in responses to anxiety producing situations but also in fear conditioning [28]. The antidepressant properties of estrogens in experimental animals and humans are further evidence of the emotional effects of these steroids [29,30]. Although some of these estrogenic effects might involve the 'arousal crescent' of neurons in the hindbrain, the actions on fear and mood are most easily envisaged as occurring in the amygdala. In addition, stimulation of ERβ in the dorsal raphe nucleus of the midbrain [31,32] could account for elevated mood, by acting through serotonergic mechanisms.

Thyroid hormones also influence states of arousal. In humans, hyperthyroid conditions are associated with tenseness and irritability, whereas hypothyroid patients are sluggish. In fact, thyroid hormone administration is used as adjunct therapy for antidepressants [33,34]. Because the genes encoding thyroid-hormone receptors (TRα and TRβ), another type of nuclear hormone receptor, are expressed widely along the neuraxis in rats [35] and mice [36], thyroid hormones could interact with ascending arousal pathways at many points.

Genetic influences

For a 'bottom up' approach to arousal, one must consider the potential sites of genetic and hormonal influences from the medullary reticular formation ascending into the forebrain. It can be postulated that genetic influences impacting generalized arousal would be manifest in the lower brainstem – the 'arousal crescent' of neurons in the medullary reticular formation described previously. In contrast, it might be expected that the highly specific behavioral influences of receptor stimulation arise in the forebrain sites devoted to those particular behavioral functions. It is clear from animal models that stimulation of either ERα or ERβ mediates different effects, which are different to those of TRα or TRβ stimulation, and that each of these receptors has different actions to that of enkephalin.

For example, the effects of estrogen on locomotor activity depend on expression of the gene encoding ERα and not ERβ [37]. This genetic influence subserves the effects of estrogens on preoptic neurons [15–18], although it could, additionally, depend on the effects of estrogens binding to the ERα in noradrenergic neurons of the cell group A1 to influence generalized arousal states. However, although our results with ER-knockout mice are most easily interpreted in terms of nuclear receptors, the possible participation of non-genomic mechanisms should not be ignored. Rapid membrane actions of estrogens have been reported [38] and may be relevant for reproductive behavior and sexual arousal [39–41].

TRα and TRβ have a different spectrum of action on arousal [42] than ERs. TRα-knockout mice have significantly lower acoustic-startle and tactile-startle responses whereas TRβ-knockout mice are less anxious; they entered open arms of the elevated plus maze more frequently and spent more time in the lighted compartment of the dark–light transition test than did wild-type animals. Also unlike ER, deletion of the genes encoding either TRα1 or TRβ had no effect on fear learning, which implies that TR-related mechanisms affect anxiety but not fear. The differences in the effects of ER and TR isoforms indicate the specificity of nuclear receptor genes in relation to arousal. Explaining these differences poses an exciting challenge for neurobiologists.

Still another spectrum of genetic influences is seen in the behaviors of enkephalin-knockout animals [43]. As responses to a fear-learning situation were elevated and measurements of anxiety were heightened, the loss of the gene encoding this opioid peptide produces mice that might comprise new models of chronic fear and anxiety. The phenotype of enkephalin-knockout mice, coupled with the neuropharmacologic data from the lab of Kang and Wilson [44,45] make it highly likely that expression of the gene encoding enkephalin in the amygdala is central to this set of results. The partial analgesia expected from expression of an opioid peptide should allow the female to accept what might otherwise be noxious somatosensory stimuli from the male during mating [46]. This likely provides the clearest example so far of an indirect contribution to a specific well understood behavior. It does account fully for the simple sensory-motor mechanisms themselves. Rather, it encourages a behavioral temperament that permits the lordosis-triggering stimuli to be applied.

In each of these cases, genetic manipulations modulate arousal responses selectively, but do not make them appear or disappear: arousal functions are too crucial for such gross alterations. Thus, operationally defined quantitative measurements of arousal will continue to be required for further explorations of the genetics of arousal pathways. It is not surprising that considerable effort needs to be spent to work out the gene–behavior relationships

across this range of mammalian social behaviors. After all, even in *Drosophila* questions are still asked about whether 'genes control behavior' [47]. Nevertheless, sophisticated experiments in mammals are yielding answers not simply about whether genes do indeed control behavior, but about the mechanisms by which proteins modulate arousal to affect the motivational states and, thus, the biologically regulated behaviors that depend upon heightened arousal.

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