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Neural, hormonal and genetic mechanisms for the activation of brain and behavior

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Abstract

Underlying all motivated behavior is the concept of brain arousal, the generalized activation of forebrain and behavior. A concrete expression of this would be sexual arousal and behavior. The sex behavior whose mechanisms are best understood is the lordosis response, a vertebral dorsiflexion by the female permitting fertilization. Estrogenic facilitation of this behavior requires new transcription and protein synthesis. The genes which are turned on by estrogens and whose products facilitate the behavior are organized in modules. Some exert direct effects, e.g. genes coding for neurotransmitter receptors in hypothalamic neurons. Other modules exert indirect effects: through neuronal growth, through facilitation of transcription from the progesterone receptor gene, and even through other preparative behaviors. An unexpected result deriving from a microarray study was the estrogenic effect on prostaglandin-D synthetase, important because of the marked actions of prostaglandin-D on arousal and sleep.

Keywords: Estrogen; Genomic; Hypothalamus; Sex; Arousal

1. Introduction

We have explained a simple sex behavior, the lordosis response, by demonstrating its neural mechanisms, hormonal drives and genetic influences (Pfaff, 1999). Now, we are beginning to analyze the underlying concepts of sexual motivation and arousal. A substantial part of any motivation depends upon 'arousal', the activation of brain and behavior. We propose the existence of a global arousal function which accounts for about 1/3 of the variance in our results with mice. Moreover, results with gene knockouts give us insights into the genomic regulation of generalized arousal, as quantified using novel protocols in mice.

2. Molecular mechanisms of a hormone-driven behavior

The simple behavior in question, lordosis, depends on defined physical signals: cutaneous stimuli, and estrogen administration followed by progesterone (E + P). In the presence of adequate sources of food and water, and in the absence of fear or anxiety-provoking conditions, females under the influence of E + P will demonstrate courtship and then mating behaviours. Knowing that transcriptional facilitations by estrogens are required for the behavior (Pfaff, 1999), here we give a brief account of the genetic/neural effector modules found so far (Mong et al., 2003a,b).

Some of the modules execute direct hormonal effects, from gene induction to neural circuit to behavioral change. These would include α_{1b}-adrenoceptors which are induced in the basomedial hypothalamus by estrogens, as well as muscarinic receptors in ventromedial hypothalamic neurons. We note that both of these receptor systems in ventromedial hypothalamic neurons are required for normal controls over lordosis behavior.

Other modules carry out indirect effects, from gene induction to downstream genes to behavioral change. These include neuronal growth; growth promotion by estrogens in ventromedial hypothalamic neurones follows from the stimulation of synthesis of ribosomal RNA, which precedes the elaboration of dendrites and synapses on ventromedial hypothalamic neurones observed after hormonal treatment. Also included is the Amplification by progesterone; admin-

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istration of progesterone 24 or 48 h after estrogen priming greatly amplifies the effect of estrogens on mating behavior. Thus, it is significant that estrogens greatly increase rate of transcription from the progesterone receptor gene, allowing progesterone, in turn, to facilitate expression from certain downstream genes.

The physiological importance of estrogenic elevation of gonadotropin releasing hormone (GnRH, also known as luteinizing hormone releasing hormone, LHRH) mRNA levels under positive feedback conditions—as well as elevation of the receptor mRNA for GnRH—must be to synchronize reproductive behavior with the ovulatory surge of luteinizing hormone (LH).

Other types of indirect effects lead from genetic induction to intermediate behaviors. The enkephalin gene is turned on rapidly by estrogens, within about 30 min, and this is proven to represent a hormone-facilitated transcriptional facilitation. The route of action upon lordosis, of the enkephalin gene product, is indirect, through other behaviors. That is, through the reduction of pain, a partial analgesic action, it helps to allow the female to engage in mating behavior despite strong stimuli she receives from the male (Bodnar et al., 2002). Also anxiety reduction: the oxytocin gene and the gene for its receptor are both expressed by hypothalamic neurones at higher levels in the presence of estrogens. The indirect route of action of this multiplicative set of gene inductions, on mating behavior, is likely through a behavioral link. That is, oxytocin has been conceived as protecting instinctive behaviors connected with reproduction, maternity and other social behaviors from the disruptive effects of stress.

2.1. Social recognition and aggression

The induction of the oxytocin gene by estrogens is an estrogen receptor-beta-dependent, behaviorally significant phenomenon, reassuring since only estrogen receptor-beta gene expression is found in oxytocinergic cells. In turn, oxytocinergic projections to the amygdala are thought to be important for social recognition in mice, which helps to prevent aggression and promote reproduction. A four-gene micronet essential for social recognition has been inferred (Choleris et al., 2003).

Among our microarray studies of genes influenced in certain forebrain structures either by estrogen treatments or by sex differences, one interesting result so far has yielded important implications: Prostaglandin D synthetase transcripts were markedly elevated by 2 or 24 h of estrogen treatment in the basomedial hypothalamus, even in the same mice where the mRNA was dramatically suppressed in the preoptic area (Mong et al., 2003a,b). This gene demanded attention because of the important roles for prostaglandin-D in control of the sleep/wake cycle, responses to pain and responses to olfactory inputs. Subsequent work (Mong et al., 2003a,b) used a new antisense DNA moiety, locked nucleic acids, to reduce the levels of Prostaglandin D synthetase in the preoptic area. Mice so treated displayed increased responses to sensory stimuli and increased voluntary motor activity compared to scrambled sequence and vehicle controls. We hypothesize that suppression of Prostaglandin D synthetase by estrogens in the preoptic area could account for increased generalized arousal (see below) and movement evident in estrogen-treated females.

2.2. Modular summary

Emerging from this series of individual gene inductions by estrogens acting in the basal forebrain, and this recounting of downstream genes and their physiological routes of action, comes a set of modules (GAPPS) which help to account for the causal relations between sex hormones and female mating behaviors. First, there is a hormone dependent Growth (G) response, which permits hormone-facilitated, behavior-directing hypothalamic neurones a greater range of input/output connections and, thus, physiological power. Second, progesterone can amplify (A) the estrogen effect, in part through the downstream genes listed above. Then, through indirect behavioral means—the reduction of anxiety and a partial analgesia—the female as an organism is prepared (P) for engaging in reproductive behavior sequences. Here the genes for oxytocin (and its receptor) as well as the genes for the opioid peptide (and its receptors) are important. Next, neurotransmitter receptor induction by estradiol permits (P) the neural circuit for lordosis behavior to be activated. The α_1-adrenoceptor and muscarinic acetylcholine receptors are key here, in the ventromedial nucleus of the hypothalamus. Finally, induction of the decapeptide which triggers ovulation, GnRH as well as its cognate receptor acts to synchronise (S) mating behavior with ovulation in a biologically adaptive fashion.

3. Arousal

Underlying all hormone-dependent sex behaviors is sexual arousal. Concepts of arousal are essential for helping to explain broad classes of behavior, but they also have been murky and ill-defined. In humans, ‘‘arousal’’ is intuitively obvious, but what about in experimental animals? We have shown that global arousal levels can be measured by an array of behavior tests quantifying sensory responsiveness, motor activity, and emotional responsiveness. Furthermore, statistical analyses support the operational definition of arousal: high arousal is reflected by high sensory responsiveness, high motor activity, and high emotional reactivity. That is, we have reanalyzed data from several experiments by principal components analysis (Garey et al., 2003). In these calculations, generalized arousal accounted for a significant amount of the data—about one third (Garey et al., 2003). To begin exploring genetic influences, we used gene knockouts for the estrogen receptors estrogen receptor-alpha and estrogen receptor-beta, two very similar transcrip-

tion factors, probably gene duplication products. In a novel assay intended to quantify components of generalized arousal, we studied female mice, individually housed, sleeping in their home cages as they do during the light phase of the daily light cycle. For all sensory modalities tested, estrogen receptor-alpha knock out (α-ERKO) female mice were less responsive to sensory stimuli than their wild-type female littermate controls. Disruption of the gene for the closely related estrogen receptor-beta did not have the same effect. In terms of locomotor activity during the dark phase of the daily light cycle, α-ERKO females were less active. This phenotype was dependent on age; older a-ERKO females were subject to the genetic effect, whereas the younger α-ERKO females were not. Differences between estrogen receptor-beta knock out (β-ERKO) females and their wild-type littermate controls were not significant.

Furthermore, we can ask whether generalized arousal, as approached above, can be shown to influence specific arousal mechanisms such as those important for sexual motivation and behavior. Histamine is a neurotransmitter involved in arousal pathways. In human behavioral pharmacology, the ability of anti-histamines to make a subject sleepy is well recognized. In animal neuropharmacology, a histamine H_1 receptor antagonist can be shown to influence mouse behaviors in a quantitative set of measures which reflect arousal (Easton and Pfaff, 2003), without inducing a sleep state. Importantly, histamine excites electrical activity in the ventromedial hypothalamic neurons which control the lordosis behavior neuronal circuit, and those responses can be influenced by estrogen treatment (Kow and Plaff, 2002).

Overall, the results mentioned above can be interpreted as evidence that a generalized arousal function exists in the mouse brain, that it can be measured precisely and that it can be altered by genetic manipulation, for example by the deletion of the nuclear receptor for a steroid sex hormone. In a more general framework, this molecular analysis of biologically regulated motivated behavior has led us to a point where we can not only explain an individual behavioral response, like lordosis, but also explain an entire state of the mouse central nervous system, arousal, which is associated with broad classes of responses. The ability to assay generalized arousal using a new approach (Easton et al., unpublished data) and to manipulate it with gene knockouts and with local microinjections of locked nucleic acid antisense oligomers likely opens up a new field of work in the neural analysis of biologically motivated behaviors.

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