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CHAPTER 3

Reproductive behaviors: new developments in concepts and in molecular mechanisms

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Abstract: New developments in the analysis of mechanisms for reproductive behaviors are reviewed. Conceptually, the concept of generalized arousal (GA) of the central nervous system (CNS) is considered. Breeding for high and low GA, we show an impact of GA on sexual arousal of male mice, and also find that the structure of GA in the CNS of males and females is not the same. Further, we propose, theoretically, that among epithelial tissues in humans, there are correlations among their innervation densities and their ability to trigger arousal. In new technical developments, we analyze transcriptional effects of estrogens in the hypothalamic neurons that regulate lordosis behavior. The rapid effect of estradiol to increase acetylation of histones in ventromedial hypothalamic neurons could be tied into transcriptional activation, but the effect of estradiol to increase methylation of histone 3, lysine 9 (H3K9) is puzzling. This work seeks to discover the coactivator dynamics underlying transcriptional effects of estrogens on sex behavior.

Keywords: arousal; sex difference; lordosis; histones; epigenetics

Introduction

Since the purpose of this volume of *Progress in Brain Research* is to review new developments in neuroendocrinology, we shall not go back over the huge bodies of fact that are covered for female sexual behaviors (Lee et al., 2009) and male sexual behaviors (Hull et al., 2009) in *Hormones, Brain and Behavior* (Academic Press/Elsevier, 2009). Instead, we'll open up a discussion about a new emphasis on concepts of the brain's regulation of hormone-dependent sexual behaviors in the female and male. This will be followed by a novel chemical approach: an introduction to methods for studying changes in the chromatin overlying genes crucial for the transcriptional changes in hypothalamic and preoptic neurons, which in turn are crucial for reproductive behaviors.

Generalized arousal and its impact on sex behaviors

The most powerful and essential force in the vertebrate central nervous system (CNS) has been proposed (Pfaff, 2006) as originating from primitive reticular neurons in the lower brainstem. We have gathered evidence for its existence using three approaches: (1) by principal components analysis, showing that about one-third of the variance of the

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data in arousal-related assays (of mouse behavior) is due to generalized arousal (GA) force (Garey et al., 2003); (2) by acknowledging the large amount of neuroanatomical, neurophysiological and genomic evidence for the detailed mechanisms supporting GA (reviewed in Pfaff, 2006); and (3) by breeding mice for high and low GA (Weil et al., 2010). Given that generalized CNS arousal actually exists, what are its impacts on female and male sexual behaviors?

In the female, new neuropharmacological results prove that arousal-enhancing drugs, particularly amphetamines, increase the expression of lordosis behavior (Holder et al., 2009). As reported in Weil et al. (2010), we have new evidence that the mechanistic structure of generalized CNS arousal mechanisms are not the same in female mice as in male mice. Mice are being bred for high GA and low GA according to the operational definition stated in Pfaff (2006). Male and female mice derived from 'high' parents are referred to as HM and HF, respectively, while the offspring of 'low' parents are LM and LF.

Males from the high line and those offspring who exhibited high levels of GA exhibited a specific pattern of sexual behavior associated with a higher level of excitability and sexual arousal (Fig. 1). High-arousal males exhibited more mounts before intromission and, then, fewer intromissions before ejaculating and rapid ejaculation after the first intromission. Additionally, the percentage of mount attempts that were successful in leading to intromission was significantly lower among male mice from the high-arousal line. The pattern of sexual behavior indicates that high-arousal males were excitable in an inappropriate manner, as indicated by the large numbers of early mounts without intromission and the very low intromission: total mount ratio.

Finally, to extract information about the most prominent feature of the data gathered from our GA assay, we used a mathematical method called principal components analysis. This method is used here to analyze the relative contributions of motor, sensory and emotional (fear) measures as they influence the largest, most elementary dimension of arousal. That is, the most generalized, elementary force operating in our arousal assay is revealed by a forced one-component

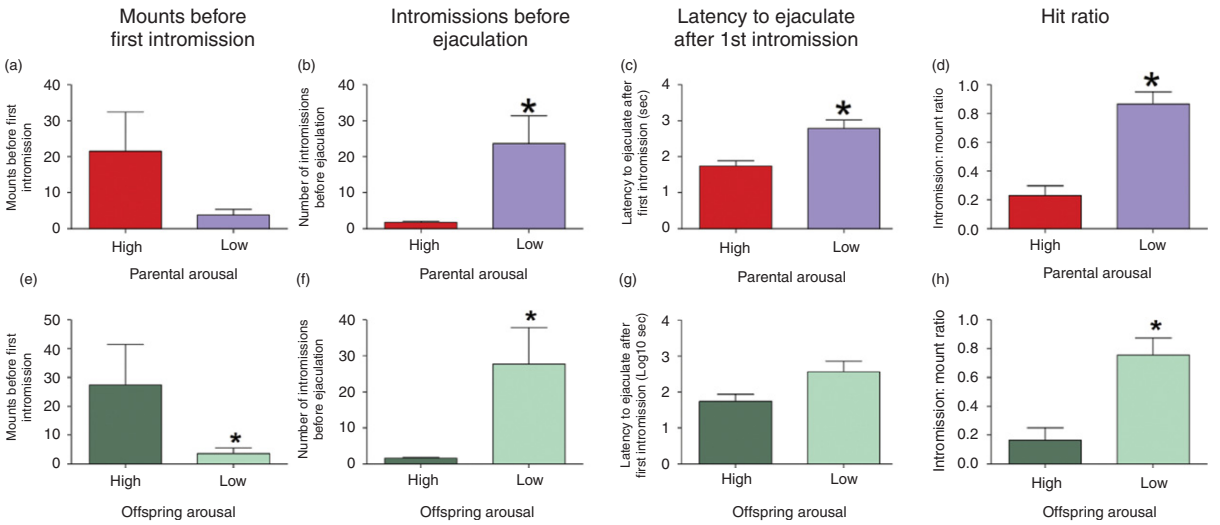

Fig. 1. Male mice bred for high levels of generalized CNS arousal were excitable, made many quick and unsuccessful mounts of the female and, once having achieved an intromission, quickly achieved ejaculation. In this figure their male sex behavior is compared to male mice bred for low levels of generalized CNS arousal (from Weil et al., 2010). Thus illustrated is a contribution of generalized CNS arousal to one specific type of arousal, sexual arousal.

solution of our data set. The most interesting comparisons to come out of the principal components analysis are illustrated in Fig. 2. It demonstrates the separate contributions of motor activity, olfactory responsivity and fear to Principal Component #1, the component that quantifies the most generalized, powerful force generating behavior in these arousal assays. In Fig. 2, when a measure has a (–) sign, it means that it was, indeed, grouped with the forces on Principal Component #1, but in the reverse direction (low values of that behavior are strongly associated with Principal Component #1's contribution to the production of arousal-related behaviors). Principal Component #1 reflects a high degree of motor activity. Our analysis raises the question of whether the structures of arousal functions are the same in males and females.

Fig. 2 shows that the major differences between HM and LM come from the large contribution of motor activity of HM to Principal Component #1, as well as a difference in the contribution of fear. In fact, it is the failure of motor activity driven by Principal Component #1 that makes those males LM rather than HM. HM have high movement rates and are skittish. Females are different. The major difference between HF and LF comes from the strong reactivity of the HF to olfactory input. With respect to sex differences between HM and HF, there are large differences in the contributions of olfactory responsiveness to Principal Component #1, as well as fear. We speculate that a HF female ready to mate, having spent much time in her burrow, will emerge from her burrow just before ovulation. She must lack fear and locomote extensively, spreading the odour of vaginal secretions, a form of courtship behavior that encourages males to mate just as she is ovulating. In turn, her powerful olfactory response will help her choose healthy vigorous males as potential fathers of her litter. Between LM and LF, the major difference is due to the fact that the LF had a large motor contribution to Principal Component #1, as well as a smaller difference between

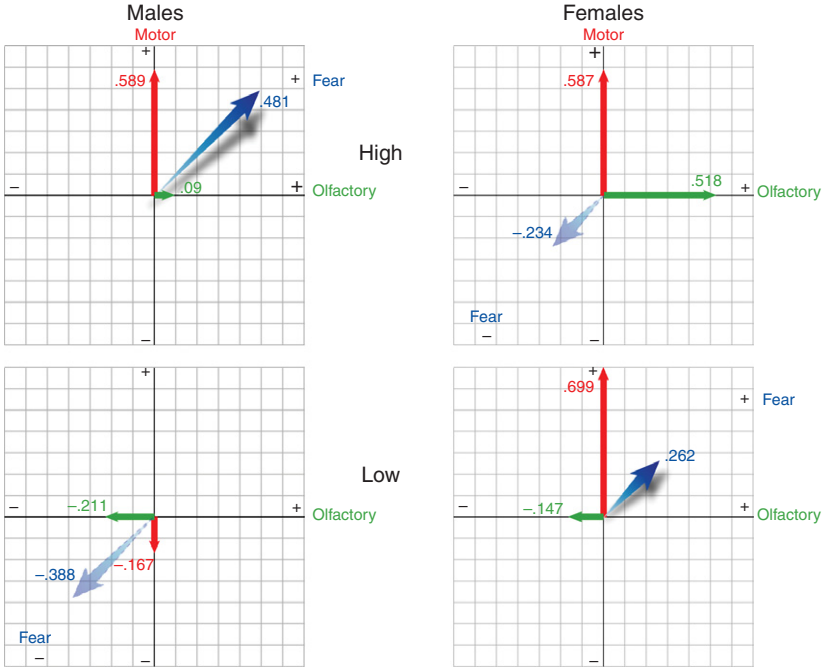

Fig. 2. The structure of generalized CNS arousal is not the same in female mice as compared to males (from Weil et al., 2010). Here the contributions of motor, sensory (olfactory) and emotional (fear) measurements to the primary component of CNS arousal are quantified. See text for explanation.

LM and LF in fear. From this application of principal components analysis, we infer that the structure of the primary arousal component is not the same in males as in females.

Mechanisms initiating generalized and sexual arousal in humans

We have summarized sources of sexual arousal from various levels of the neuraxis (Martin-Alguacil et al., 2006). Although a large number of studies with animal brain emphasize the importance of the hypothalamus and preoptic area in the regulation of sexual behavior, we clearly recognize the power of genital stimulation, in humans at least, for arousal sexual arousal. Therefore, we have begun the histochemical characterization of epithelial cells in the critical genital areas. For example, the expression of immunoreactive estrogen receptor (ER)-alpha differs significantly from that of ER-beta (Martin-Alguacil et al., 2008b; Schober et al., 2008) and has an interesting relation to the expression of the neuronal isoform of nitric oxide synthase (nNOS) (Martin-Alguacil et al., 2008a). Extrapolating from our studies with ER expression in human genital tissues, we propose (Schober et al., 2010, in preparation) the following new concept – across the entire range of human epithelia, three different functions are strictly correlated with one another: (1) a higher density of innervation and vascularization is correlated with (2) greater speed and efficiency of wound healing and (3) a more powerful ability to stimulate CNS arousal upon stimulation of the epithelial surface. New studies will (1) systematically test this theory using human tissue and (2) analyze the mechanisms by which this convergence of functions is produced.

Chromatin: steroid hormone-induced covalent modifications of histone tails

New mRNA and protein synthesis in hypothalamic neurons are required for estrogenic facilitation of lordosis behavior (reviewed in Pfaff, 1980). This discovery led to a long series of experiments in which genes that had two properties were identified: (1) their mRNA levels were increased in hypothalamic neurons after E administration and (2) their gene products would foster female reproductive behaviors (summarized in Pfaff, 1999). In turn, the emphasis on gene–behavior relationships sprung two very surprising observations: the loss of one gene (ERalpha) could cause females to be treated like males and to respond like males (Ogawa et al., 1996), and secondly, an individual gene could have opposite behavioral effects in male brains as compared to female brains (summarized in Ogawa et al., 2004). All of these and other observations (e.g. Ogawa et al., 1998a, 1998b) caused us to focus on hormone-facilitated gene transcription.

The initiation of transcription is widely understood to involve changes in chromatin, the set of proteins that guard access to DNA by transcription factors such as ERs (illustrated in Figs. 3–5). Chromatin, the principal structural protein associated with DNA molecules, has a potential regulatory role in gene expression. Chromatin is the principal structural protein around which DNA is wrapped and it is made up of four canonical proteins termed H2A, H2B, H3 and H4, the structure of which have been largely conserved over evolutionary time. Broadly, DNA exists in two states, (1) euchromatin, wherein DNA is relatively loosely coiled around chromatin and which is associated with transcriptionally active genes. This is in contrast to (2) heterochromatin where DNA is tightly wrapped around histone peptides and thus the underlying DNA is inaccessible to the transcriptional machinery. These states were long considered developmentally programmed and static. However, these broad categories are significantly complicated by dozens of covalent modifications of histone tail regions which can more subtly regulate gene expression. Modification of histone tails with the additions of acetyl, phosphate, methyl, sumo and ADP ribosyl groups at specific amino acid residues alone and in combination has specific regulatory effects on the genes associated with modified chromatin. The complexity of this system has lead Professor

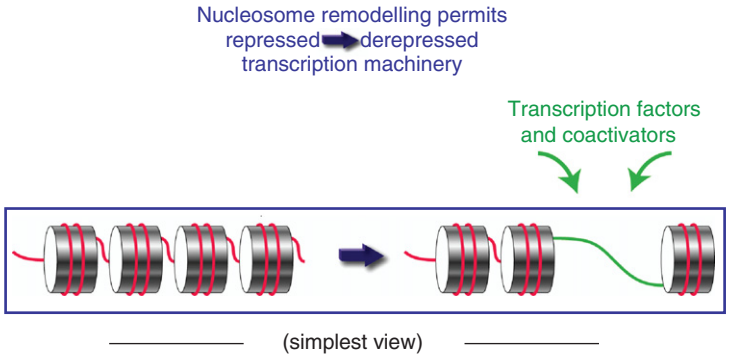

Fig. 3. Inactive DNA is tightly wound, whereas DNA accessible to transcription factors is no longer tied up in nucleosomes.

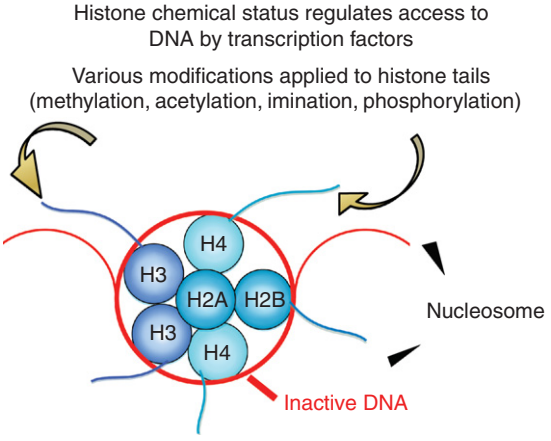

Fig. 4. Nucleosomes in which inactive DNA is wrapped are characterized by tails of histone proteins emerging in a manner that leaves them susceptible to chemical modification. In the usual case for mammalian cells, acetylation of amino acids on these tails is associated with increased rates of transcription and methylation is associated with repression of transcription. We have tied increased acetylation to estrogenic effects in the region of the ventromedial hypothalamus important for regulating lordosis behavior (Weil et al., 2009).

C. David Allis and colleagues here at Rockefeller to suggest that these covalent modifications of histone peptides taken together form a 'histone code' which provide important epigenetic regulation of gene expression. Therefore, with respect to female sex behavior, it was natural for us to try and draw histone modification into E-caused transcriptional changes in the VMH neurons essential for E-dependent lordosis behavior (Weil et al., 2009). Results showed that treatment with 17β-estradiol rapidly methylates H3K9 in the VMH, and also that treatment with 17β-estradiol rapidly increased the acetylation status of histone H4 proteins in the VMH. These changes are highly likely to be relevant to lordosis behavior because double-identified ER-alpha positive cells in the ventromedial hypothalamus are shown by our new results to exhibit H4 acetylation. We have further reason to believe that histone acetylation within VMH neurons is causally linked to female reproductive behavior, because microinjections of the histone deacetylase inhibitor into the VMH potentiates estrogen-induced lordosis

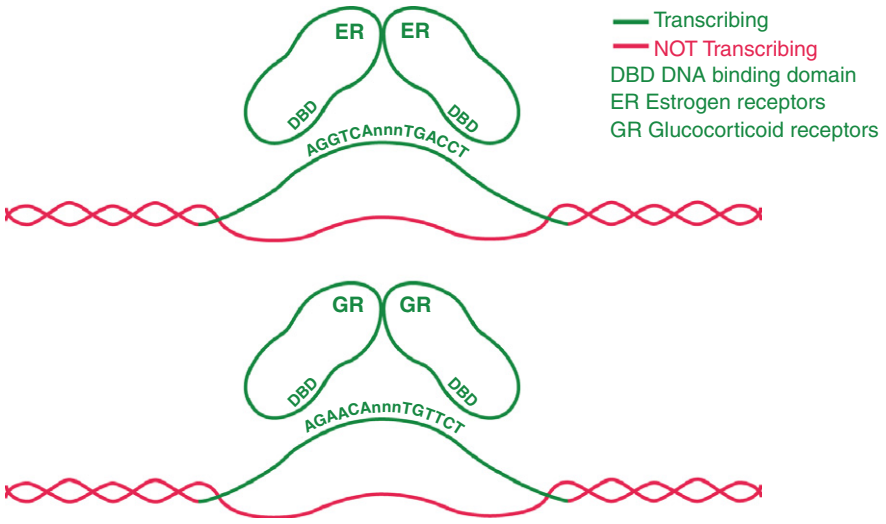

Fig. 5. The net result of loosening DNA tight connections with nucleosomes is thought to lie in freeing up transcription factor recognition elements, illustrated here by consensus nucleotide base sequences for estrogen receptors (ERs) and glucocorticoid receptors (GRs).

behavior and reduces rejection behaviors (Weil et al., 2009).

Similar considerations apply to maternal behavior. The optimal pattern of hormone exposure for the onset of maternal behaviors in female laboratory mammals comprises a sudden drop of progesterone levels in the blood coupled with high levels of estrogens in the blood. Surprisingly, however, it had not been determined exactly what duration of estrogen exposure would be minimally sufficient for the facilitation of maternal behaviors. We have just finished the appropriate experiments by ovariectomizing female mice 11 days before tests of maternal behaviors and, at the same time, implanting progesterone capsules subcutaneously, which will subsequently be removed two days before behavioral testing. The question is: how long must estrogens circulate in progesterone-withdrawn mice, for maternal behaviors to occur? The answer was surprising: estrogens administered as little as two hours before testing could enhance maternal behaviors in progesterone-withdrawn female mice (Murakami et al., unpublished data, 2010). The advantage of this short requirement of estrogen treatment is that it permits us to concentrate on a short time window during estrogen-triggered behaviorally important transcriptional changes. In turn, this knowledge tells us exactly when to look for histone chemical modifications in preoptic area neurons, a project that we are finishing now (Murakami et al., unpublished data, 2010). From unpublished experiments, we know that the schedule of E administration consistent with the initiation of maternal behaviors is associated with global changes of histone chemistry in the preoptic neurons essential for maternal behavior, and that these changes include both methylation and acetylation (Murakami et al., unpublished data, 2010), but we neither have tried to draw these histone modifications into transcriptional arguments, nor have we proven that these histone modifications are essential for the initiation of maternal behavior. Those experiments will be initiated soon in this laboratory.

Although the data and molecular concepts of histone modifications fit very clearly with access to estrogen-sensitive genes by ligand-activated transcription factors, ERs, phenomena in neuroendocrine neurons are not always so simple. The changes in methylation status of specific histones caused by acute stress in hippocampal

neurons (Hunter et al., 2009) have not, so far, been mimicked by corticosterone injections as they might have been expected to be. Alternate routes of stress effects, therefore, are being explored currently.

Our current emphasis on the chemical modifications of histone tails in hypothalamic and preoptic neurons brings us through the next mechanistic step in demonstrating how gene–behavior causal relations occur. After all, Ogawa et al. proposed that deletion of a single gene could cause a female mouse to be treated like a male and to behave like a male (Ogawa et al., 1996). Altogether, knocking out the gene for ER-alpha caused a panoply of changes in sexual, aggressive and maternal behaviors (Ogawaet al., 1997, 1998a, 1998b). Taken together, the Ogawa et al. (2004) findings proved that the same gene could have opposite behavioral effects (on aggressive behavior) in females as that same gene has in males.

In sum, because of the clear relationships between patterns of gene expression and behavior in neuroendocrine systems, we predict that sex steroid effects on reproductive behaviors – both sexual and maternal – will provide some of the most chemically detailed and functionally important examples of histone modifications in nerve cells.

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