# Patterns of Steroid Hormone Effects on Electrical and Molecular Events in Hypothalamic Neurons

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## Contents

## Abstract

Hypothalamic neurons with nuclear receptors for steroid hormones provide opportunities to relate individual biosynthetic and electrical changes to hormone-driven behaviors. Successful work with female rodent reproductive behavior has proven that it is possible to define a neural circuit for a vertebrate behavior. In contrast to what might be expected from an invertebrate system, results from several approaches to neuronal gene expression show the complexity of hypothalamic control, even over this simple mammalian behavior. This is not a 1 hormone-1 gene-1 behavior system. Neither is there just one mode of hormonal induction. Certain steroid hormone effects can multiply each other, showing how a clear endocrine signal could be discerned among other variations in neural activity.

Index Entries: Hypothalamic neurons; receptors, hypothalamic, acetylcholine, alpha-1 adrenergic; oxytocin.

## Introduction

There are at least four questions to ask about mechanisms for behaviors of adult organisms. First, what causes a particular behavioral response to occur? Second, given that this response can occur, what regulates its amplitude? Third, as that response is occurring, what prevents other responses? Fourth, as Sherrington (1906) asked, what smoothes the transition from this response to the next?

Work on cellular mechanisms for female reproductive behavior and its regulation by steroid sex hormones has answered the first and second questions in considerable detail. Completion of a neural circuit for lordosis behavior (Pfaff, 1980; Pfaff and Schwartz-Giblin, 1988) has proven that it is possible to determine mechanisms for a mammalian behavior. Successful work in this area has depended on the relative simplicity of somatosensory stimuli driving the behavior and the motor topography of the response, as well as on strong hormonal controls that bring in the experimental tools of endocrinology. The circuit is comprised of spinal, lower brainstem, midbrain, and hypothalamic modules that appear to match embryological divisions of the nervous system (Pfaff, 1980; Pfaff and Schwartz-Giblin, 1988). Notably, the most powerful regulation of lordosis behavior by estrogens and progesterone can be explained at the hypothalamic level. Here, and in the limbic system, sex steroid hormone binding cells are found in a pattern with features common among vertebrate brains (Pfaff, 1968; Pfaff and Keiner, 1973; Morrell and Pfaff, 1978). Implants of estrogens or progestins in the ventromedial nucleus of the hypothalamus are sufficient for facilitating this behavior (Lisk, 1962; Barfield and Chen, 1977; Rubin and Barfield, 1980; Davis et al., 1979; Davis et al., 1982; Rubin and Barfield, 1983a,b).

The clarity of the neural mechanisms that regulate reproductive behavior and the experimental usefulness of its steroid hormone regulation have already allowed investigations into its molecular basis. Estrogen effects on messenger RNA levels and other synthetic steps show specific ways in which the hormone acts on cells to promote reproductive behavior. Electrophysiological actions of estrogen in the medial hypothalamus, easily seen to facilitate the behavior (Kow and Pfaff, 1988), should multiply the effectiveness of the foregoing synthetic changes.

### *Not a "1 Hormone-1 Gene-1 Behavior" System*

Even with a well defined steroid effect and a simple vertebrate behavior, we are not dealing with a 1 hormone-1 gene-1 behavior system. Early responses to estrogen by cells in the ventromedial nucleus of the hypothalamus help to

prepare for later responses. For example, *in situ* hybridization for the external transcribed spacer region of the precursor form of ribosomal RNA reveals increased amounts of this short-lived nuclear product in ventromedial hypothalamic neurons merely 30 min after estrogen treatment (Jones et al., submitted). After 6 or 24 h of continuous estrogen exposure, the mature hybridizable form of ribosomal RNA is significantly elevated (Jones et al., 1986) and presumably facilitates protein synthesis. The morphological result of new ribosomal RNA synthesis, increased rough endoplasmic reticulum, endures through at least 15 d of estrogen treatment (Cohen and Pfaff, 1981), and is accompanied by changes in nucleolar surface features (Cohen et al., 1984; Chung et al., 1984). Correlations between large amounts of stacked endoplasmic reticulum, in ventromedial hypothalamic neurons, and female reproductive behavior can be seen across estradiol treatments, and where progesterone was used to augment the estrogen effect (Meisel and Pfaff, 1985; Meisel and Pfaff, 1988). Conversely, inhibition of protein synthesis, specifically in the ventromedial hypothalamus, disrupts estrogen-facilitated lordosis behavior (Meisel and Pfaff, 1985b; Kelner et al., 1980). Many of these early estrogen-induced morphological changes in ventromedial hypothalamic neurons can be related to growth—increased nuclear area, nucleolar area, somal area (Jones et al., 1985).

If estrogen leads to increases in specific hypothalamic neuronal products, and if, in turn, those products can be shown to facilitate female reproductive behavior, manufacture of that product can be one way in which the hormone facilitates the behavior (Fig. 1).

## Hypothalamic Progestin Receptors

Estrogen induces progestin receptors in ventromedial hypothalamic neurons (MacLusky and McEwen, 1978,1980). The progestin receptor gene can be shown by *in situ* hybridization to be expressed in ventromedial hypothalamic neurons (Fig. 2), and the number of cells there with progestin receptor mRNA is greatly increased by 24 h exposure to estradiol (Romano et al., 1988,1989, in press). In vitro incubation with labeled amino acid precursors indicates that the rates of synthesis of several proteins in the ventromedial hypothalamus are altered by progesterone treatment (Jones et al., 1987a,b). Strong correlations between progestin receptor concentrations and the frequency of female reproductive behavior are seen (Parsons et al., 1980,1982; Parsons and Pfaff, 1985), according to latency of activation following estradiol onset, time course of decay following estradiol offset, and tolerance for discontinuous schedules of estradiol administration. Progesterone powerfully augments estrogen actions, facilitating feminine-typical sex behavior (Beach, 1948; Fadem et al., 1979; Sodersten, 1985), even within 30–60 min (Lisk, 1960; Meyerson, 1972; Kubli-Garfias and Whalen, 1977; McGinnis et al., 1981). Antiprogestins reduce lordosis behavior, showing that limited capacity progestin receptors are, indeed, necessary for the progestin effect (Brown and Blaustein 1984; Etgen and Barfield, 1986; Vathy et al., 1987). In summary, estrogen treatment induces the mRNA for the progesterone receptor, a transcription factor. It does so in the ventromedial nucleus of the hypothalamus, a nerve cell group that mediates hormonal controls over lordosis behavior mechanisms, which comprise the only known circuit for a mammalian behavior (Pfaff, 1980; Pfaff and Schwartz-Giblin, 1988). Therefore, these results demonstrate, for the first time, a relationship between synthesis of a transcription factor and the facilitation of a specific behavior (Romano et al., 1988, 1989).

## Acetylcholine Receptors

Several lines of evidence show that estradiol can increase lordosis behavior by influencing cholinergic mechanisms. Estrogen treatment increases the number of muscarinic receptors in the ventromedial and anterior hypothalamus

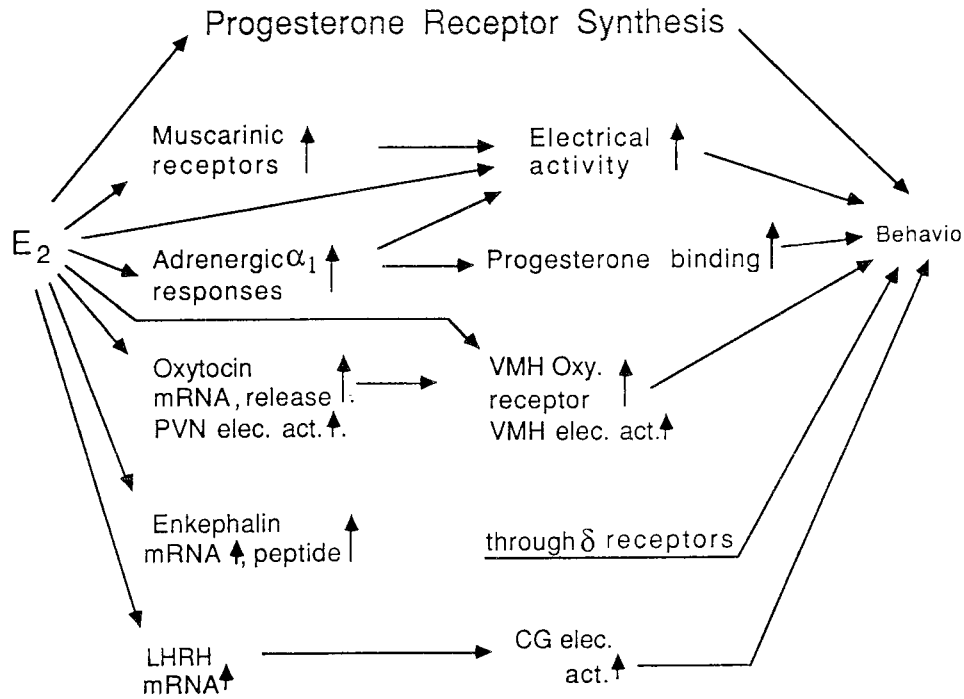


Fig. 1. Molecular and electrical responses to estradiol ($E_2$) by hypothalamic neurons show how this steroid hormone induces lordosis, a mammalian female reproductive behavior. In each case, $E_2$ leads to a cellular response which, in turn, has been shown to facilitate the behavior. References are in text. Abbreviations: CG, central gray of midbrain; LHRH, Luteinizing hormone releasing hormone; OXY, oxytocin; PVN, paraventricular nucleus of hypothalamus; VMH, ventromedial nucleus of hypothalamus.

(Dohanich et al., 1982; Olsen et al., 1982; Rainbow et al., 1980,1984), as well as the electrical responsivity of ventromedial hypothalamic neurons in vitro to acetylcholine (Kow and Pfaff, 1985). It fits well that acetylcholine acts through muscarinic receptors on ventromedial hypothalamic neurons in vitro, that its primary action is to increase neuronal activity there, and that estrogen potentiates only the excitatory electrical effects (Kow and Pfaff, 1985), since electrical stimulation of these neurons increases lordosis behavior (Pfaff and Sakuma, 1979). In turn, the work of Clemens and Dohanich showed definitively that muscarinic agonists will increase lordosis behavior, whereas muscarinic blockers reduce it (Clemens et al., 1981,1983; Dohanich and Clemens, 1981; Clemens and Dohanich, 1980; Dohanich et al., 1984; Kaufman et al., 1988), in part through ventromedial hypothalamus. The data thus fit the argument that estrogen, by increasing the number of muscarinic receptors, increases the electrical activity of ventromedial hypothalamic neurons, which facilitates lordosis behavior (Fig. 1). Since four types of muscarinic receptors have been cloned (Kubo et al., 1986; Bonner et al., 1987), estrogen effects on message levels for these receptors will be interesting to determine.

## Alpha-1 Adrenergic Receptors

Turnover of norepinephrine in the hypothalamus is increased during hormonal conditions correlated with the preovulatory LH surge and with lordosis behavior (Wise et al., 1981a,b; Rance et al., 1981; for a review, *see* Luttge, 1984). This occurs in females but not in males (Hiemke

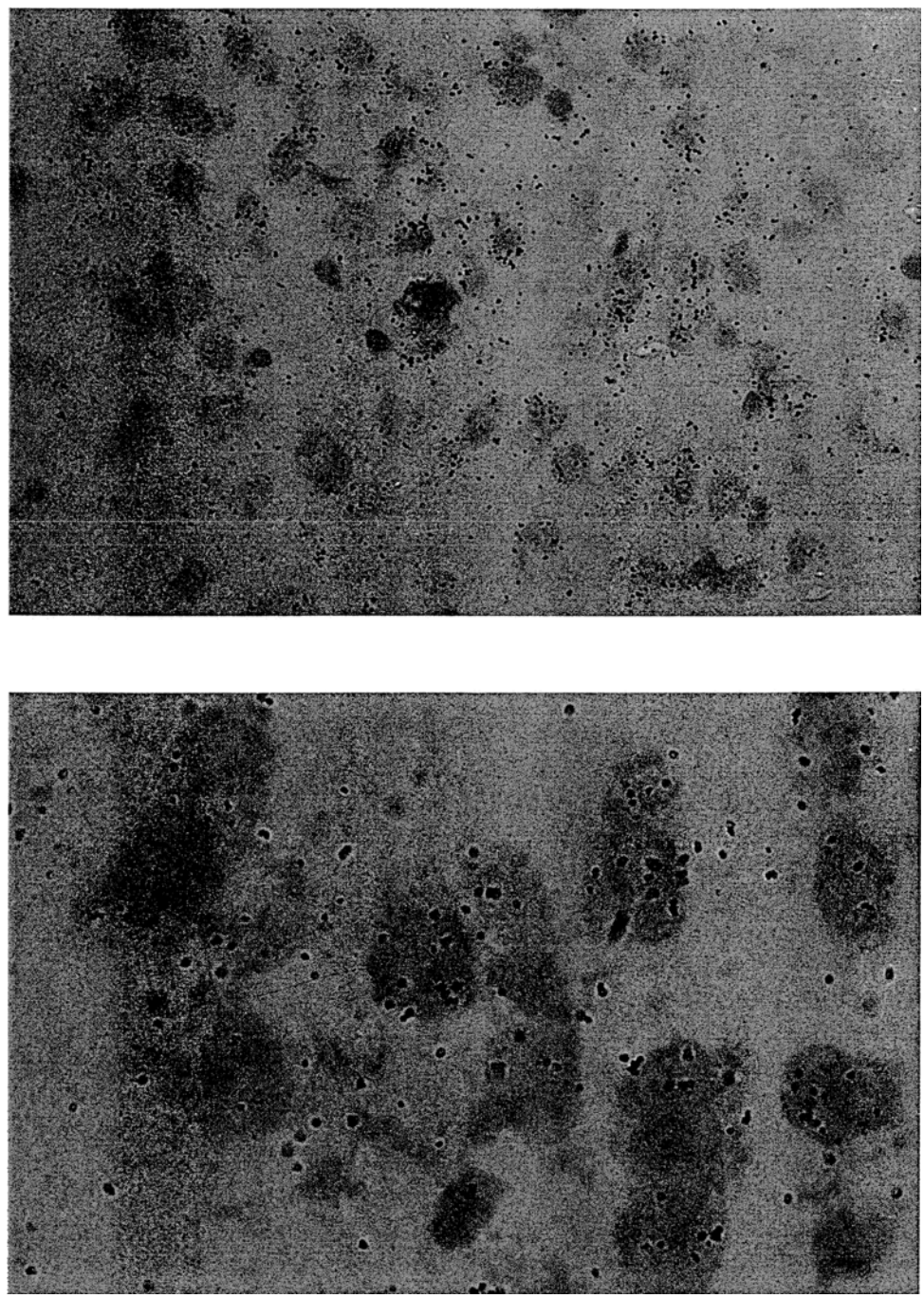

Fig. 2. *In situ* hybridization for mRNA for the progesterone receptor (PR) in female rat ventromedial hypothalamus (VMH). It is a rare message; grains per cell are low in number. Nevertheless, preferential localization of silver grains over cell bodies (purple ovals) shows neurons expressing gene for PR. Counterstain is cresyl violet. Top photo: 40× objective; bottom photo = 100× objective. Twenty-four-h exposure to estradiol resulted in a fourfold increase in the number of VMH cells expressing PR, as well as a significant increase in amount per cell (from Romano et al., 1988, 1989, in press).

et al., 1985). Moreover, treatment of ovariectomized female rats with estradiol benzoate decreases monoamine oxidase activity in the basal medial hypothalamus, which could increase effective concentrations of norepinephrine at receptor sites (Luine et al., 1975; Luine and Rhodes, 1983). In turn, increased adrenergic receptor stimulation influences the density of estrogen-induced progestin receptors (Crowley et al., 1976,1978; Nock and Feder, 1979; Nock et al., 1981), with alpha-1 but not alpha-2 ligands (Nock and Feder, 1984). Estrogen also increased the number of ventromedial hypothalamic neurons responding to norepinephrine, in vitro, (Kow and Pfaff, 1985), with excitation mediated by alpha-1 receptors (Kow and Pfaff, 1987).

The alpha adrenergic input to the basal medial hypothalamus comes from fibers ascending through the ventral noradrenergic bundle, whose damage is associated with loss of lordosis (Hansen et al., 1980; Herndon, 1976). Infusing norepinephrine into the ventromedial hypothalamic region can activate lordosis behavior in female rats given low doses of estrogen (Foreman and Moss, 1978; Nock and Feder, 1979), whereas a dopamine beta hydroxylase inhibitor or alpha adrenergic antagonist can reduce it (Foreman and Moss, 1978; Nock and Feder, 1979). In constrast, stimulation of beta adrenergic receptors inhibits lordosis in female rats (Mendelson and Gorzalka, 1988, in press).

Therefore, estrogen, by increasing opportunity for alpha-1 receptor stimulation, causes not only increased progestin receptor concentration, but also increased electrical activity of ventromedial hypothalamic neurons. Both of these results are known to increase female reproductive behavior (Fig. 1).

## Oxytocin

Steroid autoradiography combined with immunocytochemistry shows that estrogen addressed oxytocinergic cells in the paraventricular nucleus of the hypothalamus; the steroid is concentrated in the cell nucleus (Rhodes et al., 1981,1982). Estrogen binding cells are in a subdivision of the paraventricular nucleus known to project to other parts of the brain (Conrad and Pfaff, 1976; Weindl and Sofroniew, 1985; Swanson and Kuypers, 1980; Armstrong et al., 1980; Kawata et al., 1983). *In situ* hybridization reveals that combined estrogen-progesterone treatment significantly raises oxytocin mRNA levels, specifically in the paraventricular nucleus of the hypothalamus; changes in the supraoptic nucleus were not observed (Kawata et al., 1988a). With Northern blot analysis, changes in oxytocin message during the estrous cycle have been reported, consistent with an estrogen stimulation (vanTol et al., 1988), and there are estrogen response elements upstream of the oxytocin gene (Richter, 1989). As expected, estrogens stimulate oxytocin release, measured in the blood of women (Legros and Franchimont, 1972; Ogawa, 1980; Robinson, 1975), and female rats (Seif et al., 1977; Yamaguchi et al., 1979; Samson et al., 1986). This is accompanied by a decrease in oxytocin immunoreactivity in pituitary and brain (Rhodes et al., 1981a; Crowley et al., 1978). Further, estrogen leads to a large increase in the density of oxytocin receptors around the ventromedial nucleus of the hypothalamus (de Kloet et al., 1985,1986) (Fig. 3).

Electrophysiologically, estrogen activates oxytocin cells (Akaishi and Sakuma, 1985; Sakuma et al., 1985), and, in turn, oxytocin tends to activate ventromedial hypothalamic neurons (Kow and Pfaff, 1986). Following estrogen treatment, Akaishi and Sakuma observed decreased antidromic activation thresholds, shortened refractory periods, and decreased antidromic spike latencies in tonic-firing cells in the paraventricular nucleus of the hypothalamus (Akaishi and Sakuma, 1985; Sakuma et al., 1985). Since oxytocin can also excite presumed oxytocin neurons (Yamashita et al., 1987), there are opportunities for estrogen-stimulated effects to multiply (*see below*).

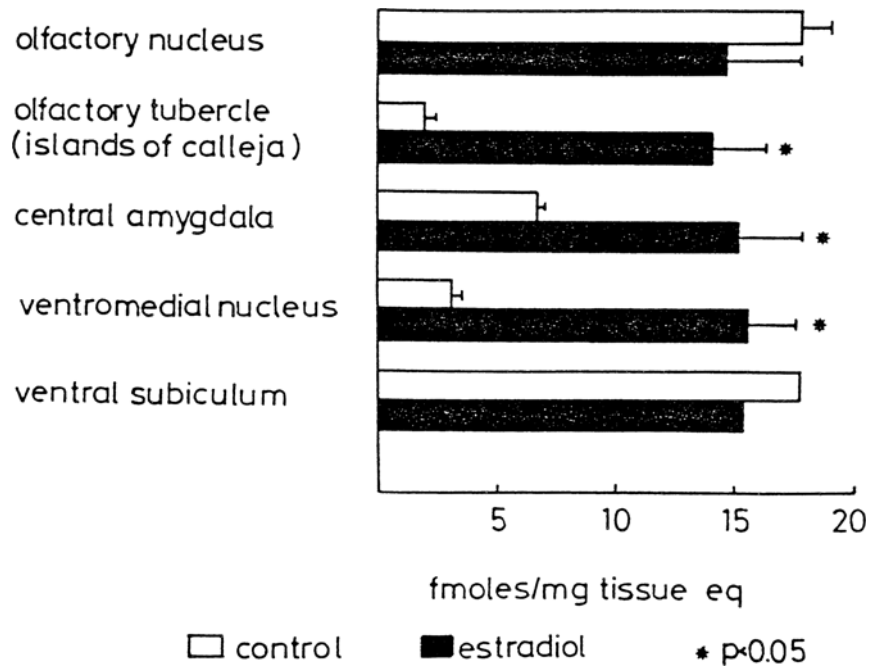


Fig. 3. Estradiol increases oxytocin binding specifically in certain brain regions, and not others. Data were obtained from rat brain sections of 6 ovariectomized and 6 estradiol-treated ovariectomized treated rats by in vitro autoradiography. Brain sections were incubated with $^3$H-labeled oxytocin and autoradiograms were generated. A strong induction was seen in the ventromedial nucleus of the hypothalamus (data from de Kloet et al., 1986; de Kloet et al., 1985).

Because of the stimulatory actions of oxytocin on ventromedial hypothalamic neurons, one would predict positive effects on lordosis behavior (Kow and Pfaff, 1988). Indeed, intraventricular oxytocin administration facilitates lordosis in female rats (Caldwell et al., 1984a,1984b; Gorzalka and Lester, 1987; Arletti and Bertolini, 1985), in part through ventromedial hypothalamic neurons (Kaufman, McEwen and Pfaff, unpublished observations; Schumacher et al., 1988,1989). Oxytocin release has been correlated with human orgasm (Newton, 1978; Carmichael et al., 1987).

Thus, estradiol followed by progesterone is associated with increased oxytocin messenger RNA, increased peptide release and increased ventromedial hypothalamic receptor density, accompanied by increased electrical activity not only in oxytocin neurons themselves, but also in ventromedial hypothalamic neurons expected to promote lordosis. In turn, oxytocin administration clearly facilitates lordosis (Fig. 1).

## *Enkephalin/Opioid Delta Receptors*

Slot blots as well as *in situ* hybridization show that two weeks of estrogen treatment induces a threefold increase of enkephalin messenger RNA levels in the ventromedial nucleus of the hypothalamus (Romano et al., 1988) (Fig. 4), beginning after only 1 h (Romano et al., 1986; Romano et al., 1989). The widespread distribution of enkephalin gene expressing cells (Harlan et al., 1987) may be subject to many hormone effects, but the ventromedial hypothalamic induction easily can be related to lordosis behavior, not primarily through recruitment of new cells but

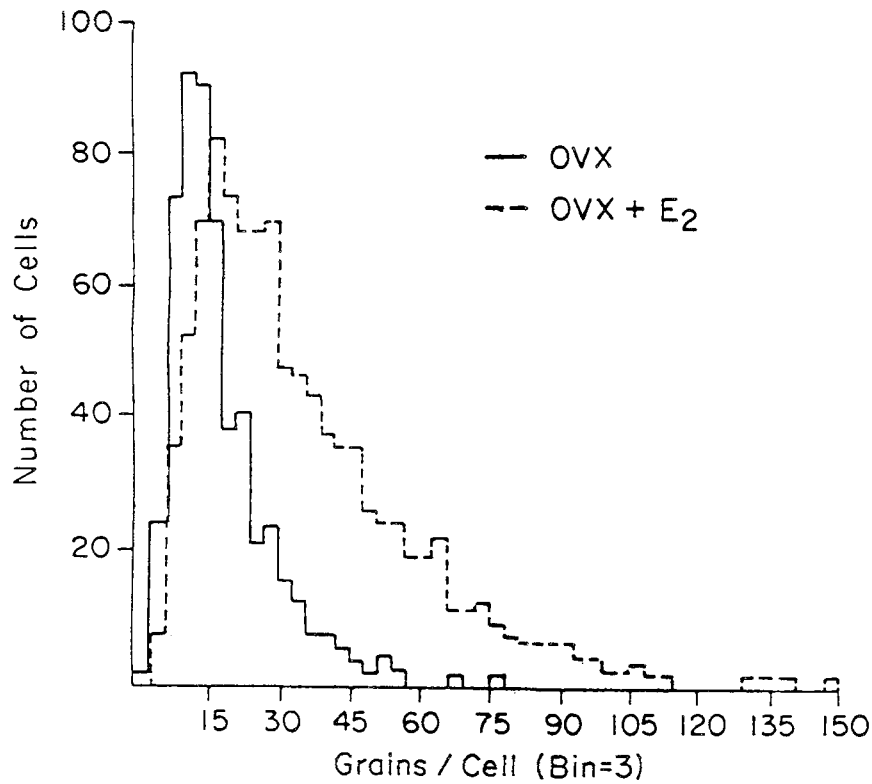


Fig. 4. *In situ* hybridization results showing induction of mRNA for preproenkephalin by estradiol ($E_2$) treatment of ovariectomized (OVX) female rats. Frequency distribution of numbers of grains per labeled cell in the ventromedial hypothalamic nucleus depends both on the numbers of neurons expressing enkephalin mRNA and the concentration per cell. Overall 3.1-fold induction by estradiol results primarily from a large increase in numbers of grains per labeled cell (from Romano et al., 1988).

through increased copies per cell (Romano et al., 1988). Furthermore, enkephalin message induction demonstrated in the female cannot be detected in the male rat, again paralleling the behavior (Romano et al., 1989, submitted). Both the enkephalin mRNA in VMN and the reproductive behavior show an orderly dose-response relation (Lauber et al., 1989). Rate of peptide synthesis can be increased by estradiol: in rats whose ventromedial hypothalamus was infused with S35-methionine and peptides extracted 4 h later, estradiol-treated rats had twice as much labeled met-enkephalin in the ventromedial nucleus as control ovariectomized animals (Mobbs and Pfaff, 1988). Enkephalin-immunoreactive neurons in the ventromedial hypothalamus project to the dorsal portion of the midbrain central gray (Yamano et al., 1986), and so may have their reproductive behavioral effect in that portion of the lordosis behavior neural circuit (Pfaff, 1980; Pfaff and Schwartz-Giblin, 1988). The large literature on opioids and sexual behavior has been reviewed (Pfaus and Gorzalka, 1987a). Met-enkephalin can bind both to mu and delta receptors, but it is more potent at delta receptors (Paterson et al., 1983). Thus, it is crucial that a delta-receptor-active peptide significantly stimulates lordosis behavior (Pfaus and Gorzalka, 1987b), and that this behavioral facilitation can be blocked by naloxone.

Therefore, estrogen increases messenger RNA and peptide levels for an opioid peptide, enkeph-

alin, which, acting through delta receptors, can facilitate female reproductive behavior (Fig. 1). In contrast, estradiol suppresses the message for beta-endorphin (Wilcox and Roberts, 1985; Schachter et al., 1986), and beta-endorphin significantly decreases lordosis behavior (Sirinathsinghji, 1984).

### Luteinizing Hormone Releasing Hormone (LHRH)

Among preoptic neurons, seven days of estradiol treatment to ovariectomized female rats is followed by increased immunocytochemical reaction product (Shivers et al., 1983). *In situ* hybridization was used to demonstrate LHRH gene expressing neurons in the preoptic area and among the fibers of the diagonal bands of Broca (Shivers et al., 1986), and four or more days of estradiol treatment to ovariectomized female rats leads to significant increases in the LHRH message (Rothfeld et al., 1989; in press; Roberts et al., 1989, in press). Some of the LHRH immunostained fibers project to the midbrain central gray (Shivers et al., 1983). There, LHRH leads to increased electrical activity (Samson et al., 1980; Koyama et al., 1987). In vitro electrophysiological recording suggests that many of these electrophysiological effects result from neuromodulation of responses to classical transmitters (Pan et al., 1989; Pan et al., 1986), owing, in part to the amphiphilic character of the LHRH decapeptide (Pan et al., 1989; Pan et al., 1986). Estrogen treatment increases responsiveness of midbrain central gray neurons to LHRH (Schiess et al., 1987). LHRH receptors have been detected in the midbrain central gray (Jennes et al., 1988). In turn, LHRH facilitates lordosis behavior (Pfaff, 1973; Moss and McCann, 1973), acting through nerve cells, since it is effective in hypophysectomized rats. An important site of action is in the midbrain central gray (Riskind and Moss, 1979; Sakuma and Pfaff, 1980). Female rat reproductive behavior can be reduced by an LHRH antagonist (Moss and Dudley, 1980; Sakuma and Pfaff, 1983) or by an LHRH antibody delivered to the midbrain central gray (Moss and Dudley, 1980; Sakuma and Pfaff, 1983). Therefore, long-term estrogen treatment leads to increased amounts of LHRH that, in part through increased electrical activity in the midbrain central gray, facilitates female reproductive behavior (Fig. 1).

### Multiple Mechanisms

In dramatic contrast to hormonally controlled behavior among some invertebrates (Kravitz, 1988), several gene expression systems and modes of hormone action already have been demonstrated to drive female reproductive behavior (Fig. 1). For example, enkephalin mRNA induction by estradiol was rapid, owing primarily to increased copies/cell and remained high with prolonged hormone treatment, whereas progesterone receptor mRNA required 24 h for induction by estradiol, was owing primarily to new cells expressing PR, and did not remain high with prolonged estrogen. What is the meaning of having more than one causal route for a hormone to influence nerve cells, thus to direct a behavior? One simple possibility is redundancy: It is hardly likely that an important form of neuroplasticity would depend on a single factor in the control of a crucial biological function. Second, different neuropeptides and neurotransmitters represent different environmental and physiological influences and constraints upon reproduction.

## Hormone Effects Can Multiply Each Other

A special feature of some cellular mechanisms by which estrogen promotes reproductive behavior is that hormone-influenced molecular and electrical changes are distributed in space and time in such a way that they can multiply effects (Pfaff, 1989). If, for the purposes of reproduction, a particular steroid hormone influence on the nervous system can be thought of

as a "signal," against the "noise" of other variations in neural activity, the impact of multiplicative steroid hormone effects would be to increase the signal/noise ratio.

### Oxytocin

Estrogen effects on mRNA levels and the peptide itself, reviewed above, would be multiplied by effects on electrical discharge, since, for hypothalamic neurosecretory neurons (Poulain and Theodosis, 1988), electrical activity determines hormone release (Fig. 5). Thus, it is important that with in vivo electrophysiological recording, Akaishi and Sakuma found that estradiol increases responsiveness in presumed paraventricular oxytocinergic cells (Akaishi and Sakuma, 1985), and, in vitro, this appears owing in part to larger responses to norepinephrine and dopamine (Akaishi and Sakuma, 1988, in press). Moreover, since released oxytocin can exert predominantly excitatory effects on other oxytocin cells, the effects of increases in electrical activity are amplified further (Yamashita et al., 1987). Hatton and his colleagues have uncovered ultrastructural, electrophysiological, and dye-coupling phenomena that indicate how synthetic and electrical increases in oxytocin neurons could achieve a self-amplifying character (Tweedle and Hatton, 1977; Hatton and Tweedle, 1982; Cobbett and Hatton, 1984; Hatton et al., 1987; Hatton, 1988; Hatton et al., 1989; Montagnese et al., 1987; Theodosis and Poulain, 1987; Montagnese et al., 1988; Hatton et al., 1988). During increased hormone production and demand, increased neuronal cell body appositions, dendritic bundling and the appearance of new double synapses are observed (Tweedle and Hatton, 1977; Hatton and Tweedle, 1982; Cobbett and Hatton, 1984; Hatton et al., 1987; Hatton, 1988; Hatton et al., 1989; Montagnese et al., 1987; Theodosis and Poulain, 1987; Montagnese et al., 1988; Hatton et al., 1988). Increased dye coupling among supraoptic neurons, with associated electrical coupling, indicates that when estrogen increases oxytocin production and electrical activity, effects would be multiplied by enhanced spread of electrical excitation across an oxytocinergic neuronal group. Moreover, effects on oxytocin gene expression and electrical activity in the same neurons would be multiplied further by effects on oxytocin receptors. DeKloet found that estradiol increased the density of oxytocin receptors in the ventromedial hypothalamus (de Kloet et al., 1986; de Kloet et al., 1985), and in vitro electrophysiological recording indicates a predominant excitation (Kow and Pfaff, 1986). Thus, this series of multiplicative steps (Fig. 5) should increase the electrical activity of ventromedial neurons that promote female reproductive behavior. As reviewed above, intraventricular oxytocin injections and applications in the ventromedial hypothalamus do, indeed, increase behavior levels.

### Ventromedial Hypothalamic Nucleus Neurons

Electrical activity of neurons in the ventromedial nucleus of the hypothalamus following estrogen treatment can be causally linked to female reproductive behavior (Kow and Pfaff, 1988). In the ventrolateral portion of the nucleus, neurons strongly accumulate radioactive estradiol. Some of these project directly to central gray (Pfaff, 1968; Pfaff and Keiner, 1973; Morrell and Pfaff, 1982), but in this nucleus, about two-thirds of the synapses are intrinsic (Nishizuka and Pfaff, 1983;1989, in press). Electrophysiological recordings from cells antidromically driven by central gray stimulation are clustered at the rostral tip of the ventromedial nucleus, and these account well for electrophysiological signs of estrogen dependence (Sakuma and Pfaff, 1982; Sakuma and Akaishi, 1987). Ventromedial hypothalamic neurons whose axons run laterally to the midbrain have lower activation thresholds and smaller refractory periods, in estrogen treated animals (Akaishi and Sakuma, 1986). Electrophysiological results agree absolutely with behavioral results,

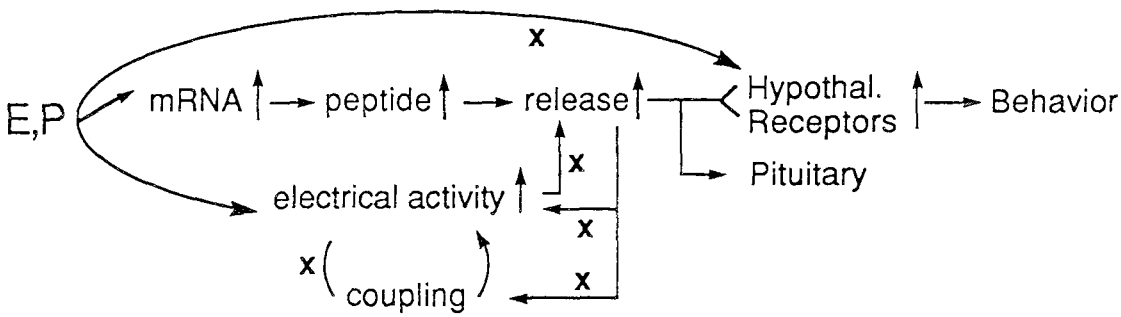


Fig. 5. Multiplicative actions of estradiol and progesterone on oxytocin neuron system. Each effect marked by (x) multiplies the primary effects of these hormones on message and peptide synthesis. Electrical effects amplify the impact of synthetic changes, as far as estrogen actions, through oxytocin, on behavior are concerned. Explanation and references are in text (from Pfaff, 1989).

in which axons running laterally from ventromedial hypothalamus are shown to be most important (Manogue et al., 1980; Malsbury and Daood, 1978; Malsbury et al., 1978).

In ventromedial hypothalamic neurons, electrophysiological effects of estrogen can multiply each other and multiply effectiveness of the hormone when it has altered mRNA and protein synthesis. Long-term estrogen treatment (>10 d) increases the electrical activity in vivo of previously silent or slowly firing neurons (Bueno and Pfaff, 1976; Pfaff, 1983). In turn, pre-exposure to estrogen is necessary for an acute estrogen effect: an immediate depolarization accompanied by a decrease in membrane conductance with a reversal potential close to the $K^+$ equilibrium potential (Oomura et al., 1986; *see also*, Nabekura et al., 1986). Owing to stimulus-secretion coupling, increased electrical activity would mean increased release of the products of hormone-altered mRNA and peptide synthesis, such as enkephalin synthesized in the ventromedial hypothalamus and transported to the midbrain (Romano et al., 1988; Yamano et al., 1986).

### Cascades

Estrogen effects can be multiplicative over time, as well; in a cascade hypothesis for hormone action on neurons (Pfaff, 1980; Pfaff and Schwartz-Giblin, 1988), early responses to temporally discrete applications of the hormone are necessary for later applications to be behaviorally effective. At least two temporally discrete requirements for estrogen nuclear receptor occupation have been detected so far with behavioral techniques (Parsons et al., 1981; 1982; Sodersten et al., 1981; Clark et al., 1983; Clark and Roy, 1987). The concept of multiplicative mechanisms for hormone actions on nerve cells fits the data especially well when "scaling up" from molecular and subcellular phenomena through observations of hormone-addressed neural systems. The marked tendency for estrogen-concentrating neuronal groups to project to other estrogen-concentrating cell groups provides the opportunity for massive amplification of hormone effects, as signals pass through the limbic forebrain and hypothalamus (Cottingham and Pfaff, 1986).

## Concepts Explained

The molecular and electrophysiological mechanisms described have their most obvious application to a simple mammalian behavior like lordosis, but the explanations of other behaviors or behavioral concepts also may be advanced. Consider the concept of motivation. It is logically required for the explanation of certain changes in well-defined responses of adult

organisms to well-defined stimuli (Pfaff, 1982). Since estrogen and progestin effects on lordosis behavior of the adult female rodent change a well-defined response to well-defined stimuli, cellular mechanisms for this behavior are, correspondingly, explanations for sexual motivation, with converging evidence from several laboratories supporting this interpretation (*reviewed in* Pfaff, 1982). Classical ethological concepts, as well, include motivation for instinctive behaviors, thought of by Lorenz (Lorenz, 1950) as a force that leads to the pressure for a particular behavioral response—clearly, mechanisms of estrogen and progestin action on hypothalamic neurons promote instinctive responses such as lordosis, and the circuitry for this behavior provides the physiological realization of Lorenz's model. Even in human experimental psychology, primitive affective reactions to stimuli have been detected (Zajonc, 1980), that are quick responses, binary (pleasant–unpleasant), and that are independent of cognitive reactions. This sort of primitive response is the type of human behavior addressed most obviously by sex hormone-dependent mechanisms in the limbic system and hypothalamus. Indeed, the psychoanalytic concept of libido (Freud, 1962, p. 83) was a quantitatively variable force for the measurement of processes occurring in the domain of sexual excitation. Sexual drive continues to be treated psychoanalytically (Compton, 1983) as belonging to the unconscious and as spanning the border between psychology and physiology. The similarity among psychoanalytic concepts of drives and ethological concepts of instincts suggests that sexual motivation may have a universal quality, analogous to those behavioral universals uncovered in the field of human ethology (Eibl-Eibesfeldt, 1971). Thus, molecular mechanisms of estrogen and progestin effects on hypothalamic neurons contribute forcefully to the explanation of a primitive sexual motivation common in the concepts of experimental behavior analysis, ethology, human psychology, and psychoanalysis.

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