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## Abstract

Research on reproductive behavior, in particular, its dependence on gonadal hormones, has a relatively short history. This chapter starts with an introduction of pioneering idea proposed by Geoffrey Harris in Cambridge in 1950s, to whom the idea of brain control of anterior pituitary can be attributed. The group of Phoenix initiated research on the opposite direction, i.e., the effects of gonadal hormones on the brain on its sexual differentiation, thus established a concept of endocrine control of the brain in enduring and acute manners. Behavioral studies began and continued in earnest by Charles Sawyer in Los Angeles and Masazumi

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Kawakami in Yokohama. A series of elegant experiments initiated by Donald W. Pfaff added substantial understanding of hypothalamic mechanism for estrogen-dependent female rat sexual behavior.

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**Keywords**

Androgen action • Aromatase hypothesis • Estradiol • Electrophysiological recording • Estrogen • Gonadotropin-releasing hormone (GnRH) • Kallmann's syndrome • Lordosis reflex • Proceptive behavior • Solicitatory behavior • Ventromedial nucleus of hypothalamus (VMN)

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**Brief History**

Research on reproductive behavior, in particular, its dependence on gonadal hormones, has a relatively short history. This chapter starts with an introduction of pioneering idea proposed by Geoffrey Harris in Cambridge in 1950s, to whom the idea of brain control of anterior pituitary can be attributed. The group of Phoenix initiated research on the opposite direction, i.e., the effects of gonadal hormones on the brain on its sexual differentiation, thus established a concept of endocrine control of the brain in enduring and acute manners. Behavioral studies began and continued in earnest by Charles Sawyer in Los Angeles and Masazumi Kawakami in Yokohama. A series of elegant experiments initiated by Donald W. Pfaff added substantial understanding of hypothalamic mechanism for estrogen-dependent female rat sexual behavior.

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**Introduction**

In mammals, females produce ova, the larger zygote containing a large amount of metabolic machinery in addition to genetic information. The machinery supports the ontogeny. Thus, females make a considerable investment over a long time period to produce mature ova and carry the burden of impregnation. Demands for males are minimal to produce spermatozoa with genetic information and a limited energy source albeit continuously and in a large number. Successful fertilization depends on the synchronism between the maturation of ova and the insemination as a result of sexual behavior. Thus, in the rat, as in other mammals, sexual behavior occurs periodically and repeatedly at the time of the production of matured ova, concomitant to the maturation of the ovarian follicle from which estrogen is secreted to induce sexual behavior. On the other hand, spermatozoa are produced continuously under the action of androgen, and thus, androgen-controlled male reproductive behavior can be observed constantly.

## Sex Hormone and Sexual Behavior

The phenomena of timed maturation of ova and behavioral estrus in females and the continuous estrus in males are due to the difference in the sensitivity of certain neural circuitry in the brain to estrogen. Besides, the difference is not genetically defined, but, as established experimentally, masculinization of the brain for male reproductive endocrinology and behavior occurs when androgen acts at a specific time during the ontogeny, with some exceptions in certain particular phenotype. This mode of androgen action was reported almost 50 years ago by Phoenix, Goy, Gerall, and Young (*Endocrinology*, September 1959) by showing prenatally administered testosterone propionate masculinizes mating behavior in the female guinea pig. They coined the term “organizing action” for this action of steroid hormones. It may be a sheer coincidence that Kawakami and Sawyer published a series of papers on the next issue of *Endocrinology* (October 1959) on the complementary “activational effects” of steroid hormones, employing, for the first time, electrophysiological methods. The activational effects have no time window and wax and wane depending on circulating titer of hormones. In rodents in general, the presence or absence of androgen, in particular aromatizable androgen like testosterone, during the perinatal period determines the sexual phenotype of the brain. The phenotype is fixed from this time onward despite the disappearance of circulating sex hormones during the juvenile period. At the time of puberty, when massive secretion of gonadal steroids revives and gametogenesis begins, sexual behaviors in both sexes emerge.

## Organizational Action of Estrogen and Aromatase Hypothesis

Sex differences in certain components of female and male sexual behaviors as well as reproductive endocrinology are determined by the endocrine environment during the perinatal period. Administration of androgens to female rats within 5 days of birth generates a male-type brain, and after sexual maturation, they show neither ovarian cyclicity nor feminine sexual behavior but exhibit mounting and territorial behaviors. On the other hand, when male neonates are orchidectomized on the day of birth, they develop female-type brain and behave as a female upon sexual maturation when supplemented with gonadal hormones. In the rodents, estradiol is the active metabolite of testosterone which establishes male phenotype of the brain. Several morphological and electrophysiological changes induced by estradiol are associated with sex-specific behaviors. During the embryonic and neonatal stages, the testis autonomously secretes a vast amount of the hormone; in the embryonic and neonatal ovaries of the rat, the activity of 3 $\beta$ -hydroxysteroid dehydrogenase is markedly lower than in the testes, and production of testosterone, hence also estradiol, is minuscule. According to the aromatase hypothesis, which has been substantiated in the rodents, testosterone is demethylated and aromatized by P450arom in neurons in

specific regions of the brain and converted to estradiol. Estradiol then masculinizes the brain through its action on estrogen receptor  $\alpha$ .

Evidence abounds in the support of aromatase hypothesis in the brain sex differentiation in rodents. Estradiol is as effective as testosterone in establishing male brain phenotype. Male mice with estrogen receptor  $\alpha$  knocked out do not exhibit male-typical aggression. Males of several strains of rats and mice, which lack androgen receptor due to mutation, exhibit male-typical behavior, albeit their external genitalia remain undeveloped. Knockout female mice of  $\alpha$ -fetoprotein, which binds estrogens of maternal and placental origin and prevents them to cross blood–brain barrier, develop male-type brain. Nonsteroidal agonists of estrogen receptor  $\alpha$ , like diethylstilbestrol, are not bound by  $\alpha$ -fetoprotein and enter the brain to masculinize the brain. On the other hand, dihydrotestosterone (DHT), a reduced metabolite of testosterone generated by steroid  $5\alpha$ -reductase, does not masculinize the brain.

It should be noted that the aromatase hypothesis does not apply to monkeys and humans. Several disorders attributed to abnormal steroidogenesis or defects in steroid hormone receptors suggest that the human brain sex may be determined by androgen actions through androgen receptor. Female brain phenotype develops in XY individuals with complete androgen receptor deficiency. This also suggests that Y chromosome itself is not involved in brain sex differentiation. It is yet to be established which of testosterone or DHT is important as the ligand for androgen receptor for the masculinization of human brain. An estrogen-receptor-deficient individual with XY chromosome, who is characterized with the lack of bone maturation, has male gender identity. Besides, human  $\alpha$ -fetoprotein does not bind estrogens. Meanwhile in the rodents, dihydrotestosterone induces territorial and aggressive behaviors through its action on androgen receptor. These phenomena are termed the organizational effects of sex hormones and are distinguished from the activational effects of sex hormones which are observed in response to the rise and fall of the blood sex hormone concentration in sexually matured animals following puberty.

## Sex Difference in Reproductive Endocrinology

For the regulation of reproductive endocrinology after the puberty, neurons producing gonadotropin-releasing hormone (GnRH) in the diagonal band/preoptic area (POA) (in the rat and mouse) or the medial basal hypothalamus (in guinea pig, monkey, and human) constitute the final common pathway for the brain control of gonadotropin secretion in both sexes. Such scheme, that molecules produced in the brain are conveyed by blood stream to the anterior pituitary and regulate its function, has been proposed by Geoffrey W. Harris (1913–1971). This proposal appeared in a series of *The Journal of Physiology* articles published in as early as 1936. They were codified in a famous 1955 monogram “*Neural Control of the Pituitary Gland*” (298 pp, Edward Arnold, London). This insight made him the “father” of neuroendocrinology, an entirely new concept on brain control of endocrine function. The

identification of molecules took many years, and many “neuroendocrinologists” competed for this endeavor. By 1971, the competition was over: GnRH, as we call the molecule for the regulation of luteinizing hormone secretion now, has been identified to be a decapeptide in the pig by Andrew Schally’s group (Matsuo et al. 1971). Roger Guillemin soon followed the similar structure for ovine GnRH. The Nobel Prize in 1977 was conferred to Schally and Guillemin, along with Rosalyn Yalow, for their identification of GnRH and other hypophysiotropic peptides. Their finding predicted that the similar molecule governs the reproductive endocrinology and behavior in pigs, sheep, and probably in man. Indeed, GnRH facilitates female rat sexual behavior (Pfaff 1973). The failure of migration of the GnRH neurons in man causes hypothalamic hypogonadism accompanied with anosmia (Kallmann’s syndrome, Schwanzel-Fukuda and Pfaff 1989). GnRH agonists are now in clinical use for diagnosis and to treat certain reproductive disorders. The major sex difference in the control of gonadotropin secretion is that in the females, the secretion is under alternate negative and positive feedbacks of gonadal steroids, while the positive feedback component is absent in the males. After maturation, when the blood estrogen concentration in an ovariectomized female rat was maintained at a high level by an implantation of indwelling Silastic capsules of estradiol, gonadotropin surge would occur at intervals of 24 h at the beginning of the dark phase in the light/dark cycle of the environment. This phenomenon, which continues for almost five consecutive days after the implantation of the capsule, is considered to be equivalent to the ovulatory surge of gonadotropin in cycling females and characterizes female pattern of gonadotropin secretion; in males, neither estrogen nor androgen induces such surge of gonadotropin.

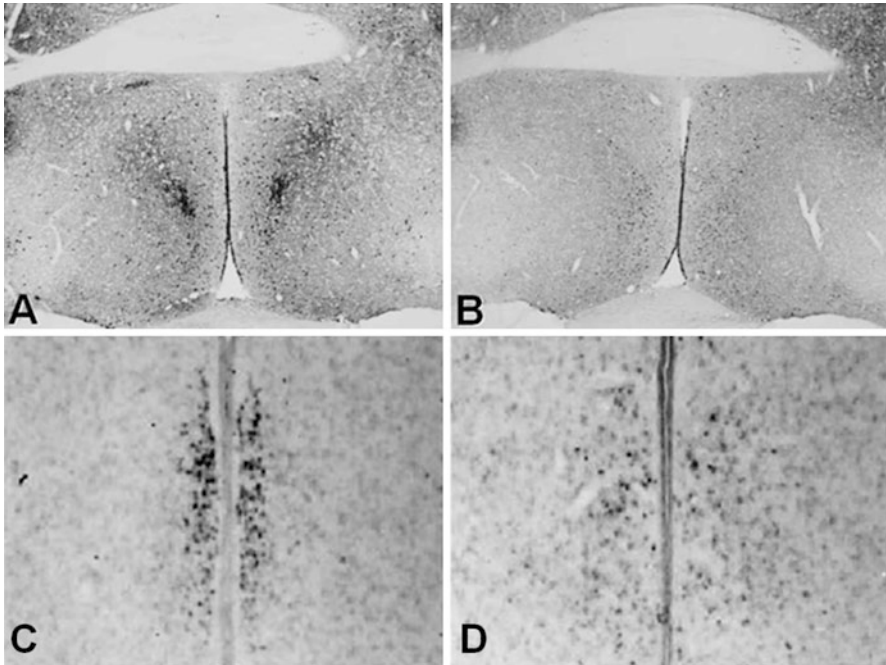
The surge of gonadotropin secretion induced by estrogen in females is thought to be a result of activation of GnRH neurons. Because GnRH neurons do not express nuclear estrogen receptor, the effect of estrogen appears to be mediated upstream by neurons which regulate GnRH neurons. Neurons in the anteroventral periventricular nucleus (AVPV) have been implicated in the ovulatory surge of gonadotropin: they express both estrogen receptor  $\alpha$  and  $\beta$ , and lesions of this structure abolish ovulation. Recently, there have been reported cases of hypogonadotropic hypogonadism with mutations in GPR54 receptor gene on chromosome 19. This drew attention to the association of GPR54 and its ligand kisspeptin in the initiation of puberty. In consideration that 75 % of kisspeptin neurons coexpress GABA, and both kisspeptin and GABA excite adult rat GnRH neurons, further experiments are needed to understand mechanism of cyclic gonadotropin surge females.

## **Morphological Sex Difference of Rat Brain**

The POA is one of the brain structures in which morphological sex differences were initially noted in the rat. The sexually dimorphic nucleus of the rat POA (SDN-POA), which is distinctively larger in males than in females, has been well characterized; its establishment depends on the presence or lack of aromatizable androgens or estrogens during the perinatal period. Structures homologous to the rat

SDN-POA, in which sex steroids play a critical role for the establishment of the sexual phenotype, have been identified in the hamster, gerbil, ferret, guinea pig, monkey, and mouse. Although there is ample evidence of sexual dimorphism in the human brain, there has not yet been any definitive proof that steroids acting early in development masculinize it directly.

On the other hand, the AVPV, which is located rostral to the SDN-POA, contains a larger number of neurons in females than in males in the rat as well as in mouse. The marked sexual dimorphism of the SDN-POA and AVPV is established during a particular period of ontogeny. The male phenotype develops in the presence of aromatizable androgen or estrogen during the neonatal period. The lack of androgen or orchidectomy in male pups results in female phenotype. Endocrine manipulations of adult rats or mice had no effect in either the SDN-POA or AVPV. Neonatal treatment with dihydrotestosterone, a nonaromatizable androgen, does not alter the adult female profile (Fig. 1).



**Fig. 1** Morphological sex differences in the two structures in the preoptic area (POA). Immunohistochemistry against calbindin D28k visualized the sexually dimorphic nucleus of the preoptic area in C57/BL6J mice as has been reported for the rat. The male structure (a) is conspicuous, while the corresponding cell aggregate is vestigial in females (b) in this as well as ddN strain (not shown) of mice (Orikasa C, Sakuma Y (2010) *J Comp Neurol* 518:3618). In situ hybridization of estrogen receptor mRNA in the rat POA visualized opposite phenotype: the anteroventral periventricular nucleus is prominent in females (c) but not in males (d) (Orikasa C et al (2002) *Proc Natl Acad Sci USA* 99:3306)

## **Behavioral Manifestation of Brain Sex Difference**

After puberty, sex hormones secreted by the gonads induce sex-specific behaviors. The differences in behaviors are not due to the difference between testicular androgen and ovarian estrogens but are caused by the different sensitivity of brain circuits to sex hormones between the sexes. This theory is evidenced by the fact that estradiol induces mounting behavior in orchidectomized male rats; likewise, in ovariectomized females, testosterone induces lordosis reflex. Combination of these hormonal treatments with brain lesions or electrical stimulation, or severance of a particular brain circuit, bespeaks involvement of specific brain structures in hormone actions on these sex-specific behaviors. Different structures in the central nervous system are involved in different aspects of sexual behaviors in each sex. Thus, it is not an anomaly that androgen is needed for intromission, ejaculation, or aggression. Similarly, odor-based sexual preference is defined by circulating sex hormone: estradiol induces female pattern of odor preference in adult orchidectomized males, whereas testosterone-treated ovariectomized females showed female-typical preference toward male odor. The existence of multiple facilitatory and inhibitory circuits for different behavioral element is suggested.

## **Courtship, Solicitatory Behavior, or Proceptivity**

Sexual interactions in the rat are initiated and paced by females in estrus through patterns of approach toward and withdrawal from sexually active males. Anestrous female rats burrow in secluded place; when highly estrous, they enter males' territory and display precopulatory hopping, darting, and ear wiggling to provoke males to initiate mounting. This cluster of female behavior, which represents anticipatory and motivational aspects, has been collectively termed proceptive or solicitatory behavior. It is predictable that females exercise their option toward sexually vigorous, healthy males over diseased or less attractive males. Odor cues play an important role to establish these preferences. In the males, olfactory signals terminate aggression toward intruder and may initiate courtship toward females in estrus.

Olfactory signals are received by the olfactory epithelium and the vomeronasal organ, relayed in the amygdala, and eventually converged in the POA. The POA and other basal forebrain structures, which include substantia innominata and accumbens, are often implicated in the regulation of motivated behaviors. Female rats with basal forebrain lesions spend less time in the vicinity of males than the nonlesioned and controls and display an unusually high incidence of rejection behavior in response to attempted mounts by male rats. POA neurons, and not axons of passage, are involved in this effect. An increased locomotion in females in estrus, which depends apparently on estrogen-sensitive POA projections to the midbrain locomotor region, has been considered to embody enhanced sexual motivation. At the same time, basal forebrain structures are targets of ascending mesolimbic dopaminergic system: dopamine has been implicated in the regulation of consummatory components of female rat sexual behavior. Indeed, dopamine

receptor stimulation in the forebrain has been associated with either increased locomotion or sexual motivation.

Increased locomotor activity in female rats in estrus embodies enhanced sexual motivation. The spinal stepping mechanism that induces locomotion is regulated by the midbrain locomotor region (MLR) in the rat as well as in the cat. In the rat, the MLR has been identified in the rostral part of the pedunculopontine and cuneiform nuclei of the midbrain. Estrogen is deafly responsible for the increased locomotion in female rats in estrus because systemic administration of estrogen to ovariectomized rats increases both open-field and wheel-running activities. The brain site for the action of estrogen on the open-field activity is not known, but the medial POA has been positively identified as a site for estrogen-induced activation of wheel running.

The POA contributes to the rostrocaudal neural axis for the locomotor synergy with its heavy projections to the MLR. The preoptic locomotor region, from which stepping can be initiated by chemical or electrical stimulation, is situated in the medial portion of the lateral POA. In contrast, the locomotor activity can be consistently reduced by cholinergic activation of the periventricular medial POA. Although the medial POA projections to the midbrain central gray and VTA are composed of estrogen-receptor-positive and estrogen-sensitive neurons, the affinity of the preoptic projections to the MLR for estrogen is not known. Estrogen-induced, diametrically opposite changes in the excitability of axons in the MLR that originate in two neuronal pools in the female rat POA have been reported.

### **The Lordosis Reflex or Receptivity**

The lordosis reflex, dorsiflexion of the vertebral column in response to touch and pressure on flank, tail base, and perineum given by mounting by male partner, is the major receptive component in the female rat in estrus. Neural circuits associated with facilitation or inhibition of the reflex and the sites of action of estrogen have been revealed, including the reasons for the lack of the reflex in the male. High blood estrogen titer is needed for female rats to exhibit the lordosis reflex. A threshold dose exists for the systemic administration of estradiol to induce the reflex, showing that certain occupancy of estrogen receptor is required. Intracerebral implantation of crystalline estradiol in the ventromedial nucleus of the hypothalamus (VMN) induces the lordosis reflex in ovariectomized female rats pretreated with subthreshold dose of systemic estradiol. The observation suggests that the VMN is one of multiple sites of the brain on which estradiol acts to induce the reflex. Indeed, implants of estradiol in the medial POA in similarly pretreated females are also effective in inducing the reflex, albeit at a larger amount of estrogen. Neurons expressing estrogen receptor (ER) have been identified in the VMN as well as in the POA. Implantation of crystalline estradiol either in the VMN or POA elicits the lordosis reflex in the ovariectomized females which were pretreated with



subthreshold amount of systemic estradiol. Mouse knockouts showed that ER $\alpha$ , and not ER $\beta$ , is needed for the induction of the lordosis reflex.

### **Facilitatory Neural Circuit for the Lordosis Reflex**

For the regulation of the lordosis reflex, estrogen acts differently on the VMN and POA. In ovariectomized animals treated with subthreshold dosage of estradiol, electrical stimulation of the ventrolateral subdivision of the VMN at a low frequency augments the lordosis reflex. This subdivision of the VMN contains a large number of ER $\alpha$ -expressing neurons. Electrolytic lesions of this structure, on the other hand, diminish the reflex. In conjunction with the results of localized implants of crystalline estradiol in the VMN, it was concluded that estrogen-sensitive efferents of the VMN facilitate the lordosis reflex. The major efferent of the VMN descends in the medial forebrain bundle to innervate neurons in the midbrain central gray via excitatory synapses. The projection, in turn, excites the neurons in the gigantocellular nucleus of the medulla, the origin of the medullospinal tract, which eventually reaches spinal motor neurons. The lordosis reflex is the contraction of the epaxial muscles caused by the excitation of spinal motor neurons.

Although the lordosis reflex can be elicited solely by estrogen in experimental settings, progesterone is needed to induce a full set of female rat sexual behavior. Progesterone receptor (PR) can be induced by estrogen in the VMN in addition to the POA. PR, in turn, can be activated by locally generated neurosteroids, like allopregnanolone or 3 $\alpha$ ,21-dihydroxy-5 $\alpha$ -pregnan-20-one (THDOC). PR activation can be also modulated by the neurotransmitter dopamine via D1 or D5 receptor. In situ hybridization visualized D1 but not D5 dopamine receptor mRNAs in the VMN; the induction of the lordosis by microinjection of D5 agonists into the VMN suggests the functional presence of D5 dopamine receptors in this structure. A cellular compartment analysis of temporal activity by fluorescent in situ hybridization (catFISH) demonstrated separate, but intermingled, subsets of neurons in the ventrolateral subdivision of the VMN of male mice, which are excited separately in aggression or during sexual behavior. Interestingly enough, optogenetic, but not electrical, stimulation of this structure induced aggression in male mice expressing channelrhodopsin-2 in this structure by stereotactic injection of adeno-associated viral vectors.

The distribution of neurons expressing ER $\alpha$  in the brain has been investigated, and a common pattern emerged across vertebrate species, which encompass many species of amphibian, lizard, and mammals, to name a few. ER $\alpha$ -positive neurons have been identified in all animals in the ventrolateral portion of the VMN, medial POA, midbrain central gray, and medial amygdala. Some theorize that these structures constitute interconnected webs of estrogen-sensitive neurons in the brain, and it is appropriate to speculate that they are involved in regulation of the endocrine

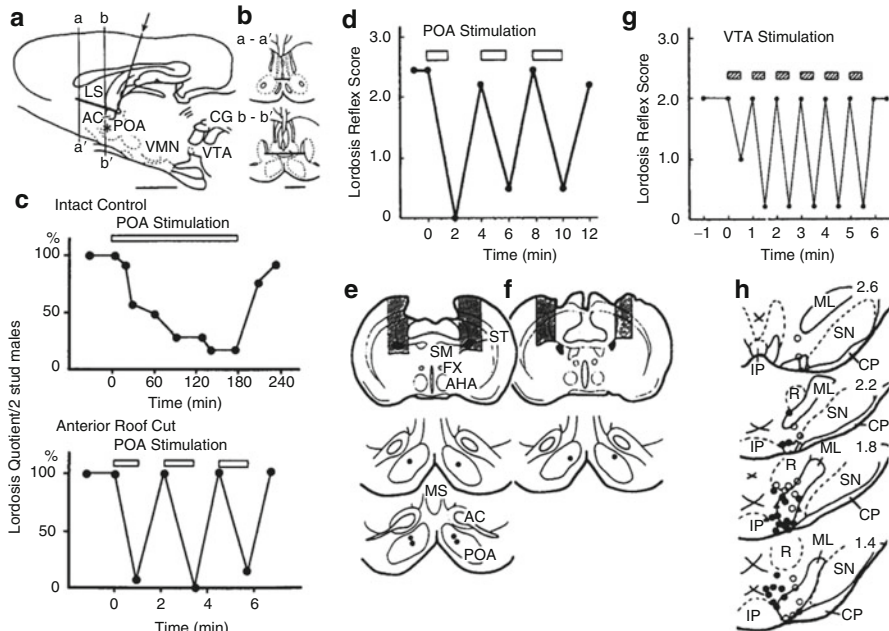
system and behavior as in the case of the VMN and central gray. The connection of the POA and the VTA may be another example. Recent addition of ER $\beta$  and other estrogen-sensitive signal pathways, however, may require additional interpretations.

### **Inhibitory Neural Circuit for the Lordosis Reflex**

The POA is a heterogeneous structure containing local neurons, projection neurons with different projection targets, and axons of passage, among others. Even among ER $\alpha$ -positive neurons, ER $\alpha$  expression is regulated by different promoters between those in the SDN-POA and those in the area surrounding this particular structure. This heterogeneity caused confusion on the role of the POA on the lordosis reflex in early studies which employed lesion of this structure. Because POA lesions diminish female rats' capability in their odor-based preference toward males, sexual motivation in general, and develop rejections, male partners are unable to apply somatosensory stimuli to females to elicit the lordosis reflex. This has been interpreted impertinently as the loss of the reflex caused by POA lesion.

In highly estrous female rats, electrical stimulation of the medial POA inhibits the lordosis reflex. The summed effect of the excitation of local neurons and axons of passage is a slow and persistent inhibition of the reflex. Involvement of local POA neurons is evidenced in animals in which the septum and medial POA were disconnected, thus removing descending afferents of the POA. Electrical stimulation of the deafferented POA caused a prompt and powerful inhibition of the lordosis reflex. Conversely, in animals given stereotaxic infusion of ibotenic acid in the POA to remove local neurons without damaging axons of passage, the threshold amount of estrogen for the induction of the reflex is decreased and the inhibitory effect of POA stimulation on the reflex persists. Additional deafferentation of the ibotenic-acid-lesioned POA diminished the entire effect of the POA stimulation. It was noted that selective disruption of the stria terminalis, but not total deafferentation of the POA, raised the amount of estradiol to induce the reflex, showing that the stria fibers, with their origin in the amygdala and which reach the POA, convey facilitatory estrogen action on the lordosis reflex. In these animals with the stria disruption, the inhibitory effect of POA stimulation on the lordosis is enhanced. Thus, while afferents of the POA from the septum and, presumably, those from the cingulate cortex are inhibitory on the lordosis reflex, those from the amygdala via the stria terminalis convey facilitatory effect of estrogen on the reflex. The deafferentation of the POA, or electrolytic lesion of this structure in the castrated male rat, when combined with a large dose of estradiol, renders them to show lordosis. These studies indicate that, not only in females but also in males, neurons and the fibers of passage in the POA are inhibitory on the reflex.

Electrical stimulation of the VTA, one of major targets of the POA efferents, also produces a quick and strong inhibition of the lordosis reflex. The magnitude and the time course of the inhibition resemble the effect of POA stimulation in which animals were deprived of stria projection to the POA. Although the VTA is widely known as the origin of the ascending mesolimbic dopaminergic projection, the



**Fig. 2** The horizontal roof cut (as shown in the sagittal section of the brain in (a); in the frontal section in (b) disrupts connection between the septum and the preoptic area (POA), diminishing axons of passage in the POA. Electrical stimulation of the POA in the control female rats caused a gradual suppression of the lordosis reflex (c, top panel); in animals carrying the roof cut, prompt and strong suppression of the reflex induced the POA stimulation (c, bottom panel, note the difference in the time scale between the two panels). POA stimulation causes the similar prompt suppression of the reflex (d) when the stria terminalis has been cut by a small knife (e vs. control in f). Electrical stimulation of the ventral tegmental area, one of the projection targets of the POA, also causes an acute suppression of the reflex (g). Stimulation sites in the VTA are shown (h) (Original data appeared in Takeo T et al (1993) *Physiol Behav* 53:831; Hasegawa T et al (1991) *Physiol Behav* 50:1033)

stimulation effect persists in female rats in which dopaminergic transmission is interrupted by pharmacological intervention. Descending projection of the VTA, which is known to bias extrapyramidal motor control and produce muscle atonia, may be involved in the stimulation effect of this structure on lordosis (Fig. 2).

### Electrophysiological Recording Studies

The results of behavioral studies employing electrical stimulation, lesion, or knife cuts suggested two independent estrogen-sensitive mechanisms for the control of the lordosis. One originates in the VMN and descends to the medulla via the midbrain central gray; another projection arises in the POA and is relayed to the VTA on its way to the dorsolateral tegmentum. Behavioral results suggest that estrogen excites

the VMN system while inhibits the POA system. As predicted, in the ovariectomized female rat, estradiol decreases antidromic activation threshold of VMN neurons in response to central gray stimulation, whereas estradiol increases the threshold for activation of POA neurons from the VTA. Estrogen treatment induced similar changes in neonatally orchidectomized males but not in females treated with testosterone as pups. Thus, behavioral capability to show lordosis in response to estradiol treatment parallels to changes in neuronal excitability.

The firing rate of POA neurons changes in association with bouts of sexual activity. At least four types of POA neurons have been identified when single unit recordings were made in female rats engaging in sexual interactions: the first group of neurons increased the firing rate when the female rats initiated proceptive behavior and ceased firing when they were penetrated, the second showed somatosensory response to the male mounts, the third fired in response to intromission, and the last group were inhibited prior to and throughout the display of lordosis reflex. Neurons in groups 1–3 are in the transitional region between the medial and lateral POAs. Group 4 neurons are located more medially in the medial POA. Disruption of dopaminergic transmission diminishes firing in group 1 neurons and abolishes proceptivity: their activity appeared to embody the motivational state of the animal with an implication for a consummatory value of penile intromission. Visceral or somatosensory inputs may be responsible for the activity of groups 2 and 3 neurons. Neurons in the last group behaved exactly as if they inhibit the execution of the lordosis reflex. Thus, recordings in free-moving animals revealed separate sets of POA neurons, each specifically associated with proceptive and receptive components of female rat sexual behavior.

## Mode of Action of Estrogen

Neuronal excitability is determined by changes in the membrane conductance or in the intracellular signal transduction pathway, which is elicited by exogenous ligands mediating second messengers, such as calcium and cyclic nucleotides. In particular, sodium and potassium channels define neuronal excitability and activity. Potassium channels in mammalian neurons are roughly classified into large conductance voltage-/Ca<sup>2+</sup>-gated BK and small and intermediate conductance Ca<sup>2+</sup>-activated SK channels. Estradiol binds to the b1 subunit of the BK and activates the channel to induce relaxation of vascular smooth muscle. In medial POA neurons, the suppression of SK channels by  $\alpha 1$  adrenergic receptors is enhanced by estrogen. SK channels regulate the phasic discharge activity often observed in hypothalamic neurons via the control of afterhyperpolarization. It has been recently shown that SK channels expressed in GnRH neurons are involved in the regulation of discharge activity that is characteristic to these neurons. In addition, there is a report showing that estrogen activates G protein-coupled inwardly rectifying potassium channels and inhibits the coupling with GABA<sub>B</sub> receptors or m opiate receptors. In the mouse GnRH neuronal cell line GT1-7, estradiol at physiological doses increases in BK current through the action on ER $\beta$ . Perhaps, It may be that estrogen-mediated

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site-specific regulation of neuronal excitability and eventually the behavioral control can be explained at the level of Na, K, and other channels.

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