PERGAMON

Journal of Steroid Biochemistry & Molecular Biology 74 (2000) 365–373

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

Estrogens, brain and behavior: studies in fundamental neurobiology and observations related to women's health

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Abstract

Mechanisms and consequences of the effects of estrogen on the brain have been studied both at the fundamental level and with therapeutic applications in mind. Estrogenic hormones binding in particular neurons in a limbic-hypothalamic system and their effects on the electrophysiology and molecular biology of medial hypothalamic neurons were central in establishing the first circuit for a mammalian behavior, the female-typical mating behavior, lordosis. Notably, the ability of estradiol to facilitate transcription from six genes whose products are important for lordosis behavior proved that hormones can turn on genes in specific neurons at specific times, with sensible behavioral consequences. The use of a gene knockout for estrogen receptor α (ERα) revealed that homozygous mutant females simply would not do lordosis behavior and instead were extremely aggressive, thus identifying a specific gene as essential for a mammalian social behavior. In dramatic contrast, ERβ knockout females can exhibit normal lordosis behavior. With the understanding, in considerable mechanistic detail, of how the behavior is *produced*, now we are also studying brain mechanisms for the biologically adaptive influences which *constrain reproductive behavior*. With respect to cold temperatures and other environmental or metabolic circumstances which are not consistent with successful reproduction, we are interested in thyroid hormone effects in the brain. Competitive relations between two types of transcription factors — thyroid hormone receptors and estrogen receptors have the potential of subserving the blocking effects of inappropriate environmental circumstances on female reproductive behaviors. TRs can compete with ERα both for DNA binding to consensus and physiological EREs and for nuclear coactivators. In the presence of both TRs and ERs, in transfection studies, thyroid hormone coadministration can reduce estrogen-stimulated transcription. These competitive relations apparently have behavioral consequences, as thyroid hormones will reduce lordosis, and a TRβ gene knockout will increase it. In sum, we not only know several genes that participate in the selective control of this sex behavior, but also, for two genes, we know the causal routes. Estrogenic hormones are also the foci of widespread attention for their potential therapeutic effects improving, for example, certain aspects of mood and cognition. The former has an efficient animal analog, demonstrated by the positive effects of estrogen in the Porsolt forced swim test. The latter almost certainly depends upon trophic actions of estrogen on several fundamental features of nerve cell survival and growth. The hypothesis is raised that the synaptic effects of estrogens are secondary to the trophic actions of this type of hormone in the nucleus and nerve cell body.

Keywords: Estrogen; Women; Hormone

1. Fundamental neurobiology: neuroendocrine mechanisms for a mammalian behavior

1.1. Hormone binding, genes activated, and neural circuitry for the execution of a behavior

1.1.1. Hormone binding phenomena

The initial entry into mechanisms by which hormones could facilitate instinctive behaviors was by the examination of radioactive estrogen binding properties expressed by neurons in the forebrain [1]. Viewed from a 1999 perspective, the retention of labeled estrogens revealed the functions of both ERα and ERβ, as well as other neuronal hormone binding components if such exist. Our autoradiographic studies demonstrated a limbic-hypothalamic system which included both estrogen-

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0960-0760/00/$ - see front matter
PII: S0960-0760(00)00114-X

binding neurons and androgen-binding neurons. Moreover, there is a distinct neuroanatomical tendency for sex hormone binding neurons to project to other neuronal groups which also contain sex hormone-binding neurons [2]; reviewed in [3]. This limbic-hypothalamic system of neurons expressing functional estrogen receptors is conserved impressively among vertebrate forms ranging from fish through amphibia, reptiles, birds, and mammals, including nonhuman primates and human brain tissue (Fig. 1; reviewed in [4]).

1.1.2. Lordosis behavior circuitry

Three features of estrogen-dependent behaviors permitted the neural circuit for the primary female reproductive behavior, lordosis, to be the first unraveled among vertebrate behaviors [5]. These were (1) the relative simplicity of the sensory determinants of lordosis behavior; (2) the relatively simple topography of the motor response; and (3) that the hormone-binding neurons referred to above allowed us to use all of the tools of biochemical and molecular endocrinology to analyze the hypothalamic portions of the circuit. In the neural circuit for lordosis behavior (Fig. 2) the sensory information ascending in an obligatory supraspinal control loop travels in the anterolateral columns of the spinal cord and distributes in certain regions of the medullary reticular formation, lateral vestibular nucleus and midbrain periaqueductal gray. Hypothalamic estrogen-facilitated neuronal activity enables the top of the circuit to operate properly. On the motor side of the circuit, neurons descending from the midbrain periaqueductal gray activate certain medullary reticular spinal neurons which, in turn, synergize with lateral vestibulospinal neurons. The combined influences of these two descending systems permit a spinal cord throughput from behaviorally adequate sensory input to activate the motoneurons for the deep back muscles which execute lordosis behavior.

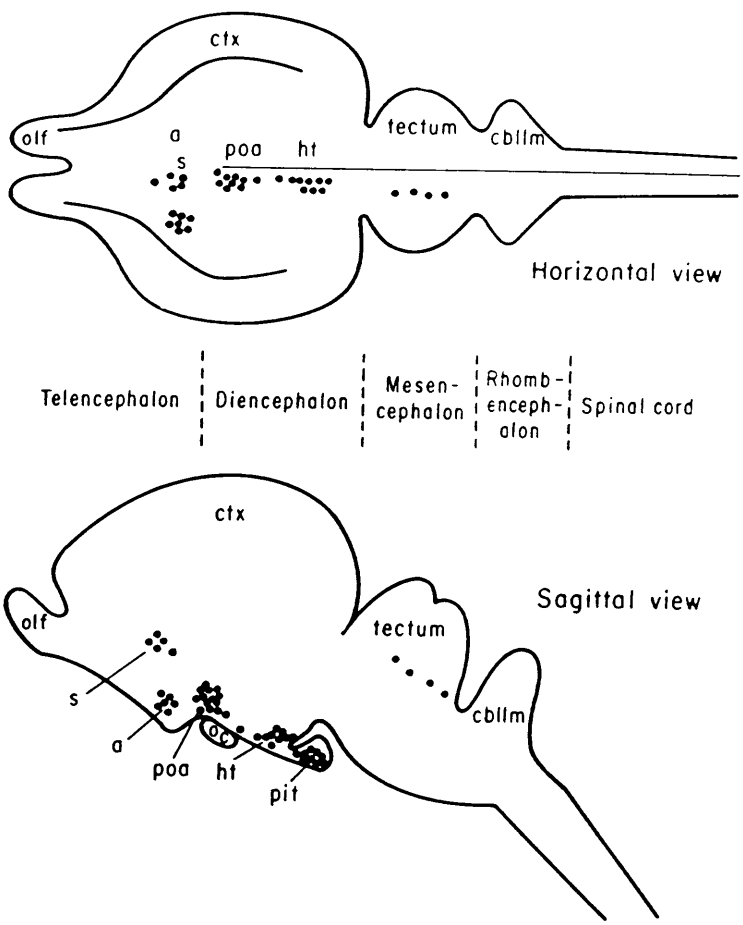

Fig. 1. Abstract representation of a 'generalized vertebrate brain' showing locations of estrogen-binding neurons common to all vertebrates so far studied. The schematic drawings, adapted from those of Walle J.H. Nauta and Harvey Karten, show a horizontal view (top drawing) and a sagittal view from the left (bottom drawing). Features of the limbic-hypothalamic system of estrogen-binding neurons [1,2] include labeled cells in the septum (s), the amygdala (a), the preoptic area (poa), the tuberal hypothalamus (ht), and the ventrolateral quadrant of the midbrain periaqueductal gray. Additionally, the anterior pituitary (pit) is well supplied with estrogen-binding cells. From [4].

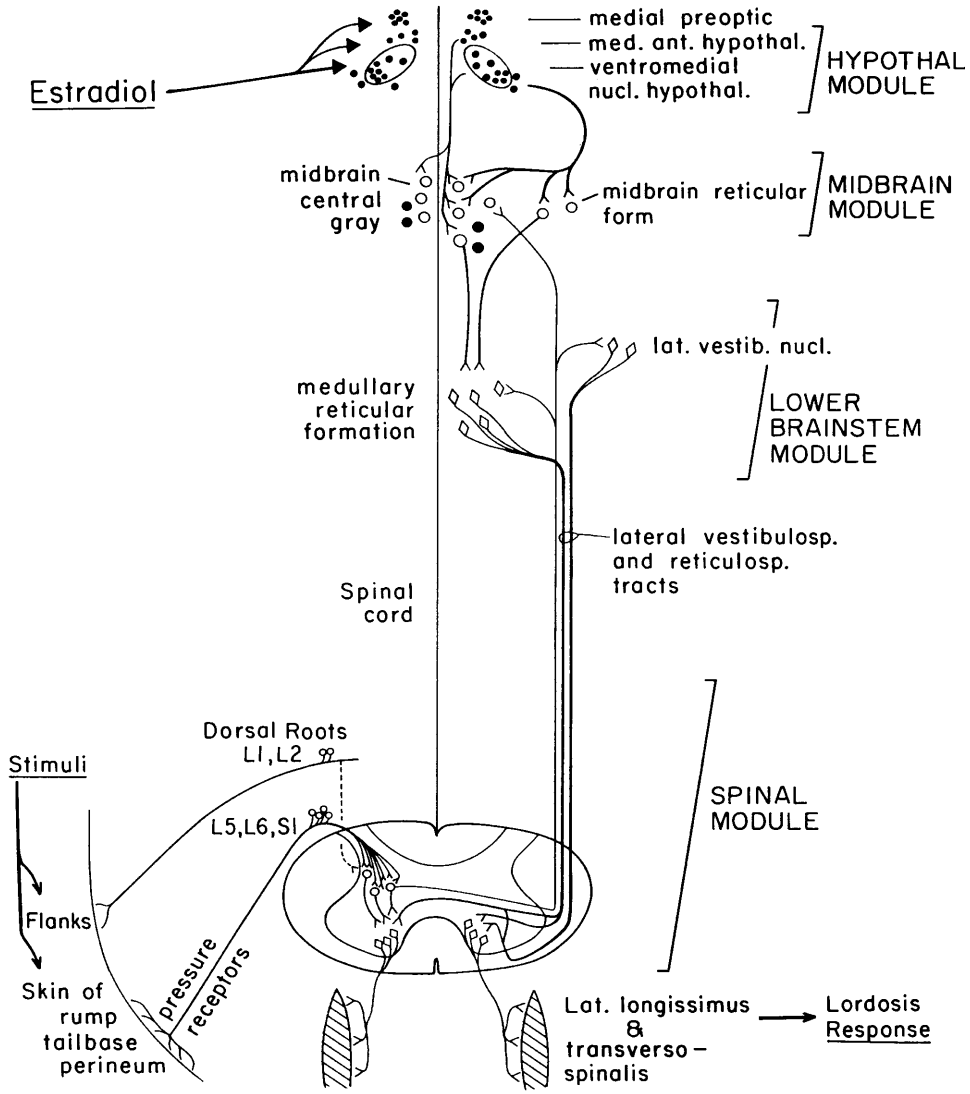

Fig. 2. The basic neural circuit required for producing the elementary, female-typical reproductive behavior, lordosis, especially prominent in female rodents [5,6]. The functional modules indicated on the right, and defined by both neuroanatomical and neurophysiological data, match fundamental embryological segments of the neuraxis.

At two different levels of detail, on two different time scales, we can see how mechanisms for this simple social behavior are fostered by 'hormone-dependent behavioral funnels' [6]. First, on a longer time scale, sex hormone-dependent communications between male and female ensure that reproductively competent conspecifics, and only they, get together. Second, on a very brief time scale, the participation of the lateral vestibular nucleus in lordosis behavior circuitry [5] gives an excellent clue as to how courtship 'proceptive' behaviors by the female rodent help to pre-activate the neural circuitry for lordosis behavior itself.

Since the facilitation of lordosis behavior by estrogens requires new RNA and protein synthesis (reviewed in [5]), the question of hormone-dependent gene expression in the brain, a subject which has occupied our lab for 15 years, became the most prominent subject for new study.

1.1.3. Genes turned on by hormones in the brain

A series of investigations using in situ hybridization, northern blots and slot blots revealed that at least six genes have the following characteristic. They are turned on by estrogens, and their products foster female reproductive behaviors. Thus, their hormone-facilitated transcription could comprise part of the mechanisms by which estrogens foster female sex behavior (Fig. 3; reviewed in [6]). These were the first genes shown to be turned on in hypothalamic neurons in a manner important for a particular behavior. As well, the large amount of data bearing on the genes coding for enkephalin and the oxytocin receptor allow us to claim

that, in these cases, hormones can turn on genes at a specific time and a specific place in the brain in a manner important for behavior. That is, under the influence of estrogens, genes for enkephalins working through opioid peptide receptors and oxytocin, working through the oxytocin receptor, facilitate the performance of a reproductive behavior sequence.

The gene for GnRH deserves special attention because of its overwhelming importance for the endocrine and behavioral aspects of sexuality. All the vertebrates use GnRH in this way and, considering all vertebrate forms, three families of peptides have been reported: GnRH as expressed in telencephalic neurons (having migrated from the olfactory placode; [7]), a hypothalamic moiety of GnRH which is important for controlling pituitary function, and a mesencephalic form of GnRH. Importantly, the ability of GnRH to foster female reproductive behavior [8,9] provided the first example of a neuropeptide whose behavioral effects are consonant with its peripheral physiological effects.

1.2. Biologically adaptive constraints on reproductive behavior mechanisms

1.2.1. 'The formula' for constraining, as well as for producing female reproductive behaviors

Since, female mammals must invest so much time and biological energy in the reproductive processes, it is crucial to understand not only the mechanisms for producing female sex behaviors, but also the biological factors constraining these behaviors in an adaptive fashion. That is, not only must nutrition be adequate, water supplies abundant, and salt available, but also nesting material must be at hand and environmental stressors must be acceptably low. In some seasonal breeders, day length is crucial, and in many species, clock time during the day is important. Finally, the factor that got our attention because of its possible linkage to an attractive molecular mechanism is the role of environmental cold temperature in reducing reproductive frequency. It seemed possible that elevated thyroid hormone levels, circulating as a result of severe

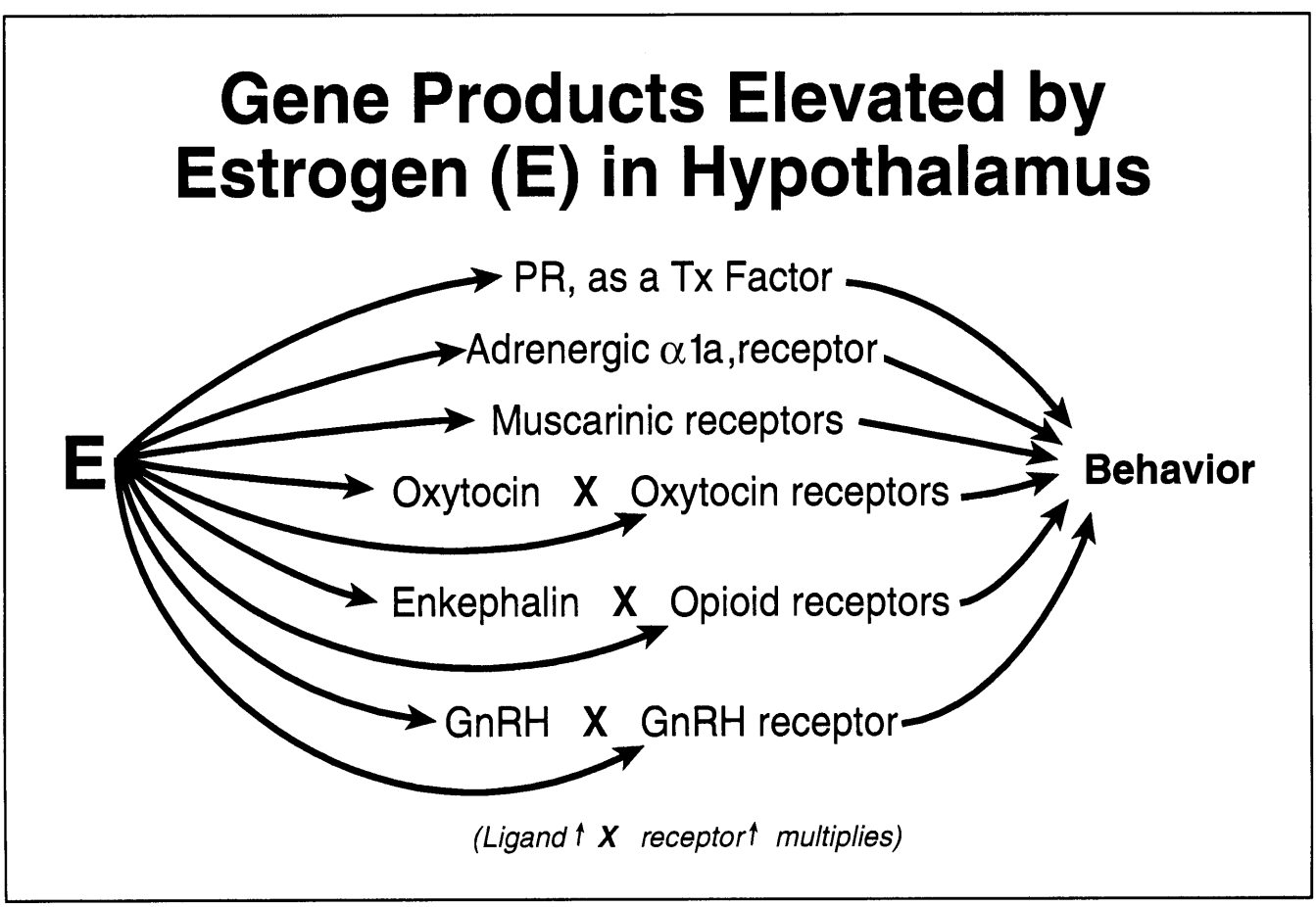

Fig. 3. More than six genes have the characteristics that their expression is elevated by estradiol (E) treatment and that their gene products promote female-typical reproductive behaviors. Note that in some cases, both the gene for a receptor ligand and the gene for the receptor itself are affected by E in this way. Thus, such E effects have the capacity to multiply each other. From [6].

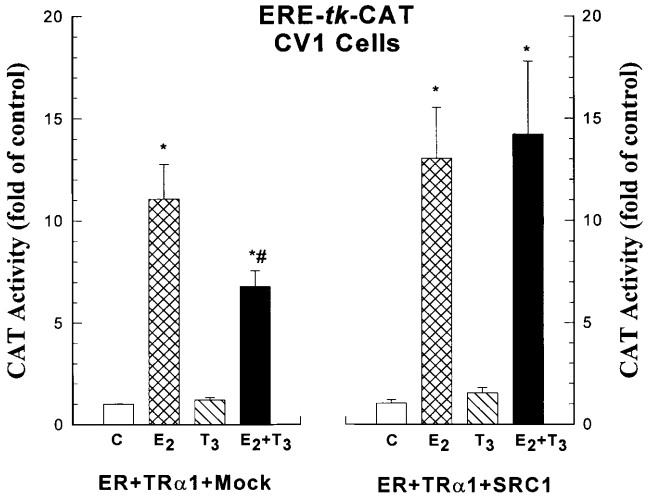

Fig. 4. The inhibition of estrogen-induced CAT activity by ligand-TRα1 in a construct containing the CAT reporter gene controlled by the minimum thymidine kinase promoter and a consensus estrogen response element (ERE-*tk*-CAT) is reversed by cotransfection of a cofactor, SRC1 [15]. Cotransfection of ERE-*tk*-CAT with ERα, TRα1, SRC1 and a β-gal expression vector in CV-1 cells were carried out as described [11]. The transfected cells were treated with vehicle control (C) or 17β-estradiol (E_2, 10^{-7} M), or triodothyronine (T_3 10^{-6} M), or E_2 plus T_3 for 48 h. The cells were harvested, cellular extracts prepared and CAT activity assayed. The CAT activity was normalized to the β-gal units and presented as fold of vehicle control. The values are the mean ± S.E.M. of four to six samples. $*P < 0.05$ compared with the corresponding control (C); # $P < 0.05$ compared with the corresponding E_2 treatment (Student–Newman–Kuels test). From [15].

cold, could lead to increased concentrations of liganded thyroid hormone receptors in neurons, which could then interfere with estrogen receptor functions, at least through competitive DNA binding. We have tested these hypotheses with a long series of molecular and behavioral studies.

1.2.2. Molecular mechanisms: competitive DNA binding of TR against ER and transcriptional interference by thyroid hormones in the presence of TR and ER

Extending previous demonstrations [10] we found that thyroid hormone receptors could bind to Estrogen Response Elements [11]. Moreover, we found that coadministration of thyroid hormones could partially inhibit transcriptional facilitation by estrogens working through ERα, if and only if TRs were present [11]. Under a variety of circumstances of assay, TR isoforms β1, β2 and α1 were able to subserve this transcriptional interference action against ER [11–13].

The theoretical requirement that TR's and ER's can be found in the same hypothalamic neuron has been satisfied [14]. That is, by the use of double in situ hybridization, Kami Kia and his colleagues have found that a high percentage of hypothalamic neurons which express the messenger RNA for ERα also express mRNA's for TRα moieties.

It is clear, however, that potential mechanisms for transcriptional interference of ER functions by TR's need not be limited to competitive DNA binding. For example, transfection studies using a p-box mutant of TRα were able to replicate the transcriptional interference effect observed with wild type TRs [11]. In this connection, therefore, it is notable that competition for a coactivator such as SRC1 [15] (see Fig. 4) provides an additional type of mechanism by which this interference could take place.

It is unlikely that the molecular mechanisms involved depend exclusively on the nuclear co-repressor NCOR, because the data above deal with transcriptional interference triggered by thyroid hormone administration, while NCOR works in the unliganded state. New questions for experiments include asking whether environmental cold is actually the physiologically relevant signal for the transcriptional and behavioral interference described above and below, and the investigation of the full range of mechanisms beyond competitive DNA binding.

1.2.3. Thyroid hormone effects on estrogen-stimulated reproductive behaviors

As predicted, both in female rats [16] and in female mice [17] thyroid hormone coadministration can significantly reduce estrogen-stimulated female reproductive behaviors. In female rats, the studies demonstrating elevated lordosis behavior in thyroidectomized animals were particularly incisive because, by definition, those data revealed the effects of thyroid hormones in their physiological range, and because one would not usually expect that removing another endocrine gland (the thyroid in addition to the ovaries) should actually make reproductive behavior better.

1.3. Genes and behavior

1.3.1. Theoretical difficulties of drawing causal routes between genes and behavior

The subtleties involved in charting causal routes between mammalian genes and mammalian behavioral responses have been illustrated in [18]. Briefly, the pleiotropy of gene action (that is, the ability of an individual gene to serve several functions, especially during different epochs of the life cycle), the redundancy (overlapping functions) among different genes, and the difficulties of understanding different degrees of penetrance among different genes. All render it extremely difficult to construct quantitative gene dose/response relationships. Furthermore, boundary constraints upon the impact of any given gene on any

given behavior, illustrated in Section 3 below, will require even more work to discover.

1.3.2. Clear examples of genes crucial for mammalian social behavior

In spite of the difficulties referred to above, it has been possible to provide an 'existence proof': the demonstration of the clear effect of an individual gene on a mammalian social behavior. In collaboration with Professor Ken Korach, we have shown that a knockout of the classical estrogen receptor gene (ERα) virtually abolishes lordosis behavior [19] and that this massive behavioral effect is not simply due to abnormalities in the circulating sex steroids [20].

In dramatic contrast, our collaboration with Professor Jan-Ake Gustafsson, using the new ERβ gene knockout, has revealed that females bearing the disrupted ERβ gene show lordosis behavior at least as good as normal [21]. It is worth noting that with this instinctive behavior and with locomotor activity both in the normal range, such assays provide a set of 'controls' from which to launch studies with Korach and Gustafsson, on the controls over cognitive and emotional behaviors.

1.3.3. Physiological specificity of gene/behavior relations

Interestingly, it is not the case that a given gene influences a given mammalian behavior under all circumstances. Instead, there are restrictions on the occasions where genes influence mammalian social behaviors. Five examples from our recent work are given. (a) Contrasting the effects of ERα gene knockout in *masculinizing* a female mouse's behavior and yet *feminizing* a male mouse's behavior [19,20,22,23], it became clear that the effect of a gene on a given behavior can depend upon the gender in which the knockout is manifest. (b) Comparing the effects of an estrogen receptor-α gene knockout, generalized throughout the body and permanent in the animal's life history [19,20,23] with the effects of an antisense DNA knockdown of ERα mRNA in the neonatal rat hypothalamus [24] shows us that the effect of a given gene on a given behavior can depend upon *when and where* the gene is expressed. (c) Effects of an oxytocin gene knockout on parental behaviors can depend upon the *level of ambient environmental stress* [25]. (d) Effects of an estrogen receptor-α gene knockout on a female mouse's aggressive behavior can depend upon the *gender of the opponent* (unpublished data); that is, ERKO females vigorously attacked intruder females but not intruder males. (e) In an ERβ knockout experiment, the quantitative comparison between the knockouts and their wild type controls with respect to level of *aggression depended upon their social experience of aggression* — that is, there was a significant interaction between social experience and genotype [21].

Indeed, it may be because of such complex dependencies that a surprising difference between the phenotypes of two particular gene knockouts has arisen. The thyroid hormone receptor beta (TRβ) knockout, achieved and analyzed by Professor Douglas Forrest and his colleagues [26] has been compared in our lab with the TRα1 gene knockout achieved and analyzed by Professor Bjorn Vennstrom and his colleagues [27]. Astoundingly, even though the TRβ knockout yielded the expected effect on reproductive behavior, a significant increase in lordosis as compared with wild type controls [28], the TRα1 knockout showed a change in the opposite direction. The reasons for this remain to be explored.

As a result of all of this work, we not only know about certain genes influencing a specific mammalian sexual behavior, but also know their causal routes, ERα facilitating lordosis behavior by acting as a transcription factor in hypothalamic neurons, whereas TRβ reduces lordosis behavior by interfering with ERα actions.

1.3.4. Genes interacting with internal signals interacting with external signals

Explicit in these results are the demonstrations of interactions between genes (G), internal signals (IN), and external or environmental signals (EX). The most brutally clear example of this is that a female but not a male (G), hormonally primed by estrogens and progestins (IN) will respond to somatosensory stimuli from the male (EX) with female-typical reproductive behaviors.

1.3.5. Physiological integration achieved

As a result of all the mechanisms reviewed above, the nervous system accomplishes physiological integrations both across its inputs and outputs (Fig. 5). That is, under permissive environmental conditions, sex hormone signals allow the animal to respond to somatosensory input from the male. This is integration across *inputs*. On the *output* side, lordosis behavior and the ovulatory discharge of luteinizing hormone are synchronized in a perfectly adaptive fashion; the female should only engage in reproductive behaviors when freshly released eggs are ready for fertilization. The autonomic outputs provide the circulatory and muscular platform, physiologically, for vigorous reproductive behaviors to occur.

1.4. Implications for underlying states of motivation and arousal

From the starkly logical definition of a motivational state, the very strong facilitation of lordosis behavior by estrogens and progestins, ipso facto, shows that these hormones strongly increase the level of sexual motivation [6]. Moreover, the experimental use of arbitrarily chosen operant responses reveals that female rats and mice do indeed display the motivation to approach males ([29], and Matthews, unpublished data). Thus, in explaining a major dichotomy of animal behavior — what makes an animal initiate an activity from a state of inactivity — revealing mechanisms of hormone effects on lordosis behavior essentially solves one version of the problem. Further, because of the tremendous degree of conservation of neuroendocrine mechanisms from animal brain into human brain, we can claim [6] that the mechanisms spelled out to date explain the biological side of the concept of libido. That is, even as Freud thought of libido as having both a physiological and a psychological underpinning, the mechanisms analyzed here deal only with the underlying primitive biological mechanisms.

A review of the literature on mechanisms of motivation [30] reveals that the generalized aspects of motivational states are coextensive with arousal. Since sex hormones do, in fact, directly address all the five brainstem neuronal systems which subserve arousal mechanisms (noradrenergic, dopaminergic, serotonergic, histaminergic, and cholinergic) the histochemical basis is demonstrated, for sex hormones to elevate generalized arousal which, in turn, plays into specific sexual motivational mechanisms [30].

2. Hormonal effects on cognitive functions, as might be related to women's health

2.1. Mood

The effects of estrogens on the elevation of mood in a subset of women has been reported widely (Schmidt and Rubinow, reviewed in [6]). These can be distinguished from the depressive effects of progesterone, often manifest in a subset of women 4 or 5 days after the onset of progesterone administration [31]. Is there an animal model that could assist in the testing of 'designer estrogens' in the future? In a recent set of experiments, Ilya Rachman [32] used the Porsolt forced swim test, in which a normal animal struggles little, swims for the duration and does not 'give up', whereas a depressed animal begins by struggling, swims little and then 'gives up'. The interpretation of these animal behavior data has been documented widely using drugs effective in treating depression. Interestingly, Rachman found that long term estrogen therapy would foster normal swimming while correspondingly reducing the struggling and the 'giving up' phases of the test.

2.2. Neuronal growth, as might contribute to learning and memory

The efficacy of estrogen therapy for reducing or delaying symptoms of Alzheimer's disease has become increasingly well recognized during the recent years [33–37]. Therefore, hormonal effects on neuronal growth processes which could contribute to the ability of neurons to demonstrate functional plasticity are of considerable interest not only with respect to this dev-

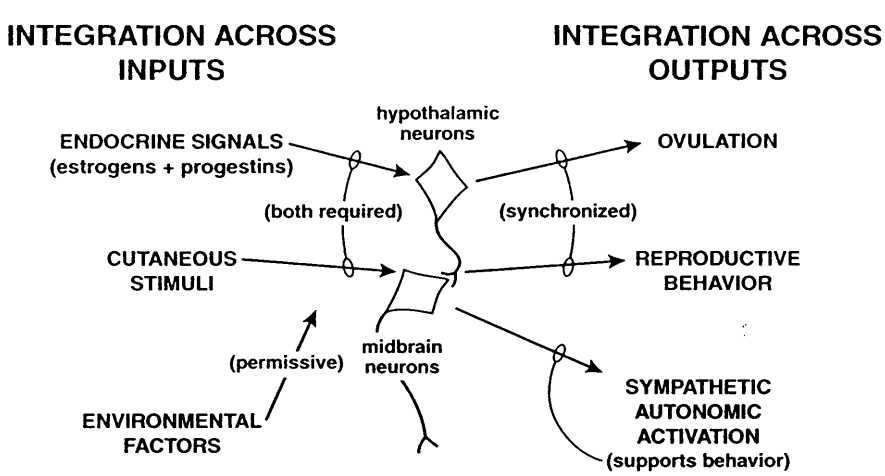

Fig. 5. Hypothalamic and midbrain neurons governing female reproductive behavior perform two separate types of neuronal integration in the Sherringtonian sense. On the input side, endocrine signals are required to facilitate cutaneous stimuli from the male, and their combined effects are constrained by limiting environmental factors. On the output side, the concerted effects of estrogens and progestins synchronize reproductive behavior with ovulation. This is biologically adaptive, as the female should only be exposed if reproduction is physiologically possible. Hypothalamic control of the autonomic nervous system provides the vascular and muscular support for reproductive behavior. From [43].

astating disease but also with respect to normal aging processes.

A long history of experimental work shows that estrogens can foster neuronal growth beginning with mechanisms resident in the nerve cell nucleus and nerve cell body [38,39]; reviewed in [40] and culminating in increased numbers of dendritic spines, axonal sprouts, and synaptic contacts [41]. While the greater majority of these demonstrations came from studies of forebrain neurons, similar data have been reported for a system descending from brainstem to the spinal cord [42]. Even as the role of the estrogen receptor ligand in cognitive performance is becoming widely accepted — depending, perhaps, on the growth reactions referred to above — recent data in our laboratory have pointed out an independent contribution of the estrogen receptor gene product, but in an *unliganded* state. (Ogawa et al., unpublished data). That is, during experiments with gonadectomized animals in the absence of hormone replacement, not only were the ERα knockout animals different from wild type controls, but also the direction of the difference depended upon gender (Ogawa et al., unpublished data). Moreover, not all of the estrogenic effects or the effects of ER itself necessarily should be linked to associative memory or mnemonic capacity. The potential effects of hormones on arousal and activation, referred to above, could also be an elementary part of cognitive changes during aging, for example. Thus, estrogenic hormones might elevate the levels of 'performance variables' such as attention, alertness and arousal, and in this manner facilitate cognitive performance.

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