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## The dual action of estrogens hypothesis

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### Abstract

Estradiol can act in the brain in a relatively fast manner (i.e. seconds to minutes) usually through signaling initiated at the cell membrane. Brain-derived estradiol has thus been considered as another type of neurotransmitter. Recent work found that behaviors indicative of male sexual motivation are activated by estrogenic metabolites of testosterone (T) in a fast manner while sexual performance (copulatory behavior *per se*) is regulated by brain estradiol in a slower manner via nuclear-initiated actions. This functional division between these two types of action appears to generalize to other behavioral systems regulated by estradiol. We propose the Dual Action Hypothesis of Estrogen Action to explain this functional distinction between these two different modes of action.

### Keywords

Estrogens membrane-initiated action; estrogens nuclear action; motivation; performance; appetitive behavior, consummatory behavior

### Introduction: Neuroestrogens vs. Ovarian estrogens

Estrogens are steroid hormones initially isolated and characterized in the late 1920ies by Adolf Butenandt (Nobel laureate for this discovery) and Edward A. Doisy [1]. They are present in all vertebrates and some invertebrates, and traditionally considered as the primary female sex hormone since they are produced by the ovary, released into the blood and play a key role in the control of female reproduction. Estrogens are secreted in the developing ovarian follicles by aromatization of androgenic substrates that have been produced locally. Aromatization is a complex enzymatic reaction catalyzed by aromatase (also known as estrogen synthase or EC 1.14.14.1, the product of gene CYP19), an enzyme of the cytochrome P450 family. Aromatization of androgens includes three successive

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hydroxylations resulting in the elimination of one methyl group and acquisition of a fully saturated (aromatic) “A” ring [2]. The main substrates of aromatization are the androgens testosterone and androstenedione that are transformed by aromatase into 17 $\beta$ -estradiol (E<sub>2</sub>; see Glossary) and estrone (E<sub>1</sub>) respectively.

In the late 1960ies, a marked level of aromatase activity was discovered in the brain of mammalian species including humans [3] and this finding was soon generalized to representative species in the major vertebrate classes (i.e., fish, reptiles, birds [4]). These claims were based on data from radioenzyme assays quantifying the transformation of radioactive testosterone into radioactive estradiol that were later supplemented by the identification of the presence of the relevant mRNA (by PCR or in situ hybridization) and of the enzymatic protein (by immunohistochemistry or Western blot). This finding of widespread brain aromatase opened an entirely new field of investigation that resulted over the years in the demonstration that estrogens produced in the brain either by the aromatization of testicular testosterone or via a series of enzymatic steps from the precursor of all steroids, cholesterol [5, 6] also called neuroestrogens, play a decisive role in a) the sexual differentiation of brain and behavior and b) the activation of male sexual behavior in rodents and many other vertebrate species [7]. Thus the notion that estrogens should be considered a “female” hormone is an over-simplification. Estrogens have important roles in males as well. During the last few decades, it was discovered that estrogens additionally control a host of physiological and pathological traits ranging from bone formation, lipid metabolism and brain plasticity and repair to tumor growth. They are now considered as pleiotropic signaling factors whose action extends well beyond the control of reproduction.

### **The dual controls of estrogen synthesis**

Ovarian and brain estrogens have the exact same chemical structure and are produced by the same enzyme, aromatase. Two fundamentally different controls of brain aromatase activity have been identified that operate in distinct time domains. In the long term, steroids increase transcription of the aromatase gene resulting in an increased enzyme concentration and thus activity (genomic control). In a shorter time scale, post-translational modifications of the enzymatic protein drastically modify its activity within minutes without changing its concentration (post-translational control) (See Box 1).

### **Fast vs. slow actions of estrogens**

Like other steroid hormones, estrogens are classically considered to exert their biological effects by binding to their cognate nuclear receptors (e.g., Estrogen receptor  $\alpha$ , ER $\alpha$ ; and Estrogen receptor  $\beta$ , ER $\beta$ ), which then act as ligand-activated transcription factors to regulate transcription of specific genes associated with an estrogen-responsive element (ERE) or other types (e.g. AP1, Sp1, NF- $\kappa$ B; [8]) of response elements. These biological effects are thus mediated via transcription of genes, translation of corresponding mRNAs into proteins, and their integration into functional pathways; they take hours to days to develop.

As early as 1976, it was however observed that in the brain estrogens exert, in addition to these relatively slow effects, electrophysiological effects with latencies of only a few

seconds [9]. This discovery was at the origin of a whole new line of research that resulted in the identification of a second mode of estrogen action usually initiated at the cell membrane and associated with shorter latencies in the second to minute range. These rapid membrane-initiated effects of E<sub>2</sub> are mimicked by E<sub>2</sub> associated with a larger molecule that prevents its entry into the cells (e.g., E<sub>2</sub>-BSA or E<sub>2</sub> biotin; [10]). They are mediated by the activation of intracellular signaling cascades resulting in changes in intracellular calcium concentration or, in a slightly slower manner, in the phosphorylation of various proteins including transcription factors such as the cyclic AMP response-element-binding protein, CREB, inducing in this way the so-called indirect genomic effects that appear with longer latencies than real non-genomic effects [11].

More recently, research has also implicated at the organismal level this mode of estrogen action in the control of a variety of behaviors including male and female sexual behavior, aggression, nociception, learning and memory, auditory signal processing (see for review [12, 13]). The first of these behavioral studies in the context of sexual behavior reported that a bolus of estradiol activates within 35 min anogenital sniffing and mounting in castrated male rats, *Rattus norvegicus* [14]. Subsequent studies extended this notion of rapid effects of E<sub>2</sub> on sexual behavior to Japanese quail (*Coturnix japonica*) and mice (*Mus musculus*) by showing that a single injection of the aromatase inhibitor rapidly (within 10-30 min) and transiently reduces various components of male sexual behavior [15, 16]. Additional experiments also demonstrated that in these two species, a single E<sub>2</sub> injection increases male sexual activity within 10-15 min [16, 17].

These experiments in quail had two important features in common. First, the rapid effects of estrogens on sexual behavior were easier to demonstrate when males were in addition exposed to a weak stimulation by androgens (a low dose of testosterone; [17]). Second, there was a clear suggestion that these rapid effects concerned mostly the initial phases of the interaction with the females rather than the copulatory performance *per se*. In the study assessing rapid behavioral effects of aromatase inhibition, the decrease in two aspects of appetitive sexual behavior, the rhythmic contractions of the cloacal sphincter muscles (RCSM) and the learned social proximity response (LSPR) in a two compartment chamber (behaviors described in Box 2), was for example more reliably inhibited (effect size = 0.408 and 0.534 respectively) than the copulatory act *per se* (effect size=0.144 or 0.251 for the low and high dose of inhibitor respectively; [15]).

### **Distinct controls of behavioral motivation and performance**

This distinction was confirmed and reinforced by more recent experiments during which brain estrogen bioavailability was pharmacologically manipulated by intracerebroventricular (ICV) injections. For these studies, castrated male quail that were chronically treated with exogenous testosterone (Subcutaneous Silastic™ implant) were implanted with an ICV cannula in the third ventricle and then used as their own control in successive behavior trials after injections of aromatase inhibitors or various forms of estrogens [18].

The frequency of RCSM was reduced by more than 50 percent, 30 minutes after a single ICV injections of either tamoxifen or ICI 182,780 (i.e., Fulvestrant or Faslodex™), two anti-estrogens, as well as of Vorozole™ or ATD (1,4,6-androstatriene-3,17-dione), two

aromatase inhibitors (Figure 1A-B). A very significant inhibition of RSCM was already observed 15 minutes after injection of tamoxifen or ICI 182,780. In contrast, the frequency of cloacal contact movements (CCM), the actual copulatory pattern (see Box 2) was completely unaffected by all these manipulations (Figure 1C-D).

The behavioral inhibitions observed after injection of aromatase inhibitors were clearly related to the depletion of local estrogen synthesis since they could be prevented by a single injection of exogenous E<sub>2</sub> (50 µg/bird 15 min. before testing i.e., 15 min after injection of the aromatase inhibitor; Figure 2A). The short latency of this behavioral effect suggested it did not rely on new protein synthesis and was thus mediated by non-genomic mechanisms and this was confirmed by the fact that the Vorozole-induced inhibition of RSCM could similarly be prevented by an acute injection of E<sub>2</sub>-biotin (Figure 2B).

The blockade of RSCM, but not copulation, following inhibition of neuroestrogens production or action suggested dissociation between mechanisms controlling sexual motivation and performance. To test whether this distinction could be generalized, we assessed the effects of these treatments on another measure of sexual motivation, the learned social proximity response (LSPR; see Box 2). Here again aromatase inhibition acutely (within 30 min) inhibited expression of the behavior and it was restored by a single injection of E<sub>2</sub> given 15 min. before the behavioral test (Figure 2C).

Finally, we wondered how copulatory behavior could be left unaltered by treatments that were markedly suppressing two independent measures of sexual motivation. One explanation could reside in the fact that copulatory behavior was tested in a small arena where the copulatory sequence was triggered reflexively due to close proximity of the partners so that decreases in motivation would not matter much. To test this interpretation, rapid effects of estrogen receptor antagonists and aromatase inhibitors were tested again but this time in a larger arena where females could more easily escape so that males had to actively pursue them to copulate successfully. In these conditions the two aromatase inhibitors Vorozole™ and ATD and the antiestrogen tamoxifen significantly inhibited within 30 min the expression of CCM and the antiestrogen ICI 182,780 reduced their frequency although the decrease was not significant.

These differences in the control by neuroestrogens of sexual motivation or performance and the influence of testing conditions on the latter behavior were confirmed in another group of subjects that were sequentially tested for RSCM and for CCM in a small and then a big arena. Vorozole™ inhibited RSCM in small arena within 30 min, an effect that was prevented by E<sub>2</sub> injected 15 min before testing. Acute aromatase inhibition had no effect on CCM frequency when tested in the small arena but inhibited this behavioral response measured in the larger arena. In this condition, E<sub>2</sub> counteracted Vorozole's effect (Figure 2D-E).

Together, these results demonstrate that acute changes in local estrogen bioavailability do not alter the ability of male quail to produce a highly coordinated sexual motor response but modulate the expression of two behaviors that reflect the sexual motivation of the subjects. Based on these data we hypothesize that estrogens have a dual action of sexual behavior.

They would act rapidly in a membrane-initiated and non-genomic manner to modulate sexual motivation but may only affect sexual performance after a longer latency, implying the existence of nuclear (genomic) mechanisms. This conclusion was further supported by an experiment performed on castrated T-treated males injected chronically with the aromatase inhibitor Vorozole™ so that both sexual motivation and performance were drastically inhibited. In these birds, a single injection of E<sub>2</sub> or of E<sub>2</sub>-BSA largely restored within 15 min. the expression of RCSM but had no effect of the CCM frequency [18].

### Towards a generalization?

We think that the Dual Action hypothesis has wide applicability. It is hard to be certain at present because investigators studying estrogen actions in other behavioral systems have not designed their studies to address questions related to the dual action view as we have done. But a consideration of some other examples of estradiol action on motivated behaviors is illustrative.

Three studies identified rapid effects of estrogens on male sexual behavior in rodents (rats: [14, 19] and mice: [16]). In all cases, the behaviors that were most markedly affected are ano-genital olfactory investigations and mounts, plus in one case intromissions [16]. For unknown reasons, one classically accepted measure of sexual motivation, the anticipatory level changes in a bi-level chamber, was however not increased after E<sub>2</sub> injections in the study of Kaufman and collaborators. Data on ejaculations are presented in only one of these studies and this behavior was not rapidly affected following injection of E<sub>2</sub> [19]. These data are thus broadly consistent with the distinction between effects of estrogens on motivation (i.e., ano-genital investigations) and performance if we accept that mounts (and possibly intromission) are at the interface between the appetitive and consummatory phase of male sexual behavior ([20]; See [21-23] for a detailed discussion on this topic). Note also that in quail the performance aspects of male sexual behavior are rapidly affected as an indirect consequence of the changes in motivation following changes in estrogens bioavailability if, and only if, the behavioral tests take place in a large arena where the male has to actively pursue the female to perform the copulatory sequence [18]. One might thus argue that this was the case for mounts and intromissions in the behavioral tests reported in rats and mice but answering this question would require additional experiments.

The separate effects of estrogens on motivational and performance aspects of behavior might also apply to other aspects of brain activity. For example, there are prominent female-biased sex differences in the response to and abuse of drugs such as amphetamine and cocaine. These differences are mediated by sex differences in estradiol secretion (e.g., [24, 25]) and estradiol seems to have differential effects on the motivational and consummatory aspects of drug seeking and consumption. Estradiol has rapid effects on striatal dopaminergic function that can be linked to some behaviors that exhibit female-biased sex differences such as stereotypic behavioral responses to amphetamine (e.g., [26, 27]).

In the case of cocaine, rats will lever press on a progressive ratio schedule of reinforcement, an appetitive response that will result in the consummatory event of cocaine self administration. This appetitive response is more pronounced in females than in males and involves rapid effects of estradiol on striatal dopamine release [27]. Female rats will also

exhibit increases in locomotor activity in response to cocaine. This occurs 30 min. after E<sub>2</sub> treatment and this effect is blocked by co-administering antagonists to mGluR5 receptors consistent with an estradiol effect on ER $\alpha$  receptors fused to the membrane [29]. Thus sex-specific effects of E<sub>2</sub> on appetitive measures of drug seeking do seem to involve fast actions of E<sub>2</sub> working at the membrane.

Behavior that involves self-administering cocaine on an FR1 schedule of reinforcement (i.e. every response immediately results in cocaine being administered) can be considered as the consummatory component of drug taking behavior ([30, 31]). The acquisition of self-administration of cocaine by rats is enhanced by estradiol but requires several days of estradiol administration and experience to be acquired [32, 33]. These data thus suggest that appetitive aspects for drug consumption are rapidly enhanced by actions of estrogens presumably initiated at the membrane level whereas the actual drug consumption itself would require slower estrogen actions presumably mediated by nuclear mechanisms. The applicability of the dual action hypothesis to estrogenic facilitation of appetitive and consummatory aspects of drug abuse is not definitive but the existing data are highly suggestive and warrant further investigation of the relevance of this hypothesis to these behaviors.

Selective aspects of other behavioral systems also are regulated by estradiol in an intriguing fashion implicating at the same time effects that seem to depend on acute membrane-initiated effects and longer term (presumably genomic) actions. This is the case, for example, for the activation of female sexual behavior in rats [34, 35], the control of auditory processing in songbirds [36, 37] and of learning and memory [38-41]. One might wonder to what extent this dichotomy in temporal characteristics may also reflect a dissociation between motivational and performance aspects of these behaviors.

### Multiple neuroanatomical targets

The separation of estrogens' effects on motivation and performance may possibly relate to a neuroanatomical specificity in the site of steroid action on sexual behavior in quail and other vertebrates. Several studies that we previously reviewed [42] have indeed suggested dissociations between the neuronal circuits underlying the expression of appetitive sexual behavior (ASB) reflecting motivation and consummatory sexual behavior (CSB) indicative of performance.

In quail, detailed analysis of the extent of stereotaxic lesion studies aimed at the medial preoptic area (mPOA) and of the related behavior deficits suggested that more rostral parts of the mPOA might be preferentially associated with the expression of ASB whereas more caudal parts of this region would control CSB [43]. This conclusion was independently supported by an immunohistological study analyzing c-Fos expression in castrated male quail treated with testosterone who had been allowed to freely copulate with a female (CSB) or to only see her and demonstrate signs of sexual motivation in the form of increased frequencies of RCSM. The performance of CSB was associated with an increased c-Fos expression compared to control un-manipulated birds throughout the medial preoptic nucleus while in contrast in the ASB group the increased c-Fos expression was limited to the rostral part of this nucleus [44].



Since aromatase-immunoreactive cells and both ER $\alpha$ - and ER $\beta$ -expressing cells are present throughout the entire rostro-caudal extent of the mPOA, even if it is with a heterogeneous density [42, 45, 46], it can be hypothesized that the anatomical specificity in the control of these two aspects of male sexual behavior correlates with a membrane-initiated action of estrogens on motivation in the rostral mPOA and a slower nuclear action on performance in its caudal part.

Such an anatomical separation between brain regions controlling motivation and performance also seems to be present in mammalian species. Lesions of the mPOA impair copulatory behavior in rats and many other mammals as well as in all species of birds, reptiles, amphibians and fishes that have been investigated (see [47] for review). Conversely, a large number of studies in a variety of species indicate that stereotaxic implants of testosterone in the mPOA activate most if not all aspects of male copulatory behavior [47]. There is however a pronounced neurochemical and functional heterogeneity within this brain region such that the whole area does not seem to be implicated in the control of copulation (see [42] for review). For example, one study showed that lesions of the caudal mPOA and anterior hypothalamus in rats block the expression of copulatory behavior but more rostral lesions in the mPOA had little or no effect [48] (see [42] for other examples). The studies of Barry Everitt and his colleagues additionally suggested a double dissociation between brain regions controlling in male rats appetitive and consummatory sexual behavior in rats. Males bearing lesions in the mPOA were no longer copulating but were still working actively in an operant conditioning procedure to get access to a female, thus indicating that they were still sexually motivated [49].

The anatomical dissociation between controls by steroids of motivational and performance aspects of behavior is also clearly illustrated in studies of the endocrine control of singing in canaries (*Serinus canaria*). It is well established that testosterone activates singing in canaries and other songbirds and that part at least of this effect results from the action of estrogenic metabolites of the androgen [50]. Because song control nuclei such as HVC contain both androgen and estrogen receptors it was initially assumed that steroids act at this level to activate behavior [50, 51]. However things are not so simple [52]. Blocking T action in HVC reduces song quality but does not affect song rate [53]. Conversely, implanting T in the HVC of castrated white-crowned sparrows does not activate singing [54]. Our recent study indicated that stereotaxic implantation of testosterone in the mPOA of castrated male canaries fully restores singing activity indicating that this brain region controls the singing motivation [51]. Three aspects of song quality, its energy, bandwidth and entropy, were however poorer in these birds than in subjects exposed to systemic testosterone indicating that the performance is controlled by steroids acting at another locus. This separation might again reflect the dual action of estrogenic metabolites of testosterone on the motivation to sing and song quality (performance aspect).

### The different estrogen receptors

The two types of behavioral effects of estrogens could also be potentially differentiated based on the different receptors mediating the responses (see Box 3). Several studies have implicated the receptors ER $\alpha$  and ER $\beta$  in the control of sexual behavior by nuclear

mechanisms but there is no published study on the type of estrogen receptor mediating rapid membrane-initiated effects on male sexual behavior. One study recently reported that the Gq protein-coupled membrane ER (Gq-mER) does not seem to play a role in the activation of male sexual behavior in rats [19]. More work on this topic is thus needed.

An important related question is whether these membrane effects are taking place in the same neural circuits as the nuclear ones, and at a finer level in the same cells. The neural distribution of nuclear sex steroid receptors has in the past been valuable in guiding research on the neural mechanisms of sexual and social behavior. Determining where, in the brain, estrogens act at the membrane level is however more problematic. Visualizing these types of receptors by immunohistochemistry has turned out to be technically challenging and correlating these neurochemical results to function is difficult because there are few specific blockers of these receptors and these often interfere with nuclear signaling as well. One option is of course to map the activation of ER-dependent intracellular signaling pathways initiated at the membrane level but since these pathways are often rather ubiquitous and not clearly identified for most behaviors; this approach would require acquisition of essential new knowledge. Furthermore even when critical brain areas will have been uncovered, one key remaining question will be whether membrane and nuclear initiated events take place, within these areas, in the same or in distinct cell populations. Both possibilities appear at this stage equally possible.

### **Conclusion: the dual action of estrogen hypothesis**

The dual action theory of estrogen action provides a useful way to organize recent somewhat surprising findings about estrogens, their regulation of behavior and their mechanisms of action. We were among those who had previously proposed that brain estrogens should be considered more like a neurotransmitter than a traditional hormone [55, 56]. These observations stimulated additional research on the various modes by which estrogens can act on behavior. These data in turn stimulated the posing of the dual action hypothesis. Using functional criteria to propose such a hypothesis at first might not seem wise. However, the functional criteria for the dual action (appetitive/motivational vs. consummatory/performance) does seem to map onto a variety of physiological criteria (fast action/membrane vs. slower action/nuclear; Figure 3). The entire goal of proposing this hypothesis is to test how consistently these criteria will separate out different aspects of estrogen action. There are many systems that one could utilize to test this theory and research conducted in light of this paradigm will be helpful in furthering our understanding of the multiple ways brain estrogens can act.

Our thinking was also influenced by why such a dual mode of action of estrogens might have evolved. One hallmark of steroid hormones, like the estrogens, is that they can coordinate suites of morphological, physiological and behavioral traits into an organized adaptive response. Sexual and other social behaviors have to be carefully coordinated with physiological and morphological changes for successful reproduction to occur. Behavior of course is regulated at several different time scales, e.g., ontogenetic, seasonal and seconds-minutes. It therefore makes sense that a chemical messenger that acts in the brain would be able to regulate behavioral activation both at a relatively fast as well a longer term time



scale. However, the well-known sluggishness of the nuclear actions of steroids made many assume that steroids could not be involved in significant short-term behavioral regulation (as Wilson famously declared in 1975; see p6 in [57]). We now know that estrogens can act in the brain on several different time scales. Is this variance functionally organized in the manner proposed by the Dual Action Hypothesis? This is an idea worth testing.

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## Glossary

<b>AA</b>	aromatase activity
<b>ASB</b>	appetitive sexual behavior
<b>ATD</b>	1,4,6-androstatriene-3,17-dione
<b>BSA</b>	bovine serum albumin
<b>E<sub>1</sub></b>	estrone
<b>CCM</b>	cloacal contact movements
<b>CREB</b>	cyclic AMP response-element-binding protein
<b>CSB</b>	consummatory sexual behavior
<b>E<sub>2</sub></b>	estradiol-17 $\beta$
<b>ER<math>\alpha</math></b>	estrogen receptor $\alpha$
<b>ER<math>\beta</math></b>	estrogen receptor $\beta$
<b>ERE</b>	estrogen-responsive element
<b>GPCR</b>	G-protein coupled seven transmembrane domains receptor
<b>HVC</b>	used as proper name for a song control nucleus in song birds ((previously High Vocal Center)
<b>ICV</b>	intracerebroventricular
<b>LPSR</b>	learned social proximity response
<b>mGluRs</b>	metabotropic glutamate receptors
<b>mPOA</b>	medial preoptic area
<b>RCSM</b>	rhythmic contractions of the cloacal sphincter muscles
<b>T</b>	testosterone

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### Box 1. Two independent controls of aromatase activity

The genomic controls of aromatase synthesis are very different from one tissue to another. The human aromatase gene is composed of 9 coding exons and multiple alternative non-coding first exons that regulate this tissue-specific expression [2, 58]. A similar situation has been identified in other species including rodents [58]. Depending on which of the alternative un-translated first exons is used for transcription, aromatase can be up-regulated by androgens (brain), cyclic AMP (ovary, testis, placenta), dexamethasone (liver, adipose and vascular tissue, skin) or retinoic acid (bone) among others [58]. Aromatase activity (AA) and estrogen production are consequently modified in parallel with changes in aromatase concentration. In the brain for example, AA increases after a few hours to a few days following exposure to testosterone [59, 60].

An alternative mechanism of aromatase activity control was identified more recently that does not involve changes in enzyme concentration and takes place more rapidly (5-15 min). We serendipitously discovered that AA in quail (*Coturnix japonica*) brain homogenates is rapidly (within min) inactivated by phosphorylating conditions (high but physiological concentrations of ATP, Mg<sup>2+</sup> and Ca<sup>2+</sup>; [61, 62]), an effect that is blocked in the presence of kinase inhibitors. Subsequent investigations performed on preoptic-hypothalamic explants maintained *in vitro* indicated a similar rapid inactivation of the enzyme activity by phosphorylations themselves under the control of glutamatergic transmission [63]. This phosphorylation-dependent inactivation of AA was also demonstrated in various cell lines transfected with the human aromatase gene as well as in quail ovaries [64]. Work conducted in the zebra finch (*Taeniopygia guttata*) telencephalon indicated that this acute modulation of AA by phosphorylating conditions occurs specifically at the level of synaptic terminals but not in the perikaryon [65]. *In vivo* dialysis in the auditory cortex of the same species provided converging evidence that glutamate rapidly decreases local estrogen concentrations [66] and this effect results from changes occurring at the level of synapses [67]. Social interactions with a congener of the opposite sex and acute stress also modulate within minutes brain AA and/or local estrogen concentration in a sex- and anatomically-specific manner [68-71].

**Box 2. Independent measures of sexual motivation and sexual performance**

Separate independent measures of sexual motivation and performance have been developed and validated for male quail. They are briefly described below (see [72] for a full description).

**Rhythmic contractions of the Cloacal Sphincter muscles (RCSM)**

When visually exposed to a female, male quail display RCSM that produce a meringue-like foam which is transferred to females during copulation and enhances the probability that sperm will fertilize the egg [73]. Several lines of evidence indicate that this behavioral response reflects the sexual motivation of the male: (1) it is rapidly upregulated in sexually active males visually exposed to a female but not a male [73], (2) it is displayed by naive males but can also be conditioned to an arbitrary stimulus previously associated with the presentation of a female [74], (3) it decreases after castration but is restored by treatments with exogenous testosterone [43], and (4) is inhibited by lesions of the preoptic area, a brain region known to control male sexual behavior [43].

**The learned social proximity response (LSPR): Approach and Look**

The LSPR is a form of associative learning in which a male learns to stand in front of a narrow window where he had previously been able to see a sexually receptive female and later allowed to copulate with her [75]. The behavior is easily quantified in a two compartment chamber where the male placed in the larger compartment is allowed to see the female placed in a smaller adjacent compartment through a narrow vertical slit (the “window”; see Figure 3C). After quantifying the time the male spends looking at the female, the separation between the two compartments is removed and the male is allowed to freely copulate with the female. The duration of looking reflects male sexual motivation since 1) it is only displayed by males exposed to testosterone [76], 2) it is inhibited by lesions of the preoptic area [43].

**The copulatory sequence *sensu stricto***

Sexual performance is then measured by the actual copulatory sequence during which the male grabs the female's neck feathers, mounts her and then performs the cloacal contact movements (CCM) during which sperm transfer occurs.

### Box 3. Multiple estrogen receptors mediating nuclear and membrane-initiated effects

Two receptors, ER $\alpha$  and ER $\beta$ , that mediate nuclear actions of estrogens have been identified [77] and implicated in the control of various aspects of estrogen action. After experiencing post-translational modifications such as palmitoylation, these receptors can also be translocated to the cell membrane [78, 79] and signal via their association with a G-protein coupled seven transmembrane domains receptor (GPCR) such as the metabotropic glutamate receptors, mGluRs [80]. One additional estrogen receptor called GPER or GPR30 has also been formally identified at the membrane level [81] and two additional ones have been postulated based on pharmacological and functional studies, Gq-mER and ER-X [82, 83] but their chemical nature has not been identified.

At the organismal level, rapid actions of E<sub>2</sub> have now been ascribed to the first 4 of these ER located at least in part at the membrane (ER $\alpha$ , ER $\beta$ , GPR30 and Gq-mER). Most advances in this context have been made in our understanding of the estrogenic regulation of learning and memory [40, 41, 84, 85] as well as nociception [86-88]. Limited information is however available concerning the specific ER implicated in the control of reproduction and it does not directly concern male sexual behavior. For example, membrane-initiated effects of estrogens on lordosis behavior in rat occurring within 30 hours or so would depend on membrane-associated ER $\alpha$  transactivating an mGluR [35] and rapid changes in LH secretions would be controlled by E<sub>2</sub> acting on GPR30 [89].

Outstanding question: Is the duality of action limited to estrogens?

Estrogens are without a doubt the steroid hormones for which the largest amount of literature has accumulated on the topic of fast action. We would like to suggest however that the dichotomy between rapid membrane-initiated and slower nucleus-initiated effect applies to several other steroids such as progesterone, corticosterone and testosterone. These steroids are indeed classically assumed to exert their effects via slow mechanisms that are mediated by nuclear receptors and presumably require regulation of the transcription of multiple genes. This was in fact formally demonstrated in a substantial number of cases [90, 91].

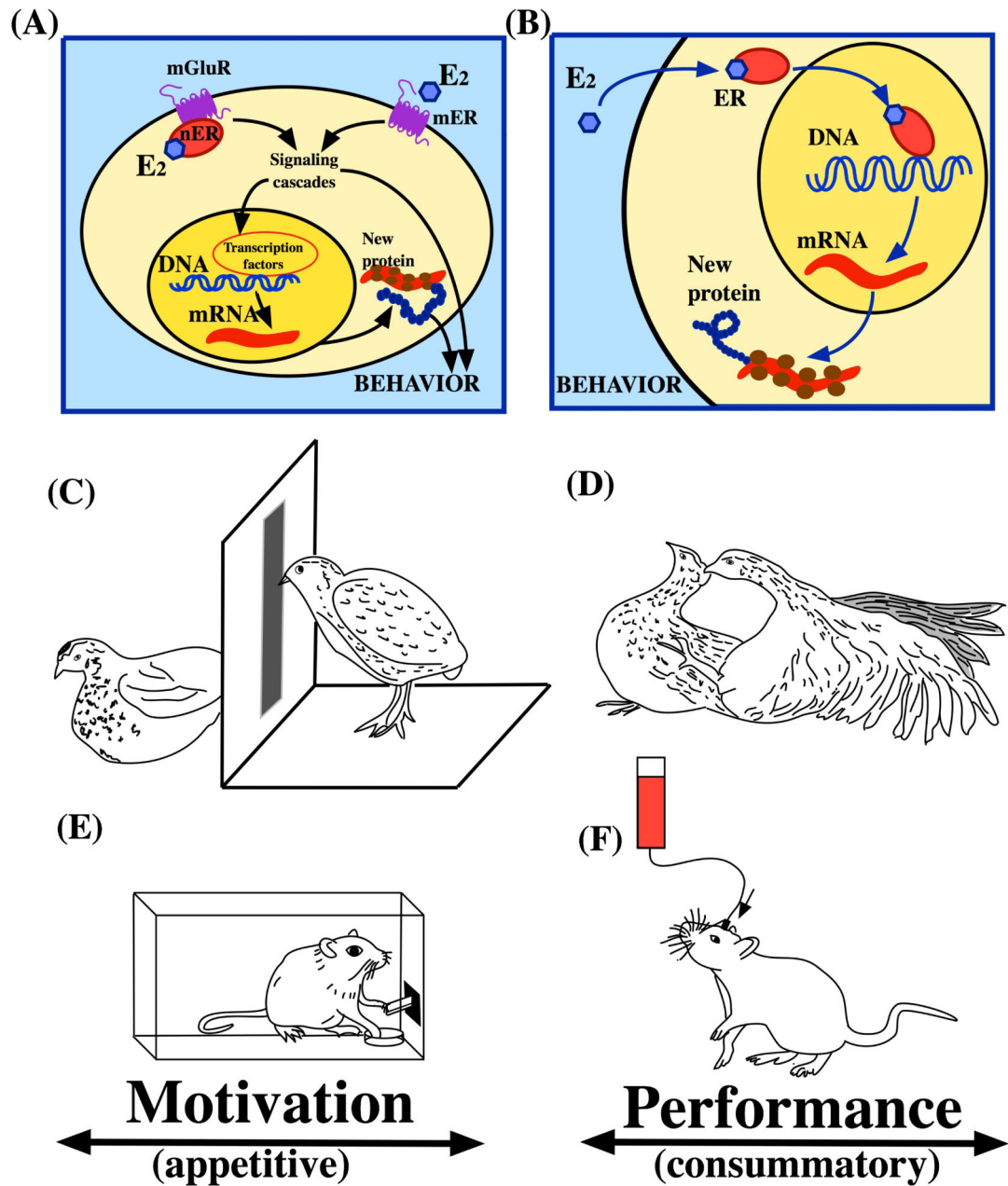
However in addition, progesterone enhances the lordosis behavior of female rodents via a fast action in the mesencephalic periaqueductal gray that synergizes with its nuclear action in the ventromedial hypothalamus ([92-94]). Testosterone has been shown to exert rapid behavioral effects on mounting behavior and on penile reflexes in rodents but also on anxiety, rewarded behaviors and pain sensitivity. Some of these effects might be mediated by the aromatization of testosterone into an estrogen but others are clearly androgen-dependent (see [95] for review and discussion). Effects of testosterone on appetitive and consummatory components of sexual behavior also seem to imply its action at different brain sites ([96]; See [42] for review). Finally, besides its classical nuclear actions, corticosterone also acts rapidly at the membrane level to modulate namely sexual behavior in male newts (see for review:[97]).

There is thus evidence concerning several other steroids for a separated action at the nuclear and at the membrane level, overlapping with an action in different time domains (slow [hours to days] vs. rapid [in the minutes range]) and in some cases with a separation in the neuroanatomical target sites of action. Data are however generally not available to decide whether this dichotomy also covers the notion of motivation versus performance presented here that would be necessary to support a broader application of the dual action of estrogens to other hormones but we certainly hope that the present opinion paper will promote research on this topic.

### Highlights

- Estrogens affect behaviors by slow nuclear- and faster membrane-initiated mechanisms
- Sexual motivation is rapidly controlled by estrogens acting at the cell membrane
- Sexual performance requires action of estrogens during a longer period
- This differential control extends to controls by estrogens of other behaviors

# The Dual Action of Estrogens



**Figure 1. Rapid effects of neuroestrogens on quail sexual behavior**

Blockade of estrogen action or of estrogen production by intracerebroventricular injections of antiestrogens (panel A; ICI 182,780, ICI or tamoxifen, TMX) or of aromatase inhibitors (panel B; 1,4,6-androstatriene-3,17-dione, ATD or Vorozole™, VOR) markedly inhibits within 30 min the expression of rhythmic contractions of the cloacal sphincter muscles (RCSM), a form of appetitive sexual behavior reflecting sexual motivation, but does not affect the frequency of copulatory behavior *sensu stricto*, represented here by the frequency of cloacal contact movements (CCM, panels C and D). \*\*= $p < 0.01$ , \*\*\*= $p < 0.001$  compared



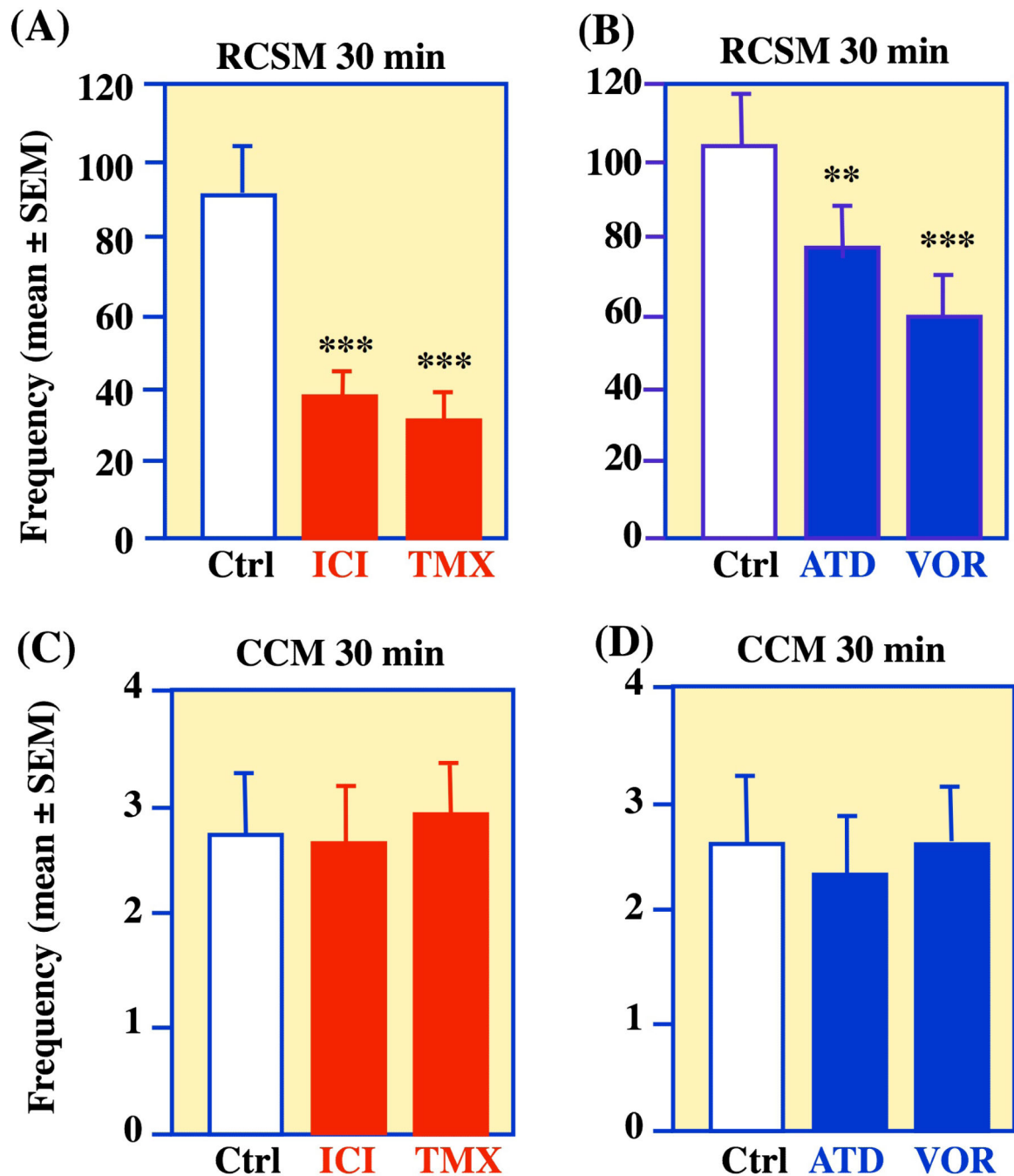
to the control (Ctrl) condition (Newman-Keuls post hoc tests following a significant overall ANOVA). Redrawn from data in [18].

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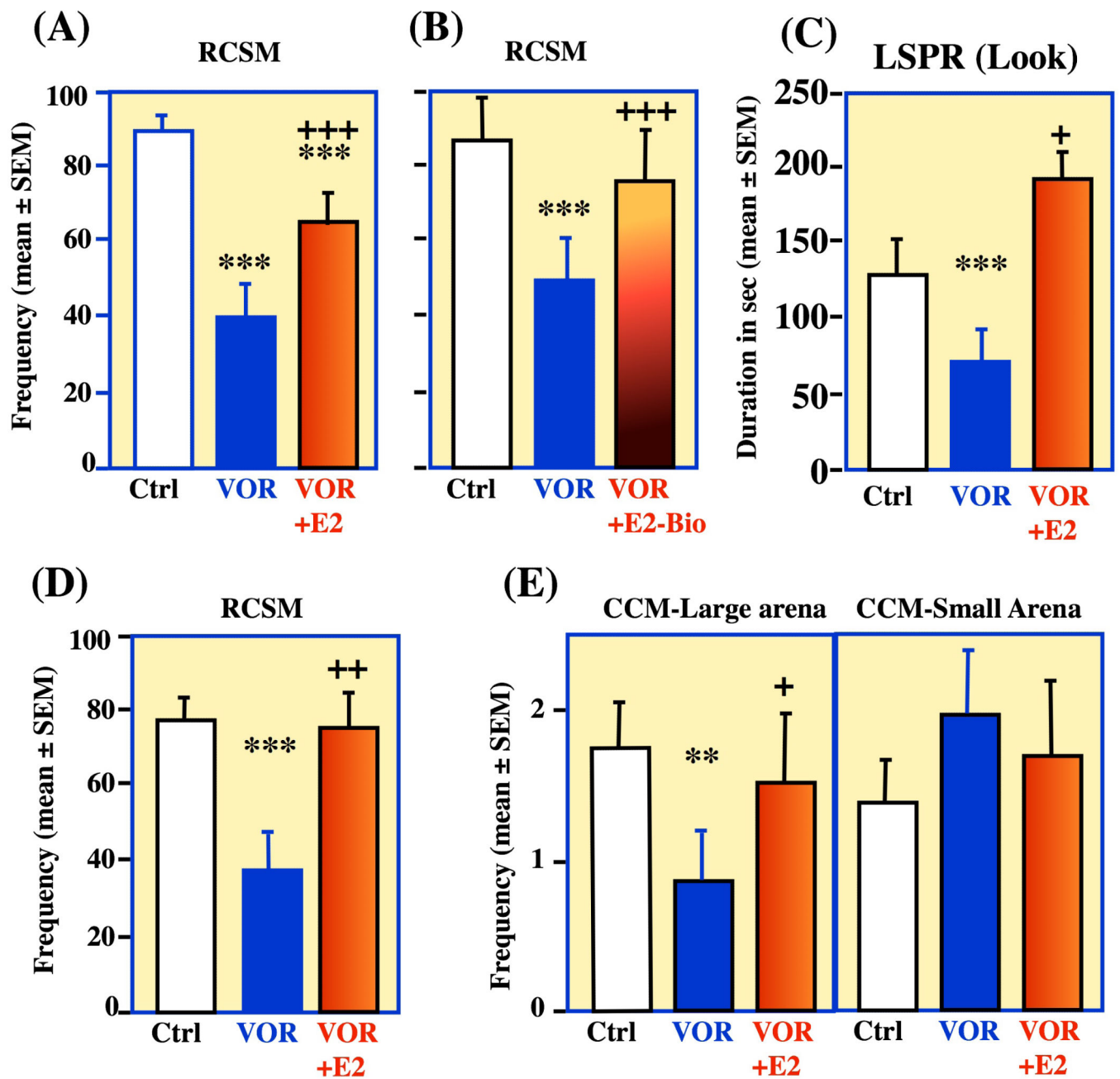
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**Figure 2. Specificity of the rapid, membrane-initiated effects of neuroestrogens on the motivational and performance aspects of male sexual behavior in quail**  
**A-B.** The aromatase inhibitor Vorozole™ (VOR) inhibits within 30 min expression of rhythmic contractions of the cloacal sphincter muscles (RCSM) and this behavior is restored by a single injection 15 min before the behavioral test of estradiol ( $E_2$ ; **A**) or estradiol coupled to biotin ( $E_2$ -Bio; **B**). **C.** A similar inhibition after VOR injections and recovery after  $E_2$  injection affects the expression of another form of appetitive sexual behavior, the learned social proximity response (LSPR) illustrated here by the time the male spends looking at the female through a narrow window. **D-E.** In another group of birds, the acute

inhibition of aromatase activity by VOR reduced RCSM frequency and recovery was observed after E<sub>2</sub> treatment (**D**). No effect of CCM frequency was again observed when tests were performed in a small arena (**E, right**), but in a large arena where male had to pursue the female to be able to copulate, the treatments modulated CCM frequency (decrease after VOR, recovery after E<sub>2</sub>; **E, left**). \*\*=p<0.01, \*\*\*=p<0.001 compared to the control (Ctrl) condition; += p<0.05, ++=p<0.01, +++=p<0.001 compared to the VOR group (Newman-Keuls post hoc tests following a significant overall ANOVA). Redrawn from data in [18].



**Figure 3. Schematic presentation of the dual action of estrogens hypothesis**

The figure illustrates the overlapping dichotomies between the cellular mechanisms mediating estrogens action (A-B), the separate controls of sexual motivation and sexual performance (C-D) in male quail, and the appetitive and consummatory phases of drug addition in rats and mice (E-F). A. Estrogens such as estradiol-17 $\beta$  (E<sub>2</sub>) signal at the membrane level by binding to estrogen receptors located at this level only (GPR30, Gq-mER) or to a receptor that is found both in the nucleus and at the membrane (ER $\alpha$ , ER $\beta$ ) but is associated at the membrane with a metabotropic glutamate receptor (mGluR). This modifies the activity of intracellular signaling cascades that affect behavior either directly or via indirect genomic effects. B. Alternatively, steroids including E<sub>2</sub> bind to intracellular

receptors that will then act as ligand-activated transcription factors to modulate production of new mRNA and new proteins that will affect behavior. C. E<sub>2</sub> rapidly modulates appetitive sexual behavior in male quail such as the rhythmic contractions of the cloacal gland or the learned social proximity response illustrated here (male looking at a female through a narrow “window”) by acting at the neuronal membrane level. D. A more prolonged action of E<sub>2</sub> presumably at the nuclear level is needed to activate copulatory behavior *sensu stricto* illustrated here by the cloacal contact of a male with a female. E. The appetitive responses displayed by rodents to have access to a drug, such as lever pressing on a progressive ratio of operant conditioning, are increased within 30 minutes by an E<sub>2</sub> treatment acting presumably via an ER associated at the membrane level with a mGluR. F. In contrast, consummatory self-administration of drug by intracerebral injections as observed in a FR1 schedule of operant conditioning (each lever pressing results in drug delivery) seems to be affected after only several days of exposure to E<sub>2</sub>, suggesting a genomic control by the steroid. See text for additional explanations.