BRAIN RESEARCH

Review

Functional significance of the rapid regulation of brain estrogen action: Where do the estrogens come from?

Charlotte A. Cornil[a],*, Gregory F. Ball[a], Jacques Balthazart[b]

[a]Department of Psychological and Brain Sciences, Johns Hopkins University, 108 Ames Hall, 3400 North Charles Street, Baltimore MD 21218-2686, USA
[b]University of Liège, Center for Cellular and Molecular Neurobiology, Liège, Belgium

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ABSTRACT

Estrogens exert a wide variety of actions on reproductive and non-reproductive functions. These effects are mediated by slow and long lasting genomic as well as rapid and transient non-genomic mechanisms. Besides the host of studies demonstrating the role of genomic actions at the physiological and behavioral level, mounting evidence highlights the functional significance of non-genomic effects. However, the source of the rapid changes in estrogen availability that are necessary to sustain their fast actions is rarely questioned. For example, the rise of plasma estrogens at pro-estrus that represents one of the fastest documented changes in plasma estrogen concentration appears too slow to explain these actions. Alternatively, estrogen can be synthesized in the brain by the enzyme aromatase providing a source of locally high concentrations of the steroid. Furthermore, recent studies demonstrate that brain aromatase can be rapidly modulated by afferent inputs, including glutamatergic afferents. A role for rapid changes in estrogen production in the central nervous system is supported by experiments showing that acute aromatase inhibition affects nociception as well as male sexual behavior and that preoptic aromatase activity is rapidly (within min) modulated following mating. Such mechanisms thus fulfill the gap existing between the fast actions of estrogen and their mode of production and open new avenues for the understanding of estrogenic effects on the brain.

1. Introduction

Gonadal steroids produce a wide range of cellular effects that are largely, but not exclusively, mediated through intracellular receptors. These steroid hormone receptors are members of a large family of ligand-activated transcription factors that bind response elements located on the DNA and regulate the transcription of genes encoding a wide variety of proteins. These proteins include synthesis enzymes (such as tyrosine hydroxylase), transporters, receptors, signal transduction proteins (phosphatases, kinases, accessory proteins, etc.) or degradation enzymes (such as monoamine oxidases), which ultimately modify neurotransmission (Maggi et al., 2004; McEwen and Alves, 1999). These effects are relatively slow and develop with latencies ranging from 1 h to several days. The role of genomic actions of steroids in adults has been extensively documented based on studies of physiology and behavior.

* Corresponding author. Fax: +1 410 516 4478.
  E-mail address: ccornil@jhu.edu (C.A. Cornil).

Steroids exert profound organizational effects on the developing brain and the increase of secretion of sex steroid hormones at puberty and their maintenance throughout the reproductive life are responsible for the activation and maintenance of secondary sex characters and behavior such as courtship, reproductive behavior, aggressive behaviors, etc. (Becker et al., 2002). Seasonal fluctuations and/or variations across the estrus cycle of circulating levels of estrogens positively correlate with variation in these reproductive responses (Ball and Balthazart, 2002; Prendergast et al., 2002; Wingfield and Silverin, 2002). A variety of effects of estrogens and other steroids on responses unrelated to reproduction have also been identified. For example, estrogens are known to influence cognitive functions, pain mechanisms, fine motor skills, mood, temperature regulation and sleep (McEwen and Alves, 1999; Wang et al., 2001).

In addition, a host of studies have also identified effects of steroids that are too rapid (seconds to minutes) to be mediated through the activation of DNA transcription and protein synthesis (Kelly and Ronnekliev, 2002; McEwen and Alves, 1999; Moss et al., 1997). This is particularly the case for 17β-estradiol (E$_2$) which can rapidly (within a few seconds to several minutes; see below) activate a wide variety of intracellular signaling pathways including modulations of intracellular calcium concentrations (Beyer et al., 1976; Mermelstein et al., 1996) and protein phosphorylations (Moss et al., 1997; Nethrapalli et al., 2004; Wade and Dorsa, 2003; Wade et al., 2001; Watters et al., 1997). These actions seem to be initiated primarily at the cellular membrane and lead to modulations of electrical activity (Kelly and Ronnekliev, 2002; Moss et al., 1997) and neuronal activation (Abraham et al., 2003, 2004; Gu et al., 1996; Wade and Dorsa, 2003; Zhou et al., 1996) in various brain regions (for review see Maggi et al., 2004; McEwen and Alves, 1999).

Although there is no longer any doubt about the existence of non-genomic effects, many uncertainties remain. One of the most debated questions is whether these effects are mediated by interactions of estrogens with the "classical" nuclear estrogen receptors (ERs) located in the cell nucleus or associated with the neuronal membrane and whether these effects are elicited by physiologically relevant concentrations of estrogens. In addition, the nature of the mechanism(s) able to modulate rapidly the bioavailability of estrogen (that is necessary to explain the existence of these fast effects) has rarely been questioned or even discussed, even though this represents a fundamental aspect of the rapid effects of steroids. Furthermore, while there are a plethora of data characterizing rapid, presumably non-genomic, effects of estrogens based on cellular measures of action, there is still little information concerning the significance of these effects at the organismal or behavioral level of analysis. This review will try to address these last two questions. Firstly, we will examine the potential sources of estrogen for the brain that are rapidly modulated and, secondly, we will summarize what is known about the functional significance of fast effects of estrogen for physiology and behavior. In addition, the question of the physiological

relevance of doses that can activate the rapid effects of estrogens will also be discussed.

2. Physiological relevance of the fast cellular effects of steroids

Rather surprisingly, while the existence of non-genomic effects of steroids is not subject to doubt anymore, our knowledge of their implications in the regulation of physiological and behavioral processes remains quite limited. Perhaps the most progress has been made for the C21 steroids corticosterone and progesterone. In the case of corticosterone, rapid effects have been identified on various measures of sexual behavior (Moore and Orchinik, 1994) and stress (Orchinik, 1998) in amphibians and on the production of vocalizations in batrachoidid fishes (Remage-Healey and Bass, 2004, 2005, in press). Furthermore, there is good evidence for a highly specific membrane-binding site of corticosteroids (Evans et al., 2000a,b; Orchinik et al., 1991). Several lines of evidence also indicate rapid, membrane-mediated effects of progesterone on sexual behavior in the ventral tegmental area of Syrian hamsters (DeBold and Frye, 1994; Frye et al., 1992, 2003; Pleim et al., 1990) and this steroid or its metabolites can clearly bind to membrane receptors such as the GABA$_A$ complex (Majewska et al., 1986), the NMDA receptor (Irwin et al., 1992, 1994) or the membrane progestin receptor recently identified in one piscine and several other vertebrate species (Zhu et al., 2003a,b). One study reported rapid effects of testosterone on striated penile muscles related to the expression of male sexual behavior (Sachs and Leipheimer, 1988). Remage-Healey and colleagues also recently demonstrated that 11-keto-testosterone (11kT, the main androgen in fishes) rapidly increases the duration of fictive vocalizations stimulated by electrical stimuli in an in vitro preparation (Remage-Healey and Bass, 2004, in press). Finally, Clifton and Andrew (1981) also suggested the existence of rapid effects of testosterone on aggressive behavior in chicks presented with foreign objects. However, these effects appeared 4 h after the injection of testosterone in animals that received an injection the previous day, so it cannot be ruled out that this effect results from the activation of genomic mechanisms.

A few rapid effects of estrogens have also been reported based on behavioral and/or physiological measurements. In rats, there is evidence that estrogens potentiate, via non-genomic mechanisms, their genomic action on lordosis behavior. For example, Kow and Pfaff (2004) showed that a pulse of E$_2$ as short as 15 min given in the hypothalamic ventromedial nucleus (VMN) potentiates the effects of a second pulse of 1 h in inducing high receptivity in ovariectomized female rats. A similar effect is obtained if purified E$_2$ conjugated with bovine serum albumin (E2-BSA, a large protein that prevents estrogens from crossing the plasma membrane) is used to replace E$_2$ in the short pulse suggesting that its effect is mediated through a non-genomic mechanism. This conclusion is further supported by the fact that the effect of the short E$_2$ pulse can be mimicked by the activation of protein kinase A or C as previously demonstrated in vitro

(Vasudevan et al., 2001). These data thus suggest that non-genomic and genomic actions of estrogens interact to activate lordosis in a synergistic manner.

Behaviors such as tonic immobility, dorsal immobility and amphetamine-elicited rotational behavior are differentially expressed across the estrus cycle (Becker et al., 1982, 1987; Meyer and Van Hartesveldt, 1992; Smith et al., 1985; Van Hartesveldt et al., 1989, 1990). The striatum has been implicated in the control of these behaviors and accordingly estrogens implanted in the striatum affect their expression within 4 h both in males and females (Van Hartesveldt et al., 1989, 1990). Given the absence of nuclear estrogen receptors in the striatum, these effects are thought to be mediated by non-genomic mechanisms. This is consistent with the observation that E_2 modulates apomorphine-induced postural deviation and amphetamine-induced rotational behavior with short latencies in the order of 1 h in ovariectomized females (Becker, 1990; Joyce and Van Hartesveldt, 1984). In addition, estrogens rapidly affect intracellular calcium concentrations in striatal neurons in vitro through a membrane-initiated non-genomic mechanism (Mermelstein et al., 1996). Thus, estrogen in the striatum appears to potentiate, acutely and probably non-genomically, dopaminergic activity that would in turn enhance sensorimotor processing. This cellular effect may be part of the mechanism controlling paced mating behavior in female rats (Xiao and Becker, 1997). Other rapid effects of steroids on measures of cell function have been described in the hypothalamus. For example, a single injection of E_2 to ovariectomized females results in CREB phosphorylation within 15 min in the medial preoptic nucleus, the medial septum and the VMN of mice (Abraham et al., 2004) and within 15–30 min the preoptic area, the bed nucleus of the stria terminalis and the anteroventral periventricular nucleus in rats (Gu et al., 1996; Zhou et al., 1996). A physiological role for these cellular effects at the level of the organism has not been reported yet, but they are likely involved in the hormonal feedback on neural systems that regulate gonadotropin secretion.

Non-genomic effects of estrogens are not limited to females. Unlike females, males of course do not exhibit a cyclical "rapid" rise of circulating concentrations of estrogens. However, they can display substantial concentrations of estrogens originating either directly from the testis or produced locally in the brain by aromatization of testosterone (Table 1; Ball and Balthazart, 2002; Hall, 1994; Hull et al., 2002). Although the existence of rapid variations in the availability of estrogen in the brain is rarely discussed (see below), fast effects of estrogens on male behaviors have been reported. For instance, Cross and Roselli (1999) showed that a subcutaneous injection of E_2, but not testosterone, stimulates the expression of male sexual behavior in castrated rats within 35 min. In Japanese quail, an intraperitoneal injection of a bolus of 17β-estradiol facilitates the expression of male sexual behavior. This effect occurs within 15 min and vanishes after 30 min. In order to prime the mechanisms involved in sexual behavior, the animals used in this experiment were castrated males that were chronically treated with a subthreshold dose of testosterone that is unable by itself to elicit sexual activity in most birds. The rapid behavioral activation observed in this experiment (Cornil et al., 2006a) thus potentially illustrates

in males the synergistic interaction between rapid and slower genomic actions of estradiol as also demonstrated by recent work on female rats (see work of Kow and Pfaff, 2004 and Vasudevan et al., 2001 described above). In plain midshipman fish, an intramuscular injection of E_2 increases within 5 min the duration of fictive vocalizations stimulated by electrical stimuli in an in vitro preparation (Remage-Healey and Bass, 2004). This effect persists for 15 to 30 min. Interestingly, 17α-estradiol or testosterone does not produce similar effects. Hayden-Hixson and Ferris (1991) also showed that a micro-injection of E_2 in the anterior hypothalamus stimulates agonistic behavior (flank marking) within 15 min in male hamsters.

Several generalizations can be derived from these observations. Firstly, these behavioral effects occur with latencies ranging from 5 min to 1 h, most effects being observed around 15–30 min. This is considerably shorter than the several days commonly required for genomic activation of behavioral responses (see below for further discussion of this issue). This rapidity of action is all the more striking that most of these effects were observed following systemic injections (Becker, 1990; Cornil et al., 2006a; Cross and Roselli, 1999; Remage-Healey and Bass, 2004) so that the hormone had first to reach the target tissue and accumulate above a certain threshold before it could activate a cellular response and trigger the neuronal circuits involved in the expression of the behavior considered. Alternatively, most of the intra-tissue applications were performed via insertion of hormone in crystals (Hayden-Hixson and Ferris, 1991; Joyce and Van Hartesveldt, 1984; Kow and Pfaff, 2004). It is thus likely that some time was required to dissolve hormone from these implants and produce a behaviorally active concentration of E_2. Together, these data therefore suggest that the effects of E_2 observed in these experiments would occur even faster if the steroid could be delivered instantaneously to its targets in effective concentrations. Secondly, some of these rapid behavioral effects appear to be transient as compared to the long lasting genomic effects. Thirdly, the rapid actions of estrogens appear to be somewhat region specific. Most of the studies available to date report fast effects of peripheral injections. But there are some examples of an estrogen implant applied only for a brief period in a specific brain area that activates the expression of complex behaviors in the absence of steroid priming, i.e., in the absence of activation of specific circuits involved in the control of these behaviors (Hayden-Hixson and Ferris, 1991; Joyce and Van Hartesveldt, 1984; Kow and Pfaff, 2004; Van Hartesveldt et al., 1989, 1990). Finally, fast effects of estrogen, and other steroids, have been identified in various vertebrate species (for review, see Remage-Healey and Bass, 2006). Therefore, these mechanisms of rapid action seem to be conserved across species.

3.　　　Non-genomic effects on cell function

Fast effects of steroids were described for the first time in 1941 by Hans Selye, who observed a rapid anesthetic and sedative effect of progesterone (Selye, 1941). In the 60s and in the beginning of the 70s, several studies suggested that estrogens

Species	Testosterone (nM)		Estradiol (pM)	
	Male	Female	Male	Female
Rat	Baseline: 1.0–19.0 (George, 1997; Graham and Desjardins, 1980; Haim et al., 2003; Kamel et al., 1975; Rittmaster et al., 1991; Torres and Ortega, 2006) After mating: 5.8–33.9 (Kamel et al., 1975; Purvis and Haynes, 1974) After social exposure: 4.2–10.4 (Kamel et al., 1977)	Baseline: 0.4 (Haim et al., 2003) Pro-estrus 1.4–1.7 (Haim et al., 2003)	Baseline: <18.35–1101.0 (Haim et al., 2003; Torres and Ortega, 2006)	Estrus to diestrus (baseline): 18.4–135.8 (Ahdieh et al., 1985; Butcher et al., 1974; Haim et al., 2003; Smith et al., 1975) Pro-estrus: 146.8–367 (Ahdieh et al., 1985; Butcher et al., 1974; Haim et al., 2003; Lu et al., 1953; Smith et al., 1975; Takeo, 1984)
Mouse	Resting levels: 6.9–41.6 (Coquelin and Bronson, 1979; Li et al., 2001) Dependent on strain and sexual activity: 3.1–48.2 (Batty, 1978) After social interaction: 27.8–86.8 (Macrides et al., 1975)	Day 12 of pregnancy: 0.7 (Mahendroo et al., 1996)	Intact: 9.4 (Fowler et al., 2000) Aromatase overexpression: 20.9–825.8 (Fowler et al., 2000; Li et al., 2001)	Intact (baseline): 41.8–165.2 (Chappell et al., 1997; Kirma et al., 2001)
Rabbit	Baseline: 0.45–34.7 (Hilliard et al., 1975) After social interaction: 13.1–26.8 (Haltmeyer and Eik-Nes, 1969)	Estrus: 0.2 (Ramirez and Soufi, 1994) After coitus: 0.9 (Ramirez and Soufi, 1994)		
Quail	Baseline: 1.6–12.2 (Balthazart et al., 1983, 1986; Delville et al., 1984a,b)	Baseline: 1.6 (Balthazart et al., 1983, 1986)	Baseline: 183.5–367 (Balthazart et al., 1986)	Morning/evening: 917.5–1101.0 (Balthazart et al., 1986; Delville et al., 1986) Midday: 367 (Balthazart et al., 1986; Delville et al., 1986)
Ring dove	0.6–2.4 (Feder et al., 1977)	<34.7 (Feder et al., 1977)		
Zebra finch	Intact: 1.0–11.8 (Adkins-Regan et al., 1990; Hutchison et al., 1984; Vockel et al., 1990) CX: 1.5–2.6 (Adkins-Regan et al., 1990; Vockel et al., 1990)	Intact: 1.7–3.5 (Hutchison et al., 1984; Vockel et al., 1990) OVX: 1.4 (Vockel et al., 1990)	Breeding: 319.3 (Schlinger Arnold, 1991) Non-breeding: 337.6 (Schlinger Arnold, 1991)	Breeding: 370.7 (Schlinger Arnold, 1991) Non-breeding: 715.7 (Schlinger Arnold, 1991)
Rhesus monkey	Baseline: 29.4 (Rose et al., 1972) After prolonged housing with female: 52.6 (Rose et al., 1972)			Baseline: 91.8–220.2 (O'Byrne et al., 1991; Wildt et al., 1980) Pre-ovulatory peak: 734–1284.5 (Knobil, 1976; O'Byrne et al., 1991; Wildt et al., 1980)
Human	Young men: 19.1–26.0 (Bremner et al., 1983) Old men: 8.7–17.4 (Bremner et al., 1983; Travison et al., 2006)	Baseline: 0.4–172.8 (Cashdan, 1995; Judd and Yen, 1973; Wong et al., 2003) After HYST or BSO: 0.001–3.2 (Aziz et al., 2005; Braunstein et al., 2005; Sherwin, 1985; Shifren et al., 2000; Simon et al., 2005)	Baseline: 0.9–157.1 (Bjornerem et al., 2004, in press; Cashdan, 1995)	Pre-ovulatory peak: 734–1570.8 (Thorneycroft et al., 1971; Wong et al., 2003) Follicular phase: 146.8–215.1 (Thorneycroft et al., 1971; Wong et al., 2003) Luteal phase: 440.4 (Thorneycroft et al., 1971) Post-menopausal or after HYST or BSO: 0.3–447.7 (Aziz et al., 2005; Bjornerem et al., 2004, in press; Braunstein et al., 2005; Sherwin, 1985; Shifren et al., 2000; Simon et al., 2005)

Table 1 – Circulating levels of testosterone and estradiol in males and females of various species

In order to facilitate the comparison with receptors affinities, concentrations that were often expressed in ng/ml or pg/ml have all been transformed in nM or pM using the following conversion ratios: 1 ng/ml = 3.47 nM for testosterone (MW = 288.48) and 1 pg/ml = 3.67 pM (MW = 272.38) for estradiol. Abbreviations: CX, castrates; HYST, hysterectomy; OVX, ovariectomized; BSO, bilateral salpingo-oophorectomy; MW, molecular weight.

modulate the electrical activity of various neuronal populations (Bueno and Pfaff, 1976; Whitehead and Ruf, 1974; Yagi, 1973). The concept of fast and non-genomic action of estrogen was first introduced in 1976 by Martin Kelly who demonstrated that E_2 applied by microiontophoresis modified within a few seconds neuronal activity recorded *in vivo* from septal and preoptic cells (Kelly et al., 1976). It is now clear that these fast effects of estrogens are diverse and involve several signaling pathways. However, the underlying mechanisms remain largely unknown and even the definition of a non-genomic action, as opposed to a genomic action, is not straightforward. As a matter of fact, these two modes of action are often compared in term of latency of onset (fast-non-genomic versus slow-genomic), but, although it is relatively easy to differentiate latencies of a few seconds from latencies of several hours or days, it is hard to define the mode of action of effects observed at intermediate latencies of about 30 min (some genomic effects are initiated within 30–45 min).

Furthermore, some non-genomic processes result in the activation of gene transcription and have for this reason been qualified as "indirect" effects (McEwen and Alves, 1999).

In order to provide simple (and probably simplistic) guidelines, it is generally considered that non-genomic effects of steroids share one or several of the following criteria: (a) they are too fast to be generated by *de novo* protein synthesis; (b) they may be reproduced in the presence of inhibitors of RNA or protein synthesis; (c) they may be reproduced following treatment with conjugated estrogen that is unable to pass the cell membrane (e.g., E_2-BSA); (d) they are observed in brain regions devoid of nuclear steroid receptors (Maggi et al., 2004).

The non-genomic effects of estrogens seem to be initiated at the cell membrane. The existence of membrane estrogen receptors was first proposed in 1977 by Pietras and Szego (Pietras and Szego, 1977). Since then, an important controversy developed concerning the nature of this (these) receptor (s) (for review, see Boulware and Mermelstein, 2005; Levin,
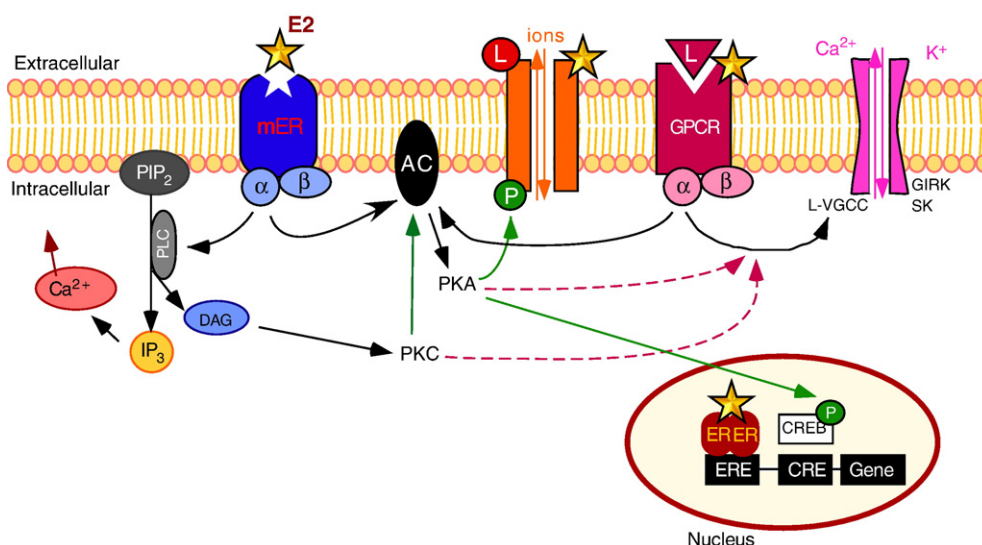
Fig. 1 – Schematic overview of some cellular pathways affected by estradiol. In order to simplify this drawing, only selected intracellular events are presented. Upon binding of their ligand (L), G-protein coupled receptors (GPCR, red receptor) such as metabotropic glutamate receptors (mGluR), 5-HT receptors, GABA$_B$ receptors, μ opioids receptors, α- and β-adrenergic receptors activate different intracellular signaling pathways through their G protein (made of α and β subunits). The activation of the G protein can lead to the activation of phospholipase C (PLC; not shown) that catalyzes the hydrolysis of membrane-associated phosphatidyl-inositol 4,5-biphosphate (PIP2) into 1,4,5 triphosphate (IP$_3$) and diacylglycerol (DAG). IP$_3$ induces calcium release from the intracellular stores (reticulum endoplasmic). DAG activates protein kinase C (PKC). In turn, PKC can stimulate, through phosphorylation (P), the activity of adenylate cyclase (AC) to produce cAMP and activate protein kinase A (PKA). PKA can also phosphorylate various proteins such as other receptors, ion channels (such as Type-L voltage-gated Ca^{2+}channels [L-VGCC], G-protein-coupled, inwardly rectifying K$^+$ channels [GIRK] or the small conductance, Ca^{2+}-dependent K$^+$ channel [SK]) or cAMP-responsive binding protein (CREB). Rapid non genomic signaling: (1) Estradiol (E$_2$, yellow star) can allosterically modulate the activity of ionotropic receptors (such as acetylcholine, kainate or NMDA receptors, orange receptors) or GPCR by directly interacting with this receptor. (2) E$_2$ can also activate a membrane-bound estrogen receptors (mER, blue receptor; the term is used here in a general way to cover both ERα and ERβ associated with the membrane and the more recently identified estrogen membrane receptor [GPR30, ER-X, etc.] specifically named mER) that is coupled to a G protein. Thereby, E$_2$ can modulate the activity of ionic conductance through phosphorylation of ionotropic receptors or uncoupling (dashed arrows) of GPCR from their ionic channels or intracellular effectors (not shown). It can also mobilize intracellular Ca^{2+} through activation of PLC or uncoupling of a GPCR from PLC (not shown). Delayed genomic signaling: E$_2$ can bind to nuclear estrogen receptors (ER) that form dimers and bind the estrogen-responsive element (ERE) on the DNA resulting in the activation of the transcription of specific genes. In addition, rapid effects of E$_2$ mediated through mER resulting in the activation of protein kinases can lead to phosphorylation of CREB, which can alter gene transcription through its interaction with the cAMP responsive element (CRE; indirect genomic effect).

2002; Nadal et al., 2001; Segars and Driggers, 2002; Toran-Allerand, 2004; Warner and Gustafsson, 2006). In brief, one hypothesis postulates the existence of novel membrane receptors, such as GPR30 (Filardo and Thomas, 2005) and ER-X (Toran-Allerand et al., 2002), that are different from the classical nuclear receptors, while the alternative hypothesis supports the involvement of the classical nuclear receptors, ERα and ERβ, that would be able to integrate the cell membrane and activate various intracellular cascades. In addition to their action on a specific membrane receptor whose nature remains to be elucidated, some steroids can act as co-agonists or allosteric antagonists of neurotransmitters's receptors. The best known example is certainly the allosteric modulation of the GABA$_A$ receptors by neurosteroids (Majewska et al., 1986). E$_2$ can also behave as an allosteric co-agonist of α$_4$β$_2$ nicotinic receptors (Paradiso et al., 2001) or as an allosteric antagonist of NMDA receptors (Weaver et al., 1997) and serotonin receptors 5-HT$_3$ (Wetzel et al., 1998). Finally, the existence of a binding site for estrogens has also been identified on an ionic voltage-dependent channel, the high conductance calcium-dependent potassium-channel called maxi-K (hSlo; Valverde et al., 1999).

Regardless of the nature of the membrane receptor involved, the action of estrogens initiated at the cell membrane activates a variety of intracellular responses (Fig. 1), including the modulation of intracellular calcium (Bologa et al., 2006; Chaban et al., 2003; Improta-Brears et al., 1999; Mermelstein et al., 1996; Morley et al., 1992; Revankar et al., 2005) and protein kinase C (PKC; Vasudevan et al., 2001; Watters et al., 1997). There is also evidence that membrane effects of estrogens can activate intracellular signaling pathways involving cyclic AMP (cAMP; Gu et al., 1999; Minami et al., 1990; Thomas et al., 2005), protein kinase A (PKA; Gu and Moss, 1998; Lagrange et al., 1997; Vasudevan et al., 2001), the "mitogen activated protein kinases" (or MAP kinases; Bi et al., 2000; Improta-Brears et al., 1999; Maggiolini et al., 2004; Razandi et al., 1999; Toran-Allerand et al., 2002; Wade and Dorsa, 2003; Watters et al., 1997) and the tyrosine kinases (Bi et al., 2000). The activation of these intracellular signaling pathways results primarily in phosphorylations/dephosphorylations producing different kinds of physiological responses such as the decoupling of a receptor from its effector system (Lagrange et al., 1997; Mize and Alper, 2000, 2002; Mize et al., 2003) or the modulation of the catalytic activity of an enzyme (Pasqualini et al., 1995). Finally, as mentioned previously, the activation of these cascades of intracellular events may result in a transcriptional activation caused, for example, by the phosphorylation of CREB (cAMP response element [CRE]-binding protein) which then acts at the level of the cAMP response element CRE; Abraham et al., 2003, 2004; Boulware et al., 2005; Gu et al., 1996; Zhou et al., 1996).

It is also the case that purely cytoplasmic effects, i.e., effects independent of the binding to a membrane receptor, have also been identified. Among others, it has been shown that the binding of estradiol to a cytoplasmic estrogen receptor, ERα or ERβ, allows an interaction between the ER and the protein kinase Src resulting in the activation of the intracellular cascade Src/Ras/ERK (Migliaccio et al., 2000). Moreover, the binding of E$_2$ to the cytoplasmic ERα activates the protein kinase Akt via a direct interaction of ER with the

protein kinase PI3K (Hisamoto et al., 2001; Simoncini et al., 2000; for more information see Driggers and Segars, 2002).

As illustrated in Table 2, the latency at which non-genomic effects of estrogen are observed varies from a few seconds to 30 min. Estrogen modulates electrical activity of neurons within a few seconds in some experiments (Mermelstein et al., 1996; Paradiso et al., 2001; Weaver et al., 1997; Wetzel et al., 1998) but with relatively longer latencies of up to 3–5 min in other biological systems (Gu et al., 1999; Gu and Moss, 1998; Kow and Pfaff, 2005; Lagrange et al., 1997; Qiu et al., 2003; Wong and Moss, 1992). The fastest of these effects appear to be associated with the allosteric modulations of ionotropic receptors (Paradiso et al., 2001; Weaver et al., 1997; Wetzel et al., 1998), but most effects on electrical activity result from a modulation of ion channel activity through G protein linkage (for review, see Kelly and Ronnekleiv, 2002; Moss et al., 1997). Estrogen-induced changes in intracellular calcium concentrations have also been identified with latencies of a few seconds (Bologa et al., 2006; Chaban et al., 2004; Morley et al., 1992; Revankar et al., 2005) to several minutes (Chaban et al., 2003; Improta-Brears et al., 1999). Finally, the time-course of protein phosphorylations and of changes in kinase activity following estradiol treatment ranges between 5 and 30 min (Bi et al., 2000; Improta-Brears et al., 1999; Maggiolini et al., 2004; Migliaccio et al., 2000; Toran-Allerand et al., 2002; Wade and Dorsa, 2003; Watters et al., 1997).

The broad spectrum of latencies associated with estrogen actions in vitro is fully consistent with the fact that they encompass a wide variety of potential mechanisms. However, independent of the nature of the effects described (potentiation/inhibition of neurotransmitter effects, protein phosphorylations, modulation of intracellular calcium levels, etc.), it appears that most of these effects result from the activation of second messengers systems. Mounting evidence also suggests that membrane estrogen receptors are coupled to G proteins (Filardo and Thomas, 2005; Kelly et al., 2003; Moss et al., 1997; Ronnekleiv and Kelly, 2005). The broad range of latencies described is thus probably explained by this diversity of mechanisms but also to a large extent by the number of successive intracellular events required for the activation of the measured effects. In addition, it should be noted that numerous studies did not report a time–response curve for the described effects. Consequently, the reported latency may simply be the result of an arbitrary choice of the experimenters when they designed their experiment. The latency of some effects also depends on technical issues that limit the temporal resolution of the data acquisition, so that the latency reported may actually be longer than the actual velocity of the effect.

Together, the range of latencies of non-genomic effects of estrogen identified in vitro is in agreement with the time-course of the fast effects of estrogens observed on measures of physiology or behavior (5 min to 1 h). If one extrapolates from the in vitro data, it is reasonable to speculate that the fastest effects observed with the use of organismal-level measurements (i.e., physiology or behavior) rely on modulations of ionotropic channel function, while slower effects result from a modulation of intracellular events through G protein activation that ultimately induce changes in ionic conductance, protein phosphorylation, enzymatic activity, etc. These effects

Table 2 – Summary of latencies and effective concentrations of estrogens that were shown to activate non-genomic effects of estrogens *in vitro*

Type of effects			Species/Tissue	Methods	Latency	[E_2]	Ref.
Electrophysiological recordings	Allo	ACh-R	Human	Expressed in *Xenopus* oocytes	A few seconds	3–15 μM	(Paradiso et al., 2001)
		NMDA-R	Rat—hippocampus	Cell culture	A few seconds	$EC_{50}=7$ μM	(Weaver et al., 1997)
		5-HT$_3$R	Human—embryonic kidney	5-HT$_3$R-transfected (HEK)293 cells	A few seconds	300 nM	(Wetzel et al., 1998)
		Synaptic	Rat—hippocampus	Slices	<2 min	10 nM	(Wong and Moss, 1992)
		Synaptic	Rat (pups)—hippocampus	Slice culture	A few minutes	1 nM	(Bi et al., 2000)
	G protein	Ca^{2+} currents	Rat—neostriatum	Slices	A few seconds	1–100 pM	(Mermelstein et al., 1996)
		KA currents	Rat—hippocampus	Slices	3 min	50–100 nM	(Gu et al., 1999; Gu and Moss, 1998)
		Depol.	Guinea pig—VMH	Slices	3–5 min	10 nM	(Minami et al., 1990)
		μ opioid-R	Rat—arcuate nucleus	Slices	A few minutes	20 nM	(Lagrange et al., 1997)
		GABA$_B$R	C57BL/6 mice—arcuate nucleus	Slices	5 min	100 nM	(Qiu et al., 2003)
		NMDA-R Hist-R	Rat—VMN	Slices	10–15 min	10 nM	(Kow and Pfaff, 2005)
Intracellular signaling	[Ca^{2+}]$_i$	L-VGCC	Rat—DRG	Primary culture—neurons	A few minutes	100 nM–1 μM	(Chaban et al., 2003)
			Mouse—DRG	Primary culture—neurons	5 min	$IC_{50}=27$ nM	(Chaban and Micevych, 2005)
		PLC	Rat	Primary culture—astrocytes	10–30 s	$EC_{50}=12.7$ nM	(Chaban et al., 2004)
		EGF/intracellular stores	Human—breast cancer	MCF-7 cells	5 min	10 nM	(Improta-Brears et al., 1999)
			Human-cervical carcinoma	Hela cells			
		PLC	Chicken, rat and pig—ovary	Isolated granulosa cells	A few seconds	100 nM	(Morley et al., 1992)
		PI$_3$K	Monkey—kidney fibroblast	GPR30-GFP-transfected COS7 cells	<2 s	$EC_{50}=0.3$ nM	(Bologa et al., 2006)
				ERα-GFP-transfected COS7 cells	<2 s	1 nM	
				ERβ-GFP-transfected COS7 cells	<2 s	1 nM	
		PLC	Monkey—kidney fibroblast	GPR30-GFP-transfected COS7 cells	1 s	$EC_{50}=0.5$ nM	(Revankar et al., 2005)
		EGF		ERα-GFP-transfected COS7 cells	1 s	1 nM	
		IP$_3$	Chinese—hamster ovary	ERα-transfected-CHO-K1 cells	15 s	10 nM	(Razandi et al., 1999)
				ERβ-transfected-CHO-K1 cells	15 s	10 nM	
	MAP-K signaling	P-ERK	Human—breast cancer	MCF-7 cells	5 min	10 nM	(Improta-Brears et al., 1999)
			Human—cervical carcinoma	HeLa cells			
		P-ERK	Human—neuroblastoma	SK-N-SH cells	5–15 min	10 nM	(Watters et al., 1997)

		P-ERK	Mouse—WT and ERαKO—Neocortex	Organotypic explant cultures	30 min	10 nM	(Toran-Allerand et al., 2002)
		P-ERK	Chinese—hamster ovary	ERα-transfected-CHO-K1 cells	10 min	10 nM	(Razandi et al., 1999)
				ERβ-transfected-CHO-K1 cells	10 min	10 nM	
		P-ERK	Rat—fibroblast	ERα-transfected-Rat-2 fibroblasts	10–15 min	0.3–10 nM	(Wade et al., 2001)
				ERβ-transfected-Rat-2 fibroblasts	10–15 min	0.3 nM	
		P-ERK	Murine—hippocampus	ERα-transfected-HT-22 cells	10 min	10 nM	(Wade and Dorsa, 2003)
				ERβ-transfected-HT-22 cells	10 min	10 nM	
		P-ERK	Human—prostate cancer	LNCaP-FGC cells	5 min	10 nM	(Migliaccio et al., 2000)
		P-CREB	Murine—hippocampus	ERα-transfected-HT-22 cells	10 min	10 nM	(Wade and Dorsa, 2003)
				ERβ-transfected-HT-22 cells	20 min	10 nM	
		cFos	Human—neuroblastoma	SK-N-SH cells	3 h	10 nM	(Watters et al., 1997)
		P-CREB	Rat—hippocampus	Cell culture—pyramidal neuron	$t=29$ s	$EC_{50}=5.5$pM	(Boulware et al., 2005)
		[cAMP]	Chinese—hamster ovary	ERα-transfected-CHO-K1 cells	20 min	10 nM	(Razandi et al., 1999)
				ERβ-transfected-CHO-K1 cells	20 min	10 nM	
		[cAMP]	Human—breast cancer	GPR30-transfected SKBR3 cells	20 min	10 nM	(Thomas et al., 2005)
			Human—embryonic kidney	GPR30-transfected (HEK)293 cells			
		eNOS	Human—vascular endothelium	Cell culture	10—15 min	$EC_{50}=0.1$ nM	(Simoncini et al., 2000)
G protein coupling	GTPγS-Binding	5-HT1$_A$ R	Rat—OVX	Hippocampus, Cortex, Amygdala	2 h	30 µg	(Mize and Alper, 2000)
		5-HT1$_B$ R		Hypothalamus		10 µg	
		CB1-R		Cortex, Hippocampus		10–30 µg	
		GABA$_B$ R		Cerebellum		10 µg	
		5-HT1$_A$ R	Rat—Hippocampus	Explant culture	60 min	50 nM	(Mize and Alper, 2002)
		5-HT1$_A$ R	Rat—Hippocampus	Explant culture	15 min	50 nM	(Mize et al., 2003)
			Rat—Frontal cortex	Explant culture	15 min	50 nM	
		G$_s$	Human—Embryonic kidney	GPR30-transfected (HEK)293 cells	20 min	100 nM	(Thomas et al., 2005)
		G$_s$ and G$_i$	Chinese—hamster ovary	ERα-transfected-CHO-K1 cells	5 min	10 nM	(Razandi et al., 1999)
				ERβ-transfected-CHO-K1 cells	5 min	10 nM	

Abbreviations: 5-HT$_3$R, 5-HT(serotonin)$_3$ receptor; Allo, allosteric modulations; ACh-R, acetylcholine receptors; [Ca^{2+}]$_i$, intracellular calcium concentration; cAMP, cyclic Adenosine 5′-monophosphate; CB1-R, cannabinoid receptor 1; DRG, dorsal root ganglia; EGF, epidermal growth factor; ERα, estrogen receptor α; ERβ, estrogen receptor β; GFP, green fluorescent protein; GPR30, G protein receptor 30; Hist, histamine; IP$_3$, inositol-1,4,5,-triphosphate; KA, kainate; L-VGCC, L-type voltage-gated calcium channel; NMDA-R, NMDA receptor; MAPK, mitogen-activated protein kinase; P-CREB, phosphorylation of cAMP response element-binding Protein(CREB); P-ERK, phosphorylation of extracellular-regulated kinases (ERK-1/ERK-2); PI3K, phosphatidylinositol triphosphate kinase; PLC, phospholipase C; VMH, ventromedial hypothalamic nucleus; VMN, hypothalamic ventromedial nucleus.

then potentially modify the firing rate of neurons and neurotransmitter release that will finally lead to measurable effects on physiology and behavior (Kelly and Wagner, 1999). The slight delay in the onset of *in vivo* as compared to *in vitro* effects is caused, in all probability, by the time needed for the steroid injected in periphery to reach an effective concentration at its target site(s). Indeed, acute intravenous injections of estradiol lead to a maximal estrogen concentration 5 min after the injection (Yamaji et al., 1972). Furthermore, an early report on fast actions of estrogen showed that it takes about 15–16 min for significant changes in the firing rate of preoptic-hypothalamic neurons to be observed following a single intravenous injection of estradiol in ovariectomized females. Interestingly, this effect persisted for 20 to 30 min and then vanished (Yagi, 1973).

In summary, it seems clear that the non-genomic effects of estrogens are quite diversified, require or not the binding to a membrane receptor and involve a variety of intracellular signaling pathways. These signaling cascades are probably not linear but act via parallel or even divergent pathways (Driggers and Segars, 2002). Estrogens may thus activate intracellular cascades without acting at the transcriptional level. These cascades could also amplify the genomic action of the hormone as suggested by the work of Pfaff and colleagues (Vasudevan et al., 2001) or influence cellular function prior to the initiation of transcription.

4.   Where does estrogen come from?

Together, these observations raise the question of the mechanisms controlling the rapid fluctuations of estrogen bioavailability that are presumably needed to sustain the fast actions of the hormone. Intuitively, it can indeed be hypothesized that, for fast non-genomic actions of steroids to be biologically effective, the endogenous ligand's availability must also change quickly. That is not to say that slow changes of steroids levels would not activate non-genomic effects. However, in this case, steroid action would result in a potential waste of the temporal-resolution provided by fast membrane responses.

What is the mechanism mediating fast behavioral effects if the concentration of the ligand (steroid) does not vary and does not vary quickly? Quite surprisingly, this aspect of brain E_2 action has rarely been considered in the literature. Although fast effects of estrogens are not limited to females, the acute effects of estrogen in the brain seem to be exclusively attributed to the pre-ovulatory peak of estrogen produced cyclically in females. In fact, in most cases, there is little discussion of the origin of the hormone that exerts these proposed effects. How then are these rapid effects triggered in males? From a physiological perspective, this question is fundamental. If estrogens have the ability to quickly trigger specific and transient cellular processes or specific behaviors, it is legitimate to postulate that their production and clearance should also be finely and rapidly tuned in order to respond appropriately to relevant stimuli. Likewise, if estrogens can activate a specific behavior through a local action only, the mechanism controlling their production should additionally be region-specific. This neuroanatomical specificity could be provided by the discrete distribution of the receptors and/or their intracellular effectors but the origin of the hormone should be envisaged as well.

As alluded to previously, estrogens can be synthesized directly in the brain by aromatization of androgens such as testosterone. The involvement of this local source of estrogens in the regulation of the fast effects of these steroids should thus be considered. Rapid changes in local estrogen production could simultaneously provide answers to the problem of the source of rapid changes in ligand and of its anatomical specificity. From a functional point of view, it would also probably be useful to produce estrogens locally where they are needed rather than flooding the entire organism with a compound that can have severe adverse effects (e.g., promote tumor growth, alter structure or function of reproductive organs, induce general toxicity, etc.; Adkins et al., 1980; Balthazart et al., 1985; Fowler et al., 2000; Gill et al., 2001; Kirma et al., 2001; Mahendroo et al., 1997) especially at the high doses that seem to be required to activate non-genomic responses (See below). Is it therefore possible that estrogens locally produced in the brain by aromatization of testosterone represent an endogenous stimulus triggering all or part of the fast non-genomic effects reviewed above. This organization would additionally provide anatomical specificity to the observed effects while avoiding inappropriate if not toxic effects potentially resulting from the activation of non-neural tissues.

5.   The fast effects of estrogens: systemic or central origin of the steroid?

In both sexes, androgens such as testosterone or androstenedione can be converted into estrogens by an enzymatic process, called aromatization, which is catalyzed by the enzyme aromatase (or estrogen synthase). This enzyme is present primarily in the ovaries (but also to a lesser extent in the testes), the placenta, the bones and the brain (Naftolin and MacLusky, 1984; Sasano and Harada, 1998; Simpson et al., 2002). Under normal conditions, aromatase expression in the avian and mammalian central nervous system is restricted to specific neuronal populations. These neuronal groups are mainly located in the hypothalamic/preoptic (medial preoptic nucleus, ventromedial nucleus) and limbic system (bed nucleus of stria terminalis, amygdala, hippocampus, septum, etc.), but more scattered populations of aromatase expressing neurons are also present in the cerebral cortex (Balthazart et al., 1998b; Matsumoto et al., 2003; Roselli and Resko, 1997, 2001; Stoffel-Wagner, 2003; Wagner and Morrell, 1997). In zebra finches and canaries, aromatase is also detected in high concentrations in parts of the nidopallium adjacent to the song control nucleus HVC (Balthazart et al., 1996; Metzdorf et al., 1999; Saldanha et al., 2000). In contrast, in the brain of teleost fishes, aromatase is expressed in radial glia (Forlano et al., 2001). In addition, in birds and mammals, aromatase is observed after *in vivo* brain injury in the glial cells within the damaged brain areas indicating that astrocytes from most brain areas have the potential for expressing aromatase and therefore produce E_2 in response to injury (Azcoitia et al., 2003; Peterson et al.,

2001, 2004). Because under normal physiological conditions circulating concentrations of testosterone produced by the gonads are fairly high in males and even females of many species (Table 1), estrogens may therefore be produced locally in the brain by aromatization of this androgen which freely enters the central nervous system. Furthermore, there is evidence that brain areas such as the rat hippocampus or the avian telencephalon are capable of producing estrogen *de novo* from cholesterol (Holloway and Clayton, 2001; Prange-Kiel and Rune, 2006). Several studies also suggest that androgen precursors, such as DHEA (dehydroepiandrosterone), and androgens are also produced *de novo* in the brain (Corpechot et al., 1981; London et al., 2003; Matsunaga et al., 2002; Mensah-Nyagan et al., 1999; Soma et al., 2004). These steroids could thus also act as a substrate for aromatase.

A prominent role for brain aromatase has been established for the control of male sexual behavior in a variety of species (Bakker et al., 2004; Balthazart et al., 1990a; Balthazart and Surlemont, 1990; Christensen and Clemens, 1974, 1975; Clancy et al., 1995; Davis and Barfield, 1979; Honda et al., 1994; Toda et al., 2001; Watson and Adkins-Regan, 1989a,b). Central aromatization also plays a role in the control of aggressive behaviors (Schlinger and Callard, 1990a,b; Schlinger and Callard, 1989a; Soma et al., 2000; Toda et al., 2001), neuroprotection (Azcoitia et al., 2003), nociception (Blomqvist, 2000; Evrard et al., 2000c; Evrard and Balthazart, 2004a,b), the development of sexual preferences (Roselli et al., 2004) and synaptic plasticity (Prange-Kiel and Rune, 2006). Most of these studies, however, concern relatively slow, presumably genomic, effects of estrogens. Whether the more rapid, presumably non-genomic, effects of estrogens on brain and behavior relate to a central or peripheral source of the steroid should be considered independently.

5.1. Gonadal production of steroids

In both sexes, estrogens may be delivered to the brain via gonadal secretion or by local production in specific regions of the central nervous system (see Fig. 2). As discussed above, it is reasonable to hypothesize that estrogen availability has to be modulated rapidly in order to take advantage of its fast non-genomic effects. One can thus ask whether gonadal steroids vary rapidly enough to achieve this goal?

In females, the estrogen surge at pro-estrus is often implicitly considered as the source of rapid changes in estrogen brain concentration. However, to our knowledge, no study has documented increases in plasma E_2 concentrations that would take place within a time frame compatible with the latency of a few seconds or minutes observed for the activation of non-genomic effects of E_2. This does not mean that this ovarian source of estrogen cannot activate non-genomic effects when estrogen reaches a threshold concentration. Rather we would argue that this peripheral source of estrogen would not be able to trigger rapid on/off switches of the response and would therefore constitute a poor use of the capacity of the membrane non-genomic response mechanisms.

Available studies indicate that, in rats, the rise of estrogen plasma concentrations at pro-estrus occurs over a period of several hours slowly rising during 24 h to "rapidly" reach a peak in about 12 h (Ahdieh et al., 1985; Butcher et al., 1974; Smith et al., 1975; Takeo, 1984). Ovulation and receptivity occur about 24 h after this peak has been reached (McCarthy and Becker, 2002). This time frame is in agreement with the activation by estrogen of the transcription of hypothalamic progesterone receptors that appear to be required for the activation of female sexual receptivity (Mani et al., 1997; McCarthy and Becker, 2002; Pleim et al., 1990). The pre-ovulation secretion of estrogen appears even slower in primates including humans (Hotchkiss and Knobil, 1994; Wildt et al., 1980). This rate of increase does not seem compatible with the dynamics of non-genomic effects of estrogens that occur with latencies of a few minutes to an hour (see above) although, this relatively abrupt rise of circulating levels could activate preparatory effects such as those described by Pfaff and colleagues (Kow and Pfaff, 2004; Sinchak et al., 2005; Vasudevan et al., 2001, 2005).

It is established that social stimuli influence endocrine function in males and females of a variety of vertebrate species (see Ball and Balthazart, 2002; Harding, 1981; Komisaruk et al., 1981; Nelson, 2000; Oliveira et al., 2002 for review). Most of these effects take place over a relatively long period (>days) and are therefore likely to influence genomic but not the more rapid non-genomic effects of steroids (fish: Liley et al., 1993; frog: Burmeister and Wilczynski, 2000; birds: Hutchison and Katongole, 1975; O'Connell et al., 1981a,b; rat: Purvis and Haynes, 1972; rhesus monkey: Rose et al., 1972).

In addition, rapid increases (in the minute range) of circulating hormone levels triggered by environmental/social stimuli have also been reported. For instance, plasma testosterone levels may be acutely altered in males by the presentation of a female and/or copulation (mice: Batty, 1978; Macrides et al., 1975; rat: Kamel et al., 1975; Purvis and Haynes, 1974; rabbit: Haltmeyer and Eik-Nes, 1969; Hilliard et al., 1975; Saginor and Horton, 1968; hamster: Kamel et al., 1977; marmoset: Ziegler et al., 2005; macaques: Cerda-Molina et al., 2006; birds: Wingfield, 1985). The fastest effects reported occurred within 5–15 min (in rat: Purvis and Haynes, 1974; in mouse: Batty, 1978; in rabbit: Saginor and Horton, 1968). A release of pituitary hormones in response to some of these stimuli has also been shown to precede the testosterone release (Coquelin and Bronson, 1979; Folman and Drori, 1966; Hilliard et al., 1975; Johnston and Bronson, 1982; Kamel et al., 1975, 1977). The nature of the stimulus eliciting these responses has been questioned. Some studies based on repeated exposures to female odors or repeated mating suggested that mating *per se* was necessary (Thomas and Neiman, 1968), while other experiments indicated that the smell or sight of an estrus female or a pre-copulatory interaction with an estrus female were sufficient to increase plasma hormone levels (Batty, 1978; Coquelin and Bronson, 1979; Macrides et al., 1974; Purvis and Haynes, 1974; Saginor and Horton, 1968; Ziegler et al., 2005). It has also been suggested that these effects may be purely social, and not sexual, given that a similar increase of hormone concentrations could be evoked by exposure to a non-receptive female (Gelez and Fabre-Nys, 2004; Johnston and Bronson, 1982; Kamel et al., 1977; Macrides et al., 1975). Interestingly, in male rats, LH and testosterone secretion can be conditioned so that after learning they are rapidly induced by a neutral
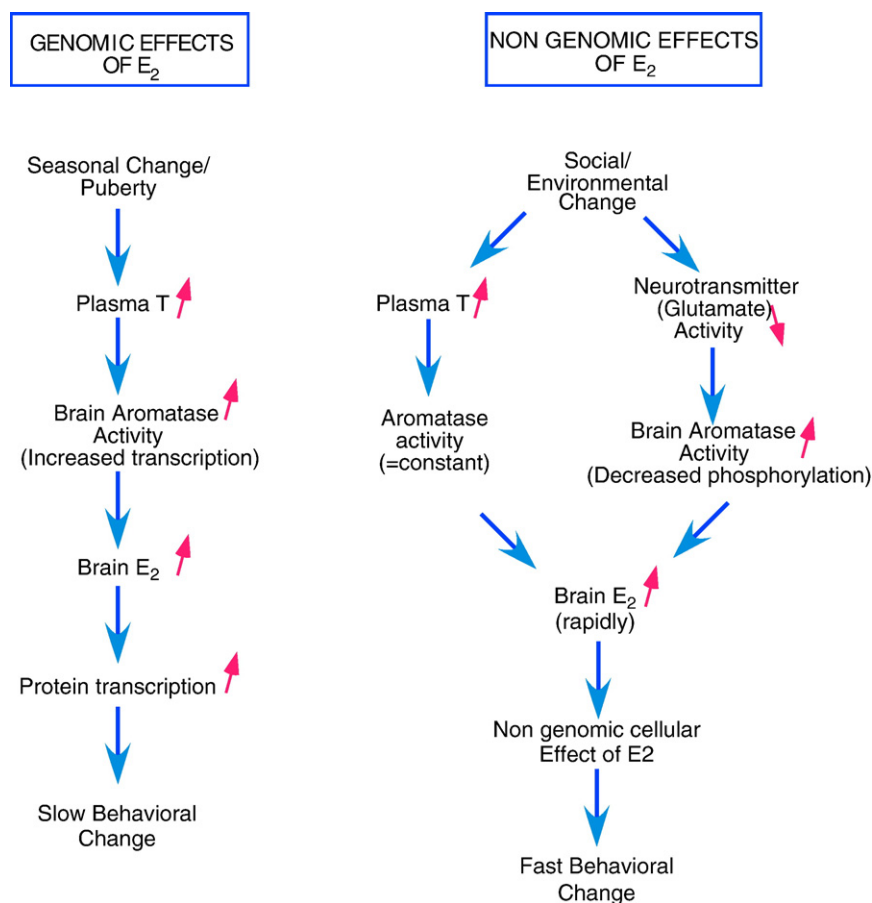
Fig. 2 – Potential sources of estrogens in males associated with genomic and non-genomic effects on physiology and behavior. On the one hand, genomic effects are associated with a slow rise of testosterone concentrations in the plasma. These changes are correlated with changes in testis function at puberty and across seasons. This rise in plasma testosterone results in an increased aromatase activity in the brain (through an activation of transcription of its gene). The estrogens produced locally by the aromatization of testosterone influence the transcription of various genes ultimately resulting in long lasting behavioral changes. On the other hand, non-genomic effects are associated with rapid changes in estrogens bio-availability. Such rapid changes in brain estrogen concentrations could result from variations of plasma testosterone that is subsequently aromatized locally into estrogen by brain aromatase as well as from variations in neurotransmitters' activity, such as glutamate or dopamine, that rapidly regulate brain aromatase activity and the subsequent production of estrogen. These fast changes in brain estrogen concentrations could in turn induce fast non-genomic actions in specific cell populations that would ultimately result in fast behavioral effects. In both cases, aromatase concentration remains constant and either the substrate of the enzyme (testosterone) rapidly becomes more concentrated resulting in an increased product formation or the substrate remains constant but the rate of conversion into estrogen (the enzymatic efficiency) is rapidly increased.

stimulus previously associated with mating (Graham and Desjardins, 1980) or by the arena where males were previously mated (Kamel et al., 1975).

Social stimuli related to aggression, stress or establishment of social rank have also been shown to affect plasma androgen levels in various species. Again, most of these effects seem to develop relatively slowly (Oliveira et al., 2002; Sapolsky, 1982), but some occur in a rather rapid fashion. For example, Harding and Follet (1979) reported that a short aggressive interaction (about 20 min) between male blackbirds results in increased levels of LH and androgens measured immediately after the encounter. Likewise, Wingfield (1985) demonstrated an increase of testosterone plasma concentrations in response to territorial intrusion in male song sparrows within 120 min.

In male teleost fish, watching a fight between two males raises the androgen levels (testosterone and 11-keto-testosterone, the main androgen in fishes) within 90 min (Oliveira et al., 2001). In the toad fish, 11-keto-testosterone was elevated 20 min after an acoustic playback challenge (Remage-Healey and Bass, 2005). In the green anole lizard, plasma testosterone is increased by 470% after agonistic encounters in winners but not in losers (Greenberg and Crews, 1990). Finally, in male guinea pig, plasma testosterone was significantly decreased 20 min after an agonistic encounter. This finding was interpreted as the expression of a relaxation phase after a physical strain associated with the encounter (Sachser, 1987).

The acute peripheral releases of testosterone that have been described in males occur within a few minutes (from

about 5 min to 2 h depending on the experiment) following the presentation of the eliciting stimuli and appear to be transient. The functional significance of these rapid endocrine changes, particularly in the context of the control of social behavior, remains, however, unclear. In the case of sexual encounters, the increased hormone levels have been proposed to enhance sexual arousal (Harding, 1981). However, the underlying mechanism of the behavioral effects of such rapid hormonal changes has not been identified. It has been shown that an injection of testosterone given to a sexually experienced and intact male rat 1 h before he was allowed to copulate with a female decreased the inter-intromission interval and facilitated ejaculation (Malmnas, 1977). Similarly, testosterone also affects penile reflexes of rats in a rapid manner (within 6 to 30 min; Sachs and Leipheimer, 1988).

The rapid behavioral effects of changes in plasma testosterone concentrations could obviously be mediated by interactions of the androgen with its cognate receptor. However, behavioral effects of increases in plasma testosterone levels could also be related to fast effects of estrogens. Indeed, contrasting with the relatively limited number of examples of fast actions of androgen reported at the cellular level (e.g., Benten et al., 1999; Lutz et al., 2003), rapid cellular effects of estrogens have been extensively documented. It is thus possible that a bolus of testosterone secreted by the testes following a social encounter is rapidly aromatized in the brain and the estrogen produced then contributes to the modulation of behavioral effects. Such a mechanism would provide anatomical specificity to the behavioral response since only aromatase-containing regions would react even though the entire body (and brain) would be exposed to increased testosterone concentrations. Whether the magnitude and time-course of these hormonal changes are compatible with the activation of non-genomic effects of estrogens is, however, unknown and more research on this topic would definitely be warranted.

5.2. Brain synthesized estrogens

Alternatively, as already discussed above, rapid changes in brain aromatase activity could generate equally rapid changes in brain estrogen concentrations that would trigger fast behavioral responses independent of changes in plasma androgen concentrations. It has traditionally been assumed that brain aromatase activity, as opposed to aromatase expressed in ovaries or adipose tissue, is regulated by sex steroids which act as transcription factors after binding to their cognate receptor to regulate the transcription of the enzyme. Treatment of castrates with testosterone markedly increases aromatase activity in several brain regions including the preoptic/hypothalamic area in birds and mammals (Roselli et al., 1997; Roselli and Resko, 1989; Schumacher and Balthazart, 1986). Testosterone similarly increases the concentration of the aromatase protein and the corresponding messenger RNA supporting the idea that testosterone induced changes in brain aromatase activity result of an increase in gene transcription (Abdelgadir et al., 1994; Balthazart and Foidart, 1993). As expected, these effects are relatively slow and take many hours if not days to be expressed (Balthazart et al., 1990b).

Besides this genomic and slow control of brain aromatase activity, recent experiments from our group have demonstrated that aromatase activity is rapidly modulated by calcium-dependent phosphorylations independently of changes in enzyme concentration. These experiments were performed in quail, taking advantage of the high concentration of the enzyme detected in the avian as compared to the mammalian brain (Schlinger and Callard, 1987; Schumacher and Balthazart, 1986; Steimer and Hutchison, 1981). They showed that the enzymatic activity measured in preoptic/hypothalamic homogenates submitted to phosphorylating conditions (i.e., in the presence of increased but physiological concentrations of ATP, Mg^{2+} and Ca^{2+}) is profoundly inhibited within 15 min (Balthazart et al., 2001). This inhibition is blocked by compounds chelating divalent ions such as EGTA and EDTA or by kinases inhibitors such as inhibitors of protein kinase A (PKA) or C (PKC) indicating that it is indeed caused by phosphorylation processes (Balthazart et al., 2001, 2003a). The identification of putative phosphorylation sites for PKC on the predicted quail aromatase sequence and Western blotting experiments on quail brain aromatase partially purified by immunoprecipitation confirmed that phosphorylation sites located on the aromatase protein itself are affected by the phosphorylating conditions that influence the enzymatic activity (Balthazart et al., 2003a,b).

Similar effects were observed in preoptic/hypothalamic explants in which the cellular integrity of the neurons and a large part of their connectivity were maintained (Balthazart et al., 2001). In these explants, aromatase activity is rapidly (within 5 min) and reversibly inhibited by conditions that increase the intracellular Ca^{2+} concentration such as a K^+-induced depolarization or the exposure to thapsigargin, a drug that mobilizes intracellular pools of Ca^{2+} (Balthazart et al., 2001). Due to technical limitations, it was impossible to assess changes in aromatase activity after latencies shorter than 5 min, but circumstantial evidence strongly supports the notion that the changes observed after 5 min take place much more rapidly at the cellular level (see Balthazart et al., 2001, 2006 and below for discussion).

Furthermore, application of glutamatergic agonists (AMPA, kainate and to a lesser extent NMDA, the effect was actually not statistically significant in this latter case) in these explants resulted in similar inhibitions. These AMPA/kainate effects are also reversible and are blocked by specific antagonists (Balthazart et al., 2006). An implication of DA in the control of aromatase activity has also been suggested but the exact mechanism of this action remains unknown (Absil et al., 2001).

Electrophysiological studies indicated that preoptic aromatase-containing cells are sensitive to glutamate, dopamine and norepinephrine (Cornil et al., 2002, 2004) and showed the existence in quail and rat preoptic area of a tonic electrical activity that can be blocked by glutamate and GABA antagonists (Cornil et al., 2002, 2004; Hoffman et al., 1994). A recent study also showed a modulation of estrogen synthesis within 30 min in hippocampal slices of male rats incubated in the presence of Mg^{2+} and NMDA. Interestingly, this effect resulted in a 2-fold increase in E_2 concentration in the preparation. The authors of this study hypothesized that this effect depends on NMDA-induced Ca^{2+} influx and thereby depends on synaptic communication (Hojo et al., 2004). Why application of NMDA

tended to decrease estrogen formation in the quail preoptic/hypothalamic explants and increase it in the rat hippocampus remains unclear at present. This difference could relate to differences in the species used, brain area investigated or specific experimental conditions (e.g., ionic, in particular Mg^{2+}, concentration in the medium). It must also be mentioned that, in addition to these rapid effects of NMDA receptor activation on estrogen synthesis, estrogens regulate, presumably by genomic mechanisms, the concentration of NMDA receptors in the songbird hippocampus (Saldanha et al., 2004). This suggests the existence of bi-directional interactions between NMDA receptors and estrogens that normally utilize two different time frames when regulating biological events. How these effects interact under intact physiological conditions remains to be investigated.

Finally, it should be noted that, in addition to its presence in cell bodies, aromatase has also been identified throughout the length of neuronal dendrites and axons, including presynaptic boutons, in a relatively large number of brain areas (Evrard et al., 2004; Foidart et al., 1995). Aromatase activity is also enriched in synaptosomes prepared from quail or rat brain (Schlinger and Callard, 1989b; Steimer, 1988) and dense aromatase immunoreactivity is observed in presynaptic boutons at the surface of synaptic vesicles (Hojo et al., 2004; Naftolin et al., 1996; Peterson et al., 2005). Together, these data suggest that aromatase activity can be rapidly (with latencies in the minute or possibly the second range) modulated at the presynaptic level by afferent inputs potentially providing a mechanism able to quickly change the local production of estrogens.

Rapid changes in estrogen availability are thus likely to occur at the synaptic level suggesting that estrogen in the brain may play a role more similar to the role of neurotransmitters or neuromodulators than previously thought. Given the lipophilic nature of estrogen, the mechanism(s) of its release and retention may be questioned. However, the definition of what should be considered a neurotransmitter has recently been extended to unconventional transmitters such as nitric oxide, a gas that is not stored in cells and is not released in an exocytotic manner (Deutch and Roth, 1999; Snyder and Ferris, 2000). More work will, of course, be necessary before estrogen produced in the brain can be fully accepted as a neurotransmitter (see Balthazart and Ball, 2006 for a further discussion). Nevertheless, these rapid changes in local estrogen concentration appear perfectly suited to mediate the rapid effects of this hormone. In addition, as pointed out previously, the intervention of brain aromatase offers anatomical specificity to the behavioral effects since estrogen is synthesized and will produce its effects only locally. Its restricted localization, in particular at the level of presynaptic boutons, could also contribute to speed up the rise of estrogen concentration since production takes place exactly where needed (presynaptic level) and does not require a transport/diffusion of the hormone to distant target site. This implies, however, that the (unidentified) brain sites mediating rapid behavioral effects of estrogens should overlap with the brain areas expressing aromatase. An important remaining question is thus whether the estrogen release/synthesis sites are localized in proximity to estrogen receptor systems that mediate fast behavioral effects and, at the cellular level,

whether these receptor systems are present in the pre/postsynaptic membrane in close association with presynaptic aromatase.

6.   What is a physiological concentration of estrogen?

The fact that estrogen production is very localized in the brain suggests that high concentrations may be rapidly attained in specialized locations such as the synaptic clef in specialized synapses. These local brain concentrations may thus exceed at discrete sites the physiological concentrations of circulating estrogens. This could then explain why some (most) of the rapid effects of estrogens that have been observed in vitro can only be activated with what has been considered so far as supra-physiological concentrations.

In a recent article, Warner and Gustafsson (2006) argued that the E_2 concentrations used in some studies are higher than physiologically relevant concentrations, classifying these concentrations as pharmacological and toxicological. Based on the maximal plasma levels measured in cycling females (±100 pg/ml, i.e., 0.4 nM in rodents; ±350 pg/ml, i.e., 1.3 nM in primates including humans, see Table 1 for details), it can be accepted that physiological concentrations of E_2 range from the high picomolar (i.e., 100 pM; rat: Ahdieh et al., 1985; Butcher et al., 1974; Smith et al., 1975) to low nanomolar concentrations (i.e., 1–2 nM; quail: Balthazart et al., 1986; Delville et al., 1985; rhesus monkey: Wildt et al., 1980; human: Hotchkiss and Knobil, 1994; see Table 1 for details). While some rapid effects of E_2 are elicited in vitro at such concentrations (Boulware et al., 2005; Mermelstein et al., 1996; Revankar et al., 2005; Vasudevan et al., 2001), numerous studies reported effects that could only be triggered by concentrations in the high nanomolar to micromolar range (Abraham et al., 2003; Chaban et al., 2003; Gu and Moss, 1998; Improta-Brears et al., 1999; Kow and Pfaff, 2005; Maggiolini et al., 2004; Qiu et al., 2003; Thomas et al., 2005; Wade and Dorsa, 2003; Watters et al., 1997; Weaver et al., 1997; Wetzel et al., 1998; Wong and Moss, 1992; see Table 2 for details). Obviously, these effects cannot be triggered by the levels of estrogen circulating in the periphery, not even by the pro-estrus surge of E_2. However, are these effects of a pharmacological nature? We do not believe that this is necessarily the case because although these fast effects cannot be elicited by peripheral concentration of estrogen, they could be activated by the potentially higher concentrations produced locally by aromatase activity.

This scenario would be in agreement with the concept that "estrogen synthesized in extragonadal compartments acts predominantly at the local tissue level in a paracrine or intracrine fashion" and consequently "the total amount of estrogen synthesized by these extragonadal sites may be small, but the local tissue concentrations achieved are probably high and exert biological influence locally" (Simpson, 2004; Simpson and Davis, 2001). This model is supported by reports of higher concentrations of estrogen in certain brain areas, such as the hypothalamus, preoptic area and the hippocampus, than in the plasma (Bixo et al., 1995; Hojo et al., 2004). It must also be noticed that these measures of tissue

concentrations are likely to be underestimations of the actual concentrations present at discrete locations close to the site of synthesis and action. Even if assays are performed in microdissected brain regions that express high level of aromatase, heterogeneity still persists in these samples and the potentially high concentration of estrogen present at specific subcellular sites (e.g., the synaptic cleft) is diluted in tissue containing lower concentrations of the steroid.

This model will remain hypothetical until technology allows us to determine estrogen concentrations at the subcellular level. The model is also currently associated with a major caveat namely that, if high concentrations of estrogens are required to activate fast physiological or behavioral responses, then (membrane?) estrogen receptor systems that are selectively sensitive to these high concentrations should be present in the brain. In contradiction with this prediction, membrane estrogen receptors have been identified during the last decade, but their affinity for estrogen is not fundamentally different from the affinity of nuclear receptors (see Table 3). Indeed, independently of their cellular localization in the nucleus or in the membrane, the so-called "classical" intra-cellular receptors (ERα and ERβ) respond to concentrations of estradiol in the high picomolar range. In addition, novel membrane receptors such as GPR30 and ER-X respond to concentrations in the low nanomolar range. This discrepancy between the doses of estrogen needed to activate fast effects and the affinity of the known

estrogen membrane receptors cannot be explained at this time. For these effects of estrogen to be considered as physiological in nature, either a novel estrogen receptor with lower affinity should be identified or some physiological mechanism (e.g., a specialized regulation of the coupling between the receptor and associated G protein) should be uncovered to explain the requirement of such high dose of steroid.

Nevertheless, based on these considerations, it is enlightening to reconsider the circulating concentrations that are presumably reached following the acute systemic injections of estrogens in behavioral studies that identified rapid effects of this steroid. These may be considered as supra-physiological when compared to endogenous circulating levels in males or even females. Indeed, assuming that distribution is immediate and the distribution volume equals the entire body (1 l/kg body weight), the average concentrations reached in these experiments in the body fluids should range between 100 and 500 ng/ml which is about 2 to 3 orders of magnitude above the endogenous concentrations of estrogens in the plasma (after an injection of 1–10 μg/mouse (Abraham et al., 2004) or an injection of 20 μg/kg (in rat: Cross and Roselli, 1999; in midshipman fish: Remage-Healey and Bass, 2004), 100 μg/kg (in rat: Cross and Roselli, 1999; Gu et al., 1996), 500 μg/kg (in rat: Zhou et al., 1996; in quail: Cornil et al., 2006a) or 1 mg/kg in midshipman fish: (Remage-Healey and Bass, 2004). The goal in these experiments is, however, not to mimic circulating levels

Table 3 – Affinity (K_d) of identified estrogen receptors as a function of their cellular localization and the system in which they have been measured

	Nuclear		Membrane	
	K_d	Origin	K_d	Origin
ERα	0.1 nM	*In vitro* synthesized human ERα (Kuiper et al., 1997)	0.30 nM	ERα-GFP-transfected COS7 cells (Bologa et al., 2006)
	0.283 nM	ERα-transfected CHO cells (Razandi et al., 1999)	0.287 nM	CHO cells (Razandi et al., 1999)
ERβ	0.6 nM	Clone 29, rat prostate cDNA library (Kuiper et al., 1996)	0.38 nM	ERβ-GFP-transfected COS7 cells (Bologa et al., 2006)
	0.6 nM	*In vitro* synthesized human ERβ (Kuiper et al., 1997)	1.17 nM	CHO cells (Razandi et al., 1999)
	1.23 nM	ERβ-transfected CHO cells (Razandi et al., 1999)		
ER-X			1.6 nM	(Toran-Allerand et al., 2002)
GPR30			5.7 nM	GPR30-GFP-transfected COS7 cells (Bologa et al., 2006)
			6.6 nM	GPR30-GFP-transfected COS7 cells (Revankar et al., 2005)
			2.7 nM	ERα−/ERβ−/GPR30+-transfected SKBR3 cells (Thomas et al., 2005)
			3.3 nM	ERα−/ERβ−/GPR30+-transfected (HEK)293 cells (Thomas et al., 2005)
E$_2$-BSA				Membrane fraction:
			4.5 nM	Hypothalamus (Ramirez and Zheng, 1996)
			8.7 nM	Olfactory bulbs (Ramirez and Zheng, 1996)
			30.2 nM	Cerebellum (Ramirez and Zheng, 1996)

Note that GPR30 was identified in the plasma membrane in the studies of Thomas and colleagues (Thomas et al., 2005) but in the endoplasmic reticulum membrane in the studies of Revankar, Bologa, Prossnitz and colleagues (Bologa et al., 2006; Revankar et al., 2005). Thomas et al. (2005) found Bmax values of 114 pM and 100 pM for SKBR3 cells and HEK-293 cells transfected with GPR30. Abbreviations: ERα, estrogen receptor α; ERβ, estrogen receptor β; GFP, green fluorescent protein; GPR30, G protein receptor 30; (HEK)293 cells, human embryonic kidney cells; CHO cells; Chinese Hamster ovary cells; COS7 cells, monkey kidney fibroblast; SKBR3 cells, human breast cancer cells.

of estrogens but to induce in a few discrete brain locations, concentrations that are presumably produced by local aromatase activity. Although much higher than concentrations observed in the plasma, these experimentally induced concentrations may thus appropriately mimic the natural situation in target areas.

In conclusion, the data summarized here suggest that estrogen concentrations that are higher than physiological circulating levels may be reached locally in the brain through the conversion of testosterone by the enzyme aromatase. This could explain why some of the rapid effects of estrogens observed *in vitro* can only be activated with what is considered as supra-physiological concentrations. The estrogen-sensitive system (receptor?) that mediates these effects of higher concentrations is, however, unclear at present and its identification should be a priority for future research.

7.   What terminates estrogen action in the brain?

Warner and Gustafsson also compared the effects of E_2 and neurotransmitters and observed that "the changes [induced by neurostransmitters] are not only rapid, they are also transient" and "there are multiple mechanisms at the synapse to terminate neurotransmitter action and rectify intracellular ion homeostasis." They stated that the case of E_2 is different because "plasma levels do not change rapidly and transiently and no extremely rapid metabolic pathways to terminate estrogen action at the cell surface have been described" (Warner and Gustafsson, 2006). Based on the data reviewed above, we would like to argue that changes in brain estrogen production can also be rapid. Furthermore, there are multiple mechanisms that could lead to the rapid termination of E_2 action based either on diffusion of the active hormone from its production/action site (thus leading to concentrations that are below the threshold for action) or on its degradation through various catabolic pathways.

Available evidence indeed is consistent with the notion that plasma concentrations of E_2 do not vary rapidly enough to support the rapid non-genomic effects of this steroid but E_2 can be synthesized in the brain and the rate of this local conversion of testosterone appears to be rapidly regulated. We have seen that aromatase activity can be rapidly modified within 5 min and probably even faster. Glutamatergic inputs seem able to rapidly turn on or off the local synthesis of E_2 depending on the brain region considered (Balthazart et al., 2006; Hojo et al., 2004). Therefore, such a mechanism should provide a source of rapid and transient changes of estrogen production and thus concentration – not in the plasma, but locally in the brain – that matches quite well the time-frame of the rapid effects described for this hormone.

Moreover, inactivation mechanisms are present in the brain to terminate estrogenic signaling when the synthesis of the steroid has been switched off. Estrogenic hormones are catabolized to hormonally inactive (or less active) water-soluble metabolites that are excreted in the urine and/or feces. The metabolic elimination of estrogens relies on oxidative metabolism (largely hydroxylations by cytochromes P450 enzymes (Zhu and Conney, 1998) and conjugations by

glucuronidation (Barbier and Belanger, 2003), sulfonation (Song, 2001; Song and Melner, 2000) and/or O-methylation (Männistö and Kaakkola, 1999). While hydroxylated products retain some estrogenic activity, conjugations appear to markedly decrease hormonal action. The metabolism of estrogens mostly takes place in the liver, but detectable levels of activity of metabolic enzymes are also expressed in other tissues including the brain. High levels of 2- and 4-hydroxylases have, for example, been identified in the brain, notably the preoptic-hypothalamic region (Balthazart et al., 1994; Lambert et al., 1999; Timmers et al., 1988; Zhu and Conney, 1998). The 2- and 4-hydroxyestrogens (also called catecholestrogens) are biologically active compounds but they are rapidly metabolized by the catecholamine-O-methyl-transferases (COMT) into methoxyestrogens that have a weaker hormonal activity (Männistö and Kaakkola, 1999). COMT is widely distributed in the brain (Männistö and Kaakkola, 1999) so that estrogens can be effectively transformed into less active compounds. Significant glucuronidase and sulfotransferase activities have also been identified in the brain (Albert et al., 2000; Miki et al., 2002; Purinton and Wood, 2000). As suggested by Song and Melner (2000), the inactivation in target tissues by these enzymes could function as a molecular switch to control local estrogen activity. In addition, aromatase itself appears to catalyze in the placenta both the conversion of testosterone into E_2 and the 2-hydroxylation of estrogens into catecholestrogens. The switch between these two enzymatic activities (catalyzed by the same protein) would dependent on the availability of the substrates and the local pH (Osawa et al., 1993). If this scenario turns out to be true in the brain, this mechanism would provide a very effective way to rapidly control local concentrations of neuro-active estrogens. Together, these data demonstrate that active pathways for estrogen synthesis and catabolism are thus present in the brain and they may proceed at speeds compatible with the rapid induction and termination of estrogenic signaling that is required to explain the fast and transient effects produced by the steroid.

8.   Fast effect of aromatase inhibition on physiology and behavior

8.1.   Effects on appetitive and consummatory aspects of male sexual behavior

If rapid modulations of brain estrogen synthesis and catabolism are involved in the non-genomic control by estrogens of physiological and behavioral processes, the blockade of aromatase activity should rapidly lead to detectable changes in these responses. Accordingly, in recent experiments conducted in quail, systemic injections of a large dose of Vorozole[TM], a non-steroidal aromatase inhibitor, significantly reduced most aspects of male copulatory behavior in sexually active males (gonadally intact males or castrates implanted with 40 mm testosterone capsules). Maximal effects were observed after 30 or 45 min (Cornil et al., 2006b). The acute effect of aromatase inhibition was even more pronounced on the expression of appetitive male sexual behavior. Appetitive sexual behavior was assessed by the frequency of rhythmic

cloacal sphincter movements (RCSM) and by the expression of the learned social proximity response, two sexually motivated behavior that have been shown previously to be dependent on the aromatization of testosterone (Balthazart et al., 1995, Balthazart et al., 1998a; Domjan and Hall, 1986; Seiwert and Adkins-Regan, 1998). A significant inhibition of RCSM was observed 30 or 45 min after the injection of Vorozole[TM] and was no longer significant after 60 min. The behavior returned to a normal rate on the next day (Cornil et al., 2006b). Androstatrienedione (ATD), another unrelated aromatase inhibitor with a very different chemical structure, induced a similar rapid inhibition of RCSM (Cornil et al., 2006b). These behavioral inhibitions correlated with inhibitions of the aromatase activity measured in the preoptic-hypothalamus area (see Cornil et al., 2006b for further details). The fact that similar effects of aromatase inhibition were observed in gonadally intact males and in castrates chronically treated with testosterone suggests that the behavioral effect resulted from a rapid modulation of aromatase activity and thus local estrogen bioavailability rather than from a rapid change in secretion of gonadal testosterone. Together, these pharmacological data thus support a role for rapid modulations of estrogen synthesis in the regulation of both appetitive and consummatory aspects of male sexual behavior.

Conversely, in other experiments, we mimicked the putative effects of a rapid activation of brain aromatase activity by injecting a large dose of E_2 to castrated male quail treated with a subthreshold dose of testosterone, unable by itself to activate a full copulatory behavior, in order to prime the mechanisms involved in sexual behavior. As expected, a marked induction of all aspects of male copulatory behavior was observed in these experiments 15 min following the estrogen injection and this effect was no longer present at 30 min post injection (Cornil et al., 2006a).

We also investigated whether preoptic aromatase activity is rapidly modulated *in vivo* during sexual activity. Gonadally intact males were allowed to see a female or copulate with her for 1, 5 or 15 min and sacrificed immediately after the interaction. Control subjects were simply handled and returned to their home cage for the duration of the test. The visual access as well as sexual interactions with a female resulted in a 20% decrease of aromatase activity within 1 min. The enzymatic inhibition reached a maximum after 5 min and was back to normal after 15 min (Cornil et al., 2005). As expected based on *in vitro* observations (see above), these data thus demonstrate that aromatase activity can be modulated very rapidly (faster than in the 5 min limit that could be tested *in vitro*) and that activity changes occur *in vivo* in physiologically relevant situations. The fact that enzymatic activity dropped following sexual interactions could, however, appear somewhat counterintuitive and an increase could have *a priori* been expected (e.g., to prepare to additional interactions with the female). It must, however, be pointed out that copulation in sexually experienced quail occurs with a time-course very different from what is observed in rodents. Adult male quail immediately engage in mating after the introduction of a female in their cage and usually achieve cloacal contact and sperm transfer within a few seconds. A successful copulation is thus usually achieved in less than one min. Sexual behavior

occurs in bouts of activity of a few minutes separated by periods during which the bird takes care of alternative needs, such as preening its plumage, feeding, etc. (Hutchison, 1978). This drop of local estrogen synthesis thus coincides with and possibly determines the transient period of refractoriness that follows sexual activity. It is thus possible that the rapid changes of aromatase activity observed in this study do not reflect what happens in the brain during the expression of sexual behavior (potentially a very short-lived increase in enzyme activity) but are rather associated with its termination.

At the mechanistic level, recent data from Dominguez et al. (2006) indicate that glutamate concentration progressively increases in the rat preoptic area in the course of mating to peak at ejaculation. If a similar release of glutamate takes place in the preoptic area of quail during copulation, this change in neurotransmitter activity could then represent the cellular mechanism controlling the decrease of aromatase activity that was observed in these experiments.

We cannot, however, exclude that the expression of the behavior is preceded in quail by an extremely fast increase in estrogen production that cannot be detected at present due to technical limitations. An *in vivo* "on line" measure of activity would be required since the time needed to dissect and freeze the brain prevents analysis to latencies shorter than one min. Both an increase and then a decrease of enzymatic activity would thus participate in the control of the onset and the termination of bouts of sexual activity within a very short period of time. Alternatively, aromatase in quail could be constantly active at (nearly) maximal levels thus allowing the immediate initiation of sexual behavior at any time. The expression of mating behavior would then turn off the enzyme for a short period of time resulting in a transitory cessation of the behavior. This would still be in agreement with our pharmacological data indicating that a bolus of estrogen is able to establish the local estrogen concentration required to allow the initiation of the behavior, while Vorozole[TM] treatment obviously mimics the natural drop of aromatase activity following mating.

8.2. Effects on nociception

Estrogen has been shown to control nociception and analgesia in both sexes in human as well as in animal models (Fillingim and Ness, 2000). Estrogen actions on nociception and analgesia involve a genomic modulation of the signaling of opioid and adrenergic systems in the peripheral and central nervous system (Amandusson et al., 1999; Dina et al., 2001; Liu and Gintzler, 2000). Moreover, recent studies suggest that estrogen also controls nociception in a non-genomic manner. Indeed, 17β-estradiol, as well 17β-estradiol conjugated with bovine serum albumin, inhibits ATP-induced intracellular calcium concentration within 5 min in the dorsal root ganglia (DRG) that contains a subset of nociceptive neurons (Chaban et al., 2003). This inhibition of ATP-induced Ca^{2+} flux by estradiol results from the blockade a voltage dependent calcium channel. The inhibition is not observed in ERαKO but persists in ERβKO mice indicating that this non-genomic modulation of DRG signaling is mediated through ERα (Chaban and Micevych, 2005). Since ATP has been implicated in sensory

transduction of noxious stimuli, these studies also provide a mechanism through which estradiol may inhibit Ca^{2+} fluxes associated with ATP-induced nociception.

Aromatase is also expressed and enzymatically active in pain-sensitive neurons in the dorsal horn of the spinal cord in quail (Evrard et al., 2000c). Locally produced estrogens appear to modulate pain responses to noxious stimuli. Indeed, the latency of withdrawal from a 54 °C hot water bath is markedly increased by castration in quail. By contrast, chronic testosterone treatment decreases this latency and it has been shown that this effect depends on its aromatization into estrogen (Evrard and Balthazart, 2004a). Rapid effects on nociception of intrathecal injections of aromatase inhibitors (vorozole™ and ATD) are also observed: the foot withdrawal latency from a hot water bath is markedly increased 1 and 5 min after an intrathecal injection of the aromatase inhibitor, ATD or Vorozole™, and this effects has disappeared 30 min after the injection. These acute effects of a systemic injection of vorozole™ are inhibited by a concurrent injection of E_2 (Evrard and Balthazart, 2004b). Similar effects were reported recently in rats (Evrard and Erskine, 2005). Together these data indicate that the rapid effects of locally produced estrogens on neuronal physiology and behavior do not only concern the brain but also the spinal cord. Such effects can be measured by behavioral responses in addition to the rapid electrophysiological effects previously identified in single neurons.

9. Conclusions

Besides their well-known long lasting action, mounting evidence indicates that estrogens also exert rapid effects on physiological and behavioral processes. These effects are activated within a few minutes and have been described in both sexes. Rapid effects of steroids, estrogens in particular, have been the subject of intense debates concerning namely the question of the existence and nature of the receptors involved. However, the fundamental issue of the source of rapid changes in estrogen concentrations required to sustain their fast effects has not received much attention. In a few rodent studies, it was assumed that the source of estrogen was the pro-estrus rise preceding receptivity and ovulation in females. Available data suggest that this relatively slow increase in plasma hormone is unable to take advantage of the rapid temporal resolution of most recently identified non-genomic effects of estrogens. In addition, fast effects of estrogens are also observed in males in which no such variation in plasma concentrations of the hormone have been described. Instead, rapid changes of testosterone levels elicited by social cues have been reported. The cerebral aromatization of the androgen may thus be one source of rapid change of local estrogen availability in males. In addition, rapid changes of aromatase activity resulting in fast regulations of estrogen availability in the brain have been identified. Such variations in brain aromatase activity provide a source of rapid and transient production of high concentrations of estrogen that seems to perfectly match the requirements for numbers of non-genomic effects. These findings thus open new avenues in the understanding of estrogen action on the brain. Of course, numerous questions still have

to be addressed. So far, rapid changes of aromatase activity have been described in the preoptic/hypothalamic area and future studies should continue to investigate the mechanisms underlying these rapid activity changes and determine whether they take place in all brain regions where aromatase is present. Likewise, although these effects may theoretically occur both in males and in females, they have been studied in males exclusively to this date. Little attention has also been devoted to the mechanism(s) that control estrogen catabolism in the brain. The characterization of these rapid changes in brain estrogen production and catabolism and the analysis of their physiological and behavioral consequences will certainly have a profound impact on the planning of future research programs and design of clinical interventions that involve actions of estrogen in the brain.

REFERENCES

Abdelgadir, S.E., Resko, J.A., Ojeda, S.R., Lephart, E.D., McPhaul, M.J., Roselli, C.E., 1994. Androgens regulate aromatase cytochrome P450 messenger ribonucleic acid in rat brain. Endocrinology 135, 395–401.

Abraham, I.M., Han, S.-K., Todman, M.G., Korach, K.S., Herbison, A.E., 2003. Estrogen receptor Beta mediates rapid estrogen actions on gonadotropin-releasing hormone neurons in vivo. J. Neurosci. 23, 5771–5777.

Abraham, I.M., Todman, M.G., Korach, K.S., Herbison, A.E., 2004. Critical in vivo roles for classical estrogen receptors in rapid estrogen actions on intracellular signalling in mouse brain. Endocrinology 145, 3055–3061.

Absil, P., Baillien, M., Ball, G.F., Panzica, G.C., Balthazart, J., 2001. The control of preoptic aromatase activity by afferent inputs in Japanese quail. Brain Res. Rev. 37, 38–58.

Adkins-Regan, E., Abdelnabi, M., Mobarak, M., Ottinger, M.A., 1990. Sex steroid levels in developing and adult male and female zebra finches (Poephila guttata). Gen. Comp. Endocrinol. 78, 93–109.

Adkins, E.K., Boop, J.J., Koutnik, D.L., Morris, J.B., Pnieswski, E.E., 1980. Further evidence that androgen aromatization is essential for the activation of in male quail. Physiol. Behav. 24, 441–446.

Ahdieh, H.B., Siegel, H.I., Wade, G.N., 1985. The role of the uterus in regulation of heat duration in cycling rats. Horm. Behav. 19, 292–303.

Albert, C., Barbier, O., Vallée, M., Beaudry, G., Bélanger, A., Hum, D.W., 2000. Distribution of uridine

diphosphate-glucuronsyltransferase (UGT) expression and activity in cynomolgus monkey tissues: evidence for differential expression of steroid-conjugating UGT enzymes in steroid target tissues. Endocrinology 141, 2472–2480.

Amandusson, A., Hallbeck, M., Hallbeck, A.L., Hermanson, O., Blomqvist, A., 1999. Estrogen-induced alterations of spinal cord enkephalin gene expression. Pain 83, 243–248.

Azcoitia, I., Sierra, A., Veiga, S., Garcia-Segura, L.M., 2003. Aromatase expression by reactive astroglia is neuroprotective. Ann. N. Y. Acad. Sci. 1007, 298–305.

Aziz, A., Bergquist, C., Brannstrom, M., Nordholm, L., Silfverstolpe, G., 2005. Differences in aspects of personality and sexuality between perimenopausal women making different choices regarding prophylactic oophorectomy at elective hysterectomy. Acta Obstet. Gynecol. Scand. 84, 854–859.

Bakker, J., Honda, S., Harada, N., Balthazart, J., 2004. Restoration of male sexual behavior by adult exogenous estrogens in male aromatase knockout mice. Horm. Behav. 46, 1–10.

Ball, G.F., Balthazart, J., 2002. Neuroendocrine mechanisms regulating reproductive cycles and reproductive behavior in birds. In: Pfaff, D.W., Arnold, A.P., Etgen, A.M., Fahrbach, S.E., Rubin, R.T. (Eds.), Hormones, Brain and Behavior, vol. 2. Academic Press, San Diego, CA, pp. 649–798.

Balthazart, J., Ball, G.F., 2006. Is brain estradiol a hormone or a neurotransmitter? Trends Neurosci 241–249.

Balthazart, J., Foidart, A., 1993. Brain aromatase and the control of male sexual behavior. J. Steroid Biochem. Mol. Biol. 44, 521–540.

Balthazart, J., Surlemont, C., 1990. Androgen and estrogen action in the preoptic area and activation of copulatory behavior in quail. Physiol. Behav. 48, 599–609.

Balthazart, J., Schumacher, M., Ottinger, M.A., 1983. Sexual differences in the Japanese quail: behavior, morphology and intracellular metabolism of testosterone. Gen. Comp. Endocrinol. 51, 191–207.

Balthazart, J., Schumacher, M., Malacarne, G., 1985. Interaction of androgens and estrogens in the control of sexual behavior in male Japanese quail. Physiol. Behav. 35, 157–166.

Balthazart, J., Delville, Y., Sulon, Y., Hendrick, J.C., 1986. Plasma levels of luteinizing hormone and of five steroids in photostimulated, castrated and testosterone-treated male and female Japanese quail (*Coturnix coturnix japonica*). Gen. Endocrinol. (Life Sci. Adv.) 5, 31–36.

Balthazart, J., Evrard, L., Surlemont, C., 1990a. Effects of the non-steroidal inhibitor R76713 on testosterone-induced sexual behavior in the Japanese quail (*Coturnix coturnix japonica*). Horm. Behav. 24, 510–531.

Balthazart, J., Foidart, A., Hendrick, C., 1990b. The induction by testosterone of aromatase activity in the preoptic area and activation of copulatory behavior. Physiol. Behav. 47, 83–94.

Balthazart, J., Stoop, R., Foidart, A., Granneman, J.C.M., Lambert, J.G.D., 1994. Distribution and regulation of estrogen-2-hydroxylase in the quail brain. Brain Res.Bull. 35, 339–345.

Balthazart, J., Reid, J., Absil, P., Foidart, A., 1995. Appetitive as well as consummatory aspects of male sexual behavior in quail are activated by androgens and estrogens. Behav. Neurosci. 109, 485–501.

Balthazart, J., Absil, P., Foidart, A., Houbart, M., Harada, N., Ball, G.F., 1996. Distribution of aromatase-immunoreactive cells in the forebrain of zebra finches (*Taeniopygia guttata*): implications for the neural action of steroids and nuclear definition in the avian hypothalamus. J. Neurobiol. 31, 129–148.

Balthazart, J., Absil, P., Gérard, M., Appeltants, D., Ball, G.F., 1998a. Appetitive and consummatory male sexual behavior in Japanese quail are differentially regulated by subregions of the preoptic medial nucleus. J. Neurosci. 18, 6512–6527.

Balthazart, J., Foidart, A., Baillien, M., Harada, N., Ball, G.F., 1998b. Anatomical relationship between aromatase and tyrosine hydroxylase in the quail brain: double-label immunocytochemical studies. J. Comp. Neurol. 391, 214–226.

Balthazart, J., Baillien, M., Ball, G.F., 2001. Rapid and reversible inhibition of brain aromatase activity. J. Neuroendocrinol. 13, 63–73.

Balthazart, J., Baillien, M., Charlier, T.D., Ball, G.F., 2003a. Calcium-dependent phosphorylation processes control brain aromatase in quail. Eur. J. Neurosci. 17, 1591–1606.

Balthazart, J., Baillien, M., Charlier, T.D., Cornil, C.A., Ball, G.F., 2003b. Multiple mechanisms control brain aromatase activity at the genomic and non-genomic level. J. Steroid Biochem. Mol. Biol. 86, 367–379.

Balthazart, J., Baillien, M., Ball, G.F., 2006. Rapid control of brain aromatase activity by glutamatergic inputs. Endocrinology 147, 359–366.

Barbier, O., Bélanger, A., 2003. The cynomolgus monkey (*Macaca fascicularis*) is the best animal model for the study of steroid glucuronidation. J. Steroid Biochem. Mol. Biol. 85, 235–245.

Batty, J., 1978. Acute changes in plasma testosterone levels and their relation to measures of sexual behaviour in the male house mouse (*Mus musculus*). Anim. Behav. 26, 349–357.

Becker, J.B., 1990. Estrogen rapidly potentiates amphetamine-induced striatal dopamine release and rotational behavior during microdialysis. Neurosci. Lett. 118, 169–171.

Becker, J.B., Robinson, T.E., Lorenz, K.A., 1982. Sex differences and estrous cycle variations in amphetamine-elicited rotational behavior. Eur. J. Pharmacol. 80, 65–72.

Becker, J.B., Snyder, P.J., Miller, M.M., Westgate, S.A., Jenuwine, M.J., 1987. The influence of estrous cycle and intrastriatal estradiol on sensorimotor performance in the female rat. Pharmacol. Biochem. Behav. 27, 53–59.

Becker, J.B., Breedlove, S.M., Crews, D., McCarthy, M.M., 2002. Behavioral Endocrinology, 2nd ed. The MIT Press, Cambridge, MA, p. 776.

Benten, W.P.M., Lieberherr, M., Stamm, O., Wrehlke, C., Guo, Z., Wunderlich, F., 1999. Testosterone signaling through internalizable surface receptors in androgen receptor-free macrophages. Mol. Biol. Cell 10, 3113–3123.

Beyer, C., Morali, G., Naftolin, F., Larsson, K., Perez-Palacios, G., 1976. Effect of some antiestrogens and aromatase inhibitors on androgen induced sexual behavior in castrated male rats. Horm. Behav. 7, 353–363.

Bi, R., Broutman, G., Foy, M.R., Thompson, R.F., Baudry, M., 2000. The tyrosine kinase and mitogen-activated protein kinase pathways mediate multiple effects of estrogen in hippocampus. Proc. Natl. Acad. Sci. U. S. A. 97, 3067–3602.

Bixo, M., Backstrom, T., Winblad, B., Andersson, A., 1995. Estradiol and testosterone in specific regions of the human female brain in different endocrine states. J. Steroid Biochem. Mol. Biol. 55, 297–303.

Bjornerem, A., Straume, B., Midtby, M., Fonnebo, V., Sundsfjord, J., Svartberg, J., Acharya, G., Oian, P., Berntsen, G.K., 2004. Endogenous sex hormones in relation to age, sex, lifestyle factors, and chronic diseases in a general population: the Tromso Study. J. Clin. Endocrinol. Metab. 89, 6039–6047.

Bjornerem, A., Straume, B., Oian, P., Berntsen, G.K., in press. Seasonal variation of estradiol, follicle stimulating hormone and dehydroepiandrosterone sulfate in women and men. J. Clin. Endocrinol. Metab. doi:10.1210/jc.2006-0866 (Electronic publication ahead of print).

Blomqvist, A., 2000. Sex hormones and pain: a new role for brain aromatase? J. Comp. Neurol. 423, 549–551.

Bologa, C.G., Revankar, C.M., Young, S.M., Edwards, B.S., Arterburn, J.B., Kiselyov, A.S., Parker, M.A., Tkachenko, S.E., Savchuck, N.P., Sklar, L.A., Oprea, T.I., Prossnitz, E.R., 2006. Virtual and biomolecular screening converge on a selective agonist for GPR30. Nat. Chem. Biol. 2, 207–212.

Boulware, M.I., Mermelstein, P.G., 2005. The influence of estradiol on nervous system function. Drug News Perspect. 18, 631–637.

Boulware, M.I., Weick, J.P., Becklund, B.R., Kuo, S.P., Groth, R.D., Mermelstein, P.G., 2005. Estradiol activates group I and II metabotropic glutamate receptor signaling, leading to opposing influences on cAMP response element-binding protein. J. Neurosci. 25, 5066–5078.

Braunstein, G.D., Sundwall, D.A., Katz, M., Shifren, J.L., Buster, J.E., Simon, J.A., Bachman, G., Aguirre, O.A., Lucas, J.D., Rodenberg, C., Buch, A., Watts, N.B., 2005. Safety and efficacy of a testosterone patch for the treatment of hypoactive sexual desire disorder in surgically menopausal women: a randomized, placebo-controlled trial. Arch. Intern. Med. 165, 1582–1589.

Bremner, W.J., Vitiello, M.V., Prinz, P.N., 1983. Loss of circadian rhythmicity in blood testosterone levels with aging in normal men. J. Clin. Endocrinol. Metab. 56, 1278–1281.

Bueno, J., Pfaff, D.W., 1976. Single unit recording in hypothalamus and preoptic area of estrogen-treated and untreated ovariectomized female rat. Brain Res. 101, 67–78.

Burmeister, S., Wilczynski, W., 2000. Social signals influence hormones independently of calling behavior in the treefrog (Hyla cinerea). Horm. Behav. 38, 201–209.

Butcher, R.L., Collins, W.E., Fugo, N.W., 1974. Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17beta throughout the 4-day estrous cycle of the rat. Endocrinology 94, 1704–1708.

Cashdan, E., 1995. Hormones, sex, and status in women. Horm. Behav. 29, 354–366.

Cerda-Molina, A.L., Hernandez-Lopez, L., Chavira, R., Cardenas, M., Paez-Ponce, D., Cervantes-De la Luz, H., Mondragon-Ceballos, R., 2006. Endocrine changes in male stumptailed macaques (Macaca arctoides) as a response to odor stimulation with vaginal secretions. Horm. Behav. 49, 81–87.

Chaban, V.V., Micevych, P.E., 2005. Estrogen receptor-alpha mediates estradiol attenuation of ATP-induced Ca2+ signaling in mouse dorsal root ganglion neurons. J. Neurosci. Res. 81, 31–37.

Chaban, V.V., Mayer, E.A., Ennes, H.S., Micevych, P.E., 2003. Estradiol inhibits atp-induced intracellular calcium concentration increase in dorsal root ganglia neurons. Neuroscience 118, 941–948.

Chaban, V.V., Lakhter, A.J., Micevych, P., 2004. A membrane estrogen receptor mediates intracellular calcium release in astrocytes. Endocrinology 145, 3788–3795.

Chappell, P.E., Lydon, J.P., Conneely, O.M., O'Malley, B.W., Levine, J.E., 1997. Endocrine defects in mice carrying a null mutation for the progesterone receptor gene. Endocrinology 138, 4147–4152.

Christensen, L.W., Clemens, L.G., 1974. Intrahypothalamic implants of testosterone or estradiol and resumption of masculine sexual behavior in long-term castrated male rats. Endocrinology 95, 984–990.

Christensen, L.W., Clemens, L.G., 1975. Blockade of testosterone-induced mounting behavior in the male rat with intracranial application of the aromatization inhibitor, androst-1,4,6,-triene-3,17-dione. Endocrinology 97, 1545–1551.

Clancy, A.N., Zumpe, D., Michael, R.P., 1995. Intracerebral infusion of an aromatase inhibitor, sexual behavior and brain estrogen receptor-like immunoreactivity in intact male rats. Neuroendocrinology 61, 98–111.

Clifton, P.G., Andrew, R.J., 1981. A comparison of the effects of testosterone on aggressive responses by the domestic chick to the human hand and to a large sphere. Anim. Behav. 29, 610–620.

Coquelin, A., Bronson, F.H., 1979. Release of luteinizing hormone in male mice during exposure to females: habituation of the response. Science 206, 1099–1101.

Cornil, C.A., Balthazart, J., Motte, P., Massotte, L., Seutin, V., 2002. Dopamine activates noradrenergic receptors in the preoptic area. J. Neurosci. 22, 9320–9330.

Cornil, C.A., Seutin, V., Motte, P., Balthazart, J., 2004. Electrophysiological and neurochemical characterization of neurons of the medial preoptic area in Japanese quail (Coturnix japonica). Brain Res. 1029, 224–240.

Cornil, C.A., Dalla, C., Papadopoulou-Daifoti, Z., Baillien, M., Dejace, C., Balthazart, J., 2005. Sexual behavior affects preoptic aromatase activity and brain monoamines' levels. Endocrinology 146, 3809–3820.

Cornil, C.A., Dalla, C., Papadopoulou-Daifoti, Z., Baillien, M., Balthazart, J., 2006a. Estradiol rapidly activates male sexual behavior and affects brain monoamine levels in the quail brain. Behav. Brain Res. 66, 110–123.

Cornil, C.A., Taziaux, M., Baillien, M., Ball, G.F., Balthazart, J., 2006b. Rapid effects of aromatase inhibition on male reproductive behaviors in Japanese quail. Horm. Behav. 49, 45–67.

Corpechot, C., Robel, P., Axelson, M., Sjovall, J., Baulieu, E.E., 1981. Characterization and measurement of dehydroepiandrosterone sulfate in rat brain. Proc. Natl. Acad. Sci. U.S.A. 78, 4704–4707.

Cross, E., Roselli, C.E., 1999. 17β-estradiol rapidly facilitates chemoinvestigation and mounting in castrated male rats. Am. J. Physiol. 276, R1346–R1350.

Davis, P.G., Barfield, R.J., 1979. Activation of masculine sexual behavior by intracranial estradiol benzoate implants in male rats. Neuroendocrinology 28, 217–227.

DeBold, J.F., Frye, C.A., 1994. Genomic and non-genomic actions of progesterone in the control of female hamster sexual behavior. Horm. Behav. 28, 445–453.

Delville, Y., Hendrick, J.C., Sulon, J., Balthazart, J., 1984a. Testosterone metabolism and testosterone-dependent characteristics in Japanese quail. Physiol. Behav. 33, 817–823.

Delville, Y., Sulon, J., Hendrick, J.C., Balthazart, J., 1984b. Effect of the presence of females on the pituitary–testicular activity in male Japanese quail (Coturnix coturnix japonica). Gen. Comp. Endocrinol. 55, 295–305.

Delville, Y., Sulon, J., Balthazart, J., 1985. Hormonal correlates of gonadal regression and spontaneous recovery in Japanese quail exposed to short day-lengths. Arch. Int. Physiol. Biochim. 93, 123–133.

Delville, Y., Sulon, J., Balthazart, J., 1986. Diurnal variations of sexual receptivity in the female Japanese quail (Coturnix coturnix japonica). Horm. Behav. 20, 13–33.

Deutch, A.Y., Roth, R.H., 1999. Neurotransmitters. In: Squire, L.R., Roberts, J.L., Spitzer, N.C., Zigmond, M.J., McConnell, S.K., Bloom, F.E. (Eds.), Fundamental Neuroscience. Academic Press, pp. 163–196.

Dina, O.A., Aley, K.O., Isenberg, W., Messing, R.O., Levine, J.D., 2001. Sex hormones regulate the contribution of PKCepsilon and PKA signalling in inflammatory pain in the rat. Eur. J. Neurosci. 13, 2227–2233.

Dominguez, J.M., Gil, M., Hull, E.M., 2006. Preoptic glutamate facilitates male sexual behavior. J. Neurosci. 26, 1699–1703.

Domjan, M., Hall, S., 1986. Sexual dimorphism in the social proximity behavior of Japanese quail (Coturnix coturnix japonica). J. Comp. Psychol. 100, 68–71.

Driggers, P.H., Segars, J.H., 2002. Estrogen action and cytoplasmic signaling pathways: Part II. The role of growth factors and phosphorylation in estrogen signaling. Trends Endocrinol. Metab. 13, 422–427.

Evans, S.J., Murray, T.F., Moore, F.L., 2000a. Partial purification and biochemical characterization of a membrane glucocorticoid receptor from an amphibian brain. J. Steroid Biochem. Mol. Biol. 72, 209–221.

Evans, S.J., Searcy, B.T., Moore, F.L., 2000b. A subset of kappa opioid ligands bind to the membrane glucocorticoid receptor in an amphibian brain. Endocrinology 141, 2294–2300.

Evrard, H.C., Balthazart, J., 2004a. Aromatization of androgens into estrogens reduces response latency to a noxious thermal stimulus in male quail. Horm. Behav. 45, 181–189.

Evrard, H.C., Balthazart, J., 2004b. Rapid regulation of pain by estrogens synthesized in spinal dorsal horn neurons. J. Neurosci. 24, 9225–9229.

Evrard, H.C., Erskine, M.S., 2005. Spinal estrogen synthesis in spinal dorsal horn neurons. Abstr. - Soc. Neurosci. 746, 16.

Evrard, H., Baillien, M., Foidart, A., Absil, P., Harada, N., Balthazart, J., 2000c. Localization and controls of aromatase in the quail spinal cord. J. Comp. Neurol. 552–564.

Evrard, H., Harada, N., Balthazart, J., 2004. Immunocytochemical localization of aromatase in sensory and integrating nuclei of the hindbrain in Japanese quail (Coturnix japonica). J. Comp. Neurol. 473, 194–212.

Feder, H.H., Storey, A., Goodwin, D., Reboulleau, C., Silver, R., 1977. Testosterone and "5alpha-dihydrotestosterone" levels in peripheral plasma of male and female ring doves (Streptopelia risoria) during and reproductive cycle. Biol. Reprod. 16, 666–677.

Filardo, E.J., Thomas, P., 2005. GPR30: a seven-transmembrane-spanning estrogen receptor that triggers EGF release. Trends Endocrinol. Metab. 16, 362–367.

Fillingim, R.B., Ness, T.J., 2000. Sex-related hormonal influences on pain and analgesic responses. Neurosci. Biobehav. Rev. 24, 485–501.

Foidart, A., Reid, J., Absil, P., Yoshimura, N., Harada, N., Balthazart, J., 1995. Critical re-examination of the distribution of aromatase-immunoreactive cells in the quail forebrain using antibodies raised against human placental aromatase and against the recombinant quail, mouse or human enzyme. J. Chem. Neuroanat. 8, 267–282.

Folman, Y., Drori, D., 1966. Effects of social isolation and of female odours on the reproductive system, kidneys and adrenals of unmated male rats. J. Reprod. Fertil. 11, 43–50.

Forlano, P.M., Deitcher, D.L., Myers, D.A., Bass, A.H., 2001. Anatomical distribution and cellular basis for high levels of aromatase activity in the brain of teleost fish: aromatase enzyme and mRNA expression identify glia as source. J. Neurosci. 21, 8943–8955.

Fowler, K.A., Gill, K., Kirma, N., Dillehay, D.L., Tekmal, R.R., 2000. Overexpression of aromatase leads to development of testicular leydig cell tumors: an in vivo model for hormone-mediated testicular cancer. Am. J. Pathol. 156, 347–353.

Frye, C.A., Mermelstein, P.G., DeBold, J.F., 1992. Evidence for a non-genomic action of progestins on sexual receptivity in hamster ventral tegmental area but not hypothalamus. Brain Res. 578, 87–93.

Frye, C.A., Petralia, S.M., Rhodes, M.E., Stein, B., 2003. Fluoxetine may influence lordosis of rats through effects on midbrain 3a,5a-THP concentrations. Ann. N. Y. Acad. Sci. 1007, 37–41.

Gelez, H., Fabre-Nys, C., 2004. The "male effect" in sheep and goats: a review of the respective roles of the two olfactory systems. Horm. Behav. 46, 257–271.

George, F.W., 1997. Androgen metabolism in the prostate of the finasteride-treated, adult rat: a possible explanation for the differential action of testosterone and 5 alpha-dihydrotestosterone during development of the male urogenital tract. Endocrinology 138, 871–877.

Gill, K., Kirma, N., Tekmal, R.R., 2001. Overexpression of aromatase in transgenic male mice results in the induction of gynecomastia and other biochemical changes in mammary glands. J. Steroid Biochem. Mol. Biol. 77, 13–18.

Graham, J.M., Desjardins, C., 1980. Classical conditioning: induction of luteinizing hormone and testosterone secretion in anticipation of sexual activity. Science 210, 1039–1041.

Greenberg, N., Crews, D., 1990. Endocrine and behavioral-responses to aggression and social-dominance in the green anole lizard, Anolis-Carolinensis. Gen. Comp. Endocrinol. 77, 246–255.

Gu, Q., Moss, R.L., 1998. Novel mechanism for non-genomic action of 17b-oestradiol on kainate-induced in isolated rat CA1 hippocampal neurones. J. Physiol. 506, 745–754.

Gu, G.B., Rojo, A.A., Zee, M.C., Yu, J.H., Simerly, R.B., 1996. Hormonal regulation of CREB phosphorylation in the anteroventral periventricular nucleus. J. Neurosci. 16, 3035–3044.

Gu, Q., Korach, K.S., Moss, R.L., 1999. Rapid action of 17beta-estradiol on kainate-induced currents in hippocampal neurons lacking intracellular estrogen receptors. Endocrinology 140, 660–666.

Haim, S., Shakhar, G., Rossene, E., Taylor, A.N., Ben-Eliyahu, S., 2003. Serum levels of sex hormones and corticosterone throughout 4- and 5-day estrous cycles in Fischer 344 rats and their simulation in ovariectomized females. J. Endocrinol. Invest. 26, 1013–1022.

Hall, P.F., 1994. Testicular steroid synthesis: organization and synthesis. In: Knobil, E., Neill, J.D., Greenwald, G.S., Markert, C.L., Pfaff, D.W. (Eds.), The Physiology of Reproduction, vol. 1. Raven Press, New York, pp. 1335–1362.

Haltmeyer, G.C., Eik-Nes, K.B., 1969. Plasma levels of testosterone in male rabbits following copulation. J. Reprod. Fertil. 19, 273–277.

Harding, C.F., 1981. Social modulation of circulating hormone levels in the male. Am. Zool. 21, 223–231.

Harding, C.F., Follett, B.K., 1979. Hormone changes triggered by aggression in a natural population of blackbirds. Science 203, 918–920.

Hayden-Hixson, D.M., Ferris, C.F., 1991. Steroid-specific regulation of agonistic responding in the anterior hypothalamus of male hamsters. Physiol. Behav. 50, 793–799.

Hilliard, J., Pang, C.N., Penardi, R., Sawyer, C.H., 1975. Effect of coitus on serum levels of testosterone and LH in male and female rabbits. Proc. Soc. Exp. Biol. Med. 149, 1010–1014.

Hisamoto, K., Ohmichi, M., Kurachi, H., Hayakawa, J., Kanda, Y., Nishio, Y., Adachi, K., Tasaka, K., Miyoshii, E., Fujiwarai, N., Taniguchii, N., Murata, Y., 2001. Estrogen induces the Akt-dependent activation of endothelial nitric-oxide synthase in vascular endothelial cells. J. Biol. Chem. 276, 3459–3467.

Hoffman, N.W., Kim, Y.I., Gorski, R.A., Dudek, F.E., 1994. Homogeneity of intracellular eletrophysiological properties in different neuronal subtypes in medial preoptic slices containing the sexually dimorphic nucleus of rat. J. Comp. Neurol. 345, 396–408.

Hojo, Y., Hattori, T.A., Enami, T., Furukawa, A., Suzuki, K., Ishii, H.T., Mukai, H., Morrison, J.H., Janssen, W.G., Kominami, S., Harada, N., Kimoto, T., Kawato, S., 2004. Adult male rat hippocampus synthesizes estradiol from pregnenolone by cytochromes P45017alpha and P450 aromatase localized in neurons. Proc. Natl. Acad. Sci. U. S. A. 101, 865–870.

Holloway, C.C., Clayton, D.F., 2001. Estrogen synthesis in the male brain triggers development of the avian song control pathway in vitro. Nat. Neurosci. 4, 170–175.

Honda, S., Harada, N., Takagi, T., 1994. Novel exon 1 of the aromatase gene specific for aromatase transcripts in human brain. Biochem. Biophys. Res. Commun. 198, 1153–1160.

Hotchkiss, J., Knobil, E., 1994. The menstrual cycle and its neuroendocrine control. In: Knobil, E., Neill, J.D., Greenwald, G.S., Markert, C.L., Pfaff, D.W. (Eds.), The Physiology of Reproduction, vol. 2. Raven Press, New York, pp. 711–750.

Hull, E.M., Meisel, R.L., Sachs, B.D., 2002. Male sexual behavior. In: Pfaff, D.W., Arnold, A.P., Etgen, A.M., Fahrbach, S.E., Rubin, R.T. (Eds.), Hormones, Brain and Behavior, vol. 1. Academic Press, San Diego, CA, pp. 3–137.

Hutchison, R.E., 1978. Hormonal differentiation of sexual behavior in Japanese quail. Horm. Behav. 11, 363–387.

Hutchison, J.B., Katongole, C.B., 1975. Plasma testosterone in courting and incubating male barbary doves (Streptopelia risoria). J. Endocrinol. 65, 275–276.

Hutchison, J.B., Wingfield, J.C., Hutchison, R.E., 1984. Sex differences in plasma concentrations of steroids during the sensitive period for brain differentiation in the zebra finch. J. Endocrinol. 103, 363–369.

Improta-Brears, T., whorton, A.R., Codazzi, F., York, J.D., Meyer, T., McDonnell, D.P., 1999. Estrogen-induced activation of mitogen-activated protein kinase requires mobilization of intracellular calcium. Proc. Natl. Acad. Sci. 96, 4686–4691.

Irwin, R.P., Maragakis, N.J., Rogawski, M.A., Purdy, R.H., Farb, D.H., Paul, S.M., 1992. Pregnenolone sulfate augments NMDA receptor mediated increases in intracellular Ca2+ in cultured rat hippocampal neurons. Neurosci. Lett. 141, 30–34.

Irwin, R.P., Lin, S.Z., Rogawski, M.A., Purdy, R.H., Paul, S.M., 1994. Steroid potentiation and inhibition of N-methyl-D-aspartate receptor-mediated intracellular Ca++ responses: structure–activity studies. J. Pharm. Exp. Ther. 271, 677–682.

Johnston, R.E., Bronson, F., 1982. Endocrine control of female mouse odors that elicit luteinizing hormone surges and attraction in males. Biol. Reprod. 27, 1174–1180.

Joyce, J.N., Van Hartesveldt, C., 1984. Estradiol application to one striatum produces postural deviation to systemic apomorphine. Pharmacol. Biochem. Behav. 20, 575–581.

Judd, H.L., Yen, S.S., 1973. Serum androstenedione and testosterone levels during the menstrual cycle. J. Clin. Endocrinol. Metab. 36, 475–481.

Kamel, F., Mock, E.J., Wright, W.W., Frankel, A.I., 1975. Alterations in plasma concentrations of testosterone, LH, and prolactin associated with mating in the male rat. Horm. Behav. 6, 277–288.

Kamel, F., Wright, W.W., Mock, E.J., Frankel, A.I., 1977. The influence of mating and related stimuli on plasma-levels of luteinizing-hormone, follicle-stimulating hormone, prolactin, and testosterone in male rat. Endocrinology 101, 421–429.

Kelly, M.J., Ronnekleiv, O.K., 2002. Rapid membrane effects of estrogen in the central nervous system. In: Pfaff, D.W. (Ed.), Hormones. Brain and Behavior, vol. 3, pp. 361–380.

Kelly, M.J., Wagner, E.J., 1999. Estrogen modulation of G-protein-coupled receptors. Trends Endocrinol. Metab. 10, 369–374.

Kelly, M.J., Moss, R.L., Dudley, C.A., 1976. Differential sensitivity of preoptic-septal neurons to microelectrophoresed estrogen during the estrous cycle. Brain Res. 114, 152–157.

Kelly, M.J., Qiu, J., Wagner, E.J., Ronnekleiv, O.K., 2003. Rapid effects of estrogen on G protein-coupled receptor activation of potassium channels in the central nervous system (CNS). J. Steroid Biochem. Mol. Biol. 83, 187–193.

Kirma, N., Gill, K., Mandava, U., Tekmal, R.R., 2001. Overexpression of aromatase leads to hyperplasia and changes in the expression of genes involved in apoptosis, cell cycle, growth, and tumor suppressor functions in the mammary glands of transgenic mice. Cancer Res. 61, 1910–1918.

Knobil, E., 1976. On the control of gonadotropin secretion in Rhesus monkey. Recent Prog. Horm. Res. 30, 1–36.

Komisaruk, B.R., Terasawa, E., Rodriguez-Sierra, J.F., 1981. How the brain mediates ovarian responses to environmental stimuli. Neuroanatomy and neurophysiology. In: Adler, N.T. (Ed.), Neuroendocrinology of Reproduction. Plenum Press, New York, pp. 349–376.

Kow, L.-M., Pfaff, D.W., 2004. The membrane actions of estrogens can potentiate their lordosis behavior-facilitating genomic actions. Proc. Natl. Acad. Sci. 101, 11235–12354.

Kow, L.-M., Pfaff, D.W., 2005. Acute estrogen potentiates excitatory responses of neurons in rats hypothalamic ventromedial nucleus. Brain Res. 1043, 124–131.

Kuiper, G.G.J.M., Enmark, E., Pelto-Huikko, M., Nilsson, S., Gustafsson, J.Å., 1996. Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc. Natl. Acad. Sci. U. S. A. 93, 5925–5930.

Kuiper, G.G.J.M., Carlsson, B., Grandien, K., Enmark, E., Häggblad, J., Nilsson, S., Gustafsson, J.Å., 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138, 863–870.

Lagrange, A.H., Ronnekleiv, O.K., Kelly, M.J., 1997. Modulation of G protein-coupled receptors by an estrogen receptor that activates protein kinase A. Mol. Pharmacol. 51, 605–612.

Lambert, J.J., Belelli, D., Shepherd, S.E., Pistis, M., Peters, J.A., 1999. The selective interaction of neurosteroids with the GABAa receptor. In: Baulieu, E.E., Robel, P., Schumacher, M. (Eds.), Neurosteroids. A New Regulatory Function in the Nervous System. Humana Press, pp. 125–142.

Levin, E.R., 2002. Cellular functions of plasma membrane estrogen receptors. Steroids 67, 471–475.

Li, X., Nokkala, E., Yan, W., Streng, T., Saarinen, N., Warri, A., Huhtaniemi, I., Santti, R., Makela, S., Poutanen, M., 2001. Altered structure and function of reproductive organs in transgenic male mice overexpressing human aromatase. Endocrinology 142, 2435–2442.

Liley, N.R., Olsen, K.H., Foote, C.J., Van der Kraak, G.J., 1993. Endocrine changes associated with spawning behavior in male kokanee salmon (Oncorhynchus nerka) and the effects of anosmia. Horm. Behav. 27, 470–487.

Liu, N.J., Gintzler, A.R., 2000. Prolonged ovarian sex steroid treatment of male rats produces antinociception: identification of sex-based divergent analgesic mechanisms. Pain 85, 273–281.

London, S.E., Boulter, J., Schlinger, B.A., 2003. Cloning of the zebra finch androgen synthetic enzyme CYP17: a study of its neural expression throughout posthatch development. J. Comp. Neurol. 467, 496–508.

Lu, J.K., LaPolt, P.S., Nass, T.E., Matt, D.W., Judd, H.L., 1985. Relation of circulating estradiol and progesterone to gonadotropin secretion and estrous cyclicity in aging female rats. Endocrinology 116, 1953–1959.

Lutz, L.B., Jamnongjit, M., Yang, W.-H., Jahani, D., Gill, A., Hammes, S.R., 2003. Selective modulation of genomic and nongenomic androgen responses by androgen receptor ligands. Mol. Endocrinol. 17, 1106–1116.

Macrides, F., Bartke, A., Fernandez, F., D'Angelo, W., 1974. Effects of exposure to vaginal odor and receptive females on plasma testosterone in the male hamster. Neuroendocrinology 15, 355–364.

Macrides, F., Bartke, A., Dalterio, S., 1975. Strange females increase plasma testosterone levels in male mice. Science 189, 1104–1106.

Maggi, A., Ciana, P., Belcredito, S., Vegeto, E., 2004. Estrogens in the nervous system: mechanisms and nonreproductive functions. Annu. Rev. Physiol. 66, 291–313.

Maggiolini, M., Vivacqua, A., Fasanella, G., Recchia, A.G., Sisci, D., Pezzi, V., Montanaro, D., Musti, A.M., Picard, D., Ando, S., 2004. The G protein-coupled receptor GPR30 mediates c-fos up-regulation by 17β-estradiol and phytoestrogens in breast cancer cells. J. Biochem. Chem. 279, 27008–27016.

Mahendroo, M.S., Cala, K.M., Russell, D.W., 1996. 5 alpha-reduced androgens play a key role in murine parturition. Mol. Endocrinol. 10, 380–392.

Mahendroo, M.S., Cala, K.M., Landrum, D.P., Russell, D.W., 1997. Fetal death in mice lacking 5alpha-reductase type 1 caused by estrogen excess. Mol. Endocrinol. 11, 917–927.

Majewska, M.D., Harrison, N.L., Schwartz, R.D., Barker, J.L., Paul, S.M., 1986. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptors. Science 232, 1004–1008.

Malmnas, C.O., 1977. Short-latency effect of testosterone on copulatory behaviour and ejaculation in sexually experienced intact male rats. J. Reprod. Fertil. 51, 351–354.

Mani, S.K., Blaustein, J.D., O'Malley, B.W., 1997. Progesterone

receptor function from a behavioral perspective. Horm. Behav. 31, 244–255.

Matsumoto, T., Honda, S., Harada, N., 2003. Alteration in sex-specific behaviors in male mice lacking the aromatase gene. Neuroendocrinology 77, 416–424.

Matsunaga, M., Ukena, K., Tsutsui, K., 2002. Androgen synthesis in the quail brain. Brain Res. 948, 180–185.

McCarthy, M.M., Becker, J.B., 2002. Neuroendocrinology of sexual behavior in the female. In: Becker, J.B., Breedlove, S.M., Crews, D., McCarthy, M.M. (Eds.), Behavioral Endocrinology. MIT press, Cambridge, pp. 117–151.

McEwen, B.S., Alves, S.E., 1999. Estrogen actions in the central nervous system. Endocr. Rev. 20, 279–307.

Mensah-Nyagan, A.G., Do-Rego, J.-L., Beaujean, D., Luu-The, V., Pelletier, G., Vaudry, H., 1999. Neurosteroids: expression of steroidogenic enzymes and regulation of steroid biosynthesis in the central nervous system. Pharmacol. Rev. 51, 63–81.

Mermelstein, P.G., Becker, J.B., Surmeier, D.J., 1996. Estradiol reduces calcium currents in rat neostriatal neurons via a membrane receptor. J. Neurosci. 16, 595–604.

Metzdorf, R., Gahr, M., Fusani, L., 1999. Distribution of aromatase, estrogen receptor, and androgen receptor mRNA in the forebrain of songbirds and nonsongbirds. J. Comp. Neurol. 407, 115–129.

Meyer, M.E., Van Hartesveldt, C., 1992. Differential effects of intrastriatal estradiol on the dorsal immobility response in male rats. Pharmacol. Biochem. Behav. 43, 303–306.

Migliaccio, A., Castoria, G., Di Domenico, M., de Falco, A., Bilancio, A., Lombardi, M., Barone, M.V., Ametrano, D., Zannini, M.S., Abbondanza, C., Auricchio, F., 2000. Steroid-induced androgen receptor-oestradiol receptor beta-src complex triggers prostate cancer cell proliferation. EMBO J. 19, 5406–5417.

Miki, Y., Nakata, T., Suzuki, T., Darnel, A.D., Moriya, T., Kaneko, C., Hidaka, K., Shiotsu, Y., Kusaka, H., Sasano, H., 2002. Systemic distribution of steroid sulfatase and estrogen sulfotransferase in human adult and fetal tissues. J. Clin. Endocrinol. Metab. 87, 5760–5768.

Minami, T., Oomura, Y., Nabekura, J., Fukuda, A., 1990. 17b-estradiol depolarization of hypothalamic neurons is mediated by cyclic AMP. Brain Res. 519, 301–307.

Mize, A.L., Alper, R.H., 2000. Acute and long-term effects of 17b-estradiol on Gi/o coupled neurotransmitter receptor function in the female rat brain as assessed by agonist-stimulated [35S]GTPgS binding. Brain Res. 859, 326–333.

Mize, A.L., Alper, R.H., 2002. Rapid uncoupling of serotonin-1A receptors in rat hippocampus by 17b-estradiol in vitro requires protein kinases A and C. Neuroendocrinology 76, 339–347.

Mize, A.L., Young, L.J., Alper, R.H., 2003. Uncoupling of 5-HT1A receptors in the brain by estrogens: regional variations in antagonism by ICI 182,780. Neuropharmacology 44, 584–591.

Moore, F.L., Orchinik, M., 1994. Membrane receptors for corticosterone: a mechanism for rapid behavioral responses in an amphibian. Horm. Behav. 28, 512–519.

Morley, P., Whitfield, J.F., Vanderhyden, B.C., Tsang, B.K., Schwartz, J.-L., 1992. A new, non genomic estrogen action: the rapid release of intracellular calcium. Endocrinology 131, 1305–1312.

Moss, R.L., Gu, Q., Wong, M., 1997. Estrogen: nontranscriptional signaling pathway. Recent Prog. Horm. Res. 52, 33–69.

Männistö, P.T., Kaakkola, S., 1999. Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacity of the new selective COMT inhibitors. Pharmacol. Rev. 51, 593–628.

Nadal, A., Diaz, M., Valverde, M.A., 2001. The estrogen trinity: membrane, cytosolic, and nuclear effects. News Physiol. Sci. 16, 251–255.

Naftolin, F., MacLusky, N.J., 1984. Aromatization hypothesis revisited. In: Serio, M., Motta, M., Zanisi, M., Martini, L. (Eds.), Sexual Differentiation: Basic and Clinical Aspects. Raven Press, New York, pp. 79–91.

Naftolin, F., Horvath, T.L., Jakab, R.L., Leranth, C., Harada, N., Balthazart, J., 1996. Aromatase immunoreactivity in axon terminals of the vertebrate brain. Neuroendocrinology 63, 149–155.

Nelson, R.J., 2000. An Introduction to Behavioral Endocrinology, 2 ed. Sinauer Associates Inc., Sunderland, MA, p. 0.

Nethrapalli, I.S., Singh, M., Guan, X., Guo, Q., Lubahn, D.B., Korach, K.S., Toran-Allerand, C.D., 2001. Estradiol (E2) elicits Src phosphorylation in the mouse neocortex: the initial event in E2 activation of the MAPK cascade? Endocrinology 142, 5145–5148.

O'Byrne, K.T., Thalabard, J.C., Grosser, P.M., Wilson, R.C., Williams, C.L., Chen, M.D., Ladendorf, D., Hotchkiss, J., Knobil, E., 1991. Radiotelemetric monitoring of hypothalamic gonadotropin-releasing hormone pulse generator activity throughout the menstrual cycle of the rhesus monkey. Endocrinology 129, 1207–1214.

O'Connell, M.E., Reboulleau, C., Feder, H.H., Silver, R., 1981a. Social interactions and androgen levels in birds: I. Female characteristics associated with increased plasma androgen levels in the male ring dove (*Streptopelia risoria*). Gen. Comp. Endocrinol. 44, 454–463.

O'Connell, M.E., Silver, R., Feder, H.H., Reboulleau, C., 1981b. Social interactions and androgen levels in birds: II. Social factors associated with a decline in plasma androgen levels in male ring doves (*Streptopelia risoria*). Gen. Comp. Endocrinol. 44, 464–469.

Oliveira, R.F., Lopes, M., Carneiro, L.A., Canario, A.V., 2001. Watching fights raises fish hormone levels. Nature 409, 475.

Oliveira, R.F., Hirschenhauser, K., Carneiro, L.A., Canario, A.V., 2002. Social modulation of androgen levels in male teleost fish. Comp. Biochem. Physiol., B Biochem. Mol. Biol. 132, 203–215.

Orchinik, M., 1998. Glucocorticoids, stress, and behavior: shifting the timeframe. Horm. Behav. 34, 320–327.

Orchinik, M., Murray, T.F., Moore, F.L., 1991. A corticosteroid receptor in neuronal membranes. Science 252, 1848–1851.

Osawa, Y., Higashiyama, T., Shimizu, Y., Yarborough, C., 1993. Multiple functions of aromatase and the active site structure; aromatase is the placental estrogen 2-hydroxylase. Biochem. Mol. Biol. 44, 469–480.

Paradiso, K., Zhang, J., Steinbach, J.H., 2001. The C terminus of the human nicotinic a4b2 receptor forms a binding site required for potentiation by an estrogenic steroid. J. Neurosci. 21, 6561–6568.

Pasqualini, C., Olivier, V., Guibert, B., Frain, O., Leviel, V., 1995. Acute stimulatory effect of estradiol on striatal dopamine synthesis. J. Neurochem. 65, 1651–1657.

Peterson, R.S., Saldanha, C.J., Schlinger, B.A., 2001. Rapid upregulation of aromatase mRNA and protein following neural injury in the zebra finch (*Taeniopygia guttata*). J. Neuroendocrinol. 13, 317–323.

Peterson, R.S., Lee, D.W., Fernando, G., Schlinger, B.A., 2004. Radial glia express aromatase in the injured zebra finch brain. J. Comp. Neurol. 475, 261–269.

Peterson, R.S., Yarram, L., Schlinger, B.A., Saldanha, C.J., 2005. Aromatase is pre-synaptic and sexually dimorphic in the adult zebra finch brain. Proc. Biol. Sci. 272, 2089–2096.

Pietras, R.J., Szego, C.M., 1977. Specific binding site for oestrogen at the outer surfaces of isolated endometrial cells. Nature 265, 69–72.

Pleim, E.T., Lisciotto, C.A., DeBold, J.F., 1990. Facilitation of sexual receptivity in hamsters by simultaneous progesterone implants into the VMH and ventral mesencephalon. Horm. Behav. 24, 139–151.

Prange-Kiel, J., Rune, G.M., 2006. Direct and indirect effects of estrogen on rat hippocampus. Neuroscience 138, 765–772.

Prendergast, B.J., Nelson, R.J., Zucker, I., 2002. Mammalian

seasonal rhythms: behavior and neuroendocrine substrates. In: Pfaff, D.W., Arnold, A.P., Etgen, A.M., Fahrbach, S.E., Rubin, R.T. (Eds.), Hormones, Brain and Behavior, vol. 2. Academic Press, San Diego, CA, pp. 93–156.

Purinton, S.C., Wood, C.E., 2000. Ovine fetal estrogen sulfotransferase in brain regions important for hypothalamus–pituitary–adrenal axis control. Neuroendocrinology 71, 237–242.

Purvis, K., Haynes, N.B., 1972. The effect of female rat proximity on the reproductive system of male rats. Physiol. Behav. 9, 401–407.

Purvis, K., Haynes, N.B., 1974. Short-term effects of copulation, human chorioninc gonadotrophin injection and non-tactile association with a female on testosterone levels in the male rat. J. Endocrinol. 60, 429–439.

Qiu, J., Bosch, M.A., Tobias, S.C., Grandy, D.K., Scanlan, T.S., Ronnekleiv, O.K., Kelly, M.J., 2003. Rapid signalling of estrogen in hypothalamic neurons involves a novel G-protein-coupled estrogen receptor that activates protein kinase C. J. Neurosci. 23, 9529–9540.

Ramirez, V.D., Soufi, W.L., 1994. The neuroendocrine control of the rabbit ovarian cycle. In: Knobil, E., Neill, J.D., Greenwald, G.S., Markert, C.L., Pfaff, D.W. (Eds.), The Physiology of Reproduction, vol. 2. Raven Press, New York, pp. 585–611.

Ramirez, V.D., Zheng, J., 1996. Membrane sex-steroid receptors in the brain. Front. Neuroendocrinol. 17, 402–439.

Razandi, M., Pedram, A., Greene, G.L., Levin, E.R., 1999. Cell membrane and nuclear estrogen receptors (ERs) originate from a single transcript: studies of ERa and ERb expressed in Chinese hamster ovary cells. Mol. Endocrinol. 13, 307–319.

Remage-Healey, L., Bass, A.H., 2004. Rapid, hierarchical modulation of vocal patterning by steroid hormones. J. Neurosci. 24, 5892–5900.

Remage-Healey, L., Bass, A.H., 2005. Rapid elevations in both steroid hormones and vocal signaling during playback challenge: a field experiment in Gulf toadfish. Horm. Behav. 47, 297–305.

Remage-Healey, L., Bass, A.H., 2006. A rapid neuromodulatory role for steroid hormones in the control of reproductive behavior. Brain Res. 1126, 27–35, doi:10.1016/j.brainres.2006.06.049 (Electronic publication ahead of print).

Remage-Healey, L., Bass, A.H., in press. From social behavior to neural circuitry: Steroid hormones rapidly modulate advertisement calling via a vocal pattern generator. Horm. Behav. doi:10.1016/j.yhbeh.2006.05.007.

Revankar, C.M., Cimino, D.F., Sklar, L.A., Arterburn, J.B., Prossnitz, E.R., 2005. A transmembrane intracellular estrogen receptor mediates rapid cell signalling. Science 307, 1625–1630.

Rittmaster, R.S., Magor, K.E., Manning, A.P., Norman, R.W., Lazier, C.B., 1991. Differential effect of 5 alpha-reductase inhibition and castration and androgen-regulated gene expression in rat prostate. Mol. Endocrinol. 5, 1023–1029.

Ronnekleiv, O.K., Kelly, M.J., 2005. Diversity of ovarian steroid signaling in the hypothalamus. Front. Neuroendocrinol. 26, 65–84.

Rose, R.M., Gordon, T.P., Bernstein, I.S., 1972. Plasma testosterone levels in the male rhesus: influences of sexual and social stimuli. Science 178, 643–645.

Roselli, C.E., Resko, J.A., 1989. Testosterone regulates aromatase activity in discrete brain areas of male rhesus macaques. Biol. Reprod. 40, 929–934.

Roselli, C.E., Resko, J.A., 1997. Sex differences in androgen-regulated expression of cytochrome P450 aromatase in the rat brain. J. Steroid Biochem. Mol. Biol. 61, 365–374.

Roselli, C.E., Resko, J.A., 2001. Cytochrome P450 aromatase (CYP19) in the non-human primate brain: distribution, regulation, and functional significance. J. Steroid Biochem. Mol. Biol. 79, 247–253.

Roselli, C.E., Abdelgadir, S.E., Resko, J.A., 1997. Regulation of aromatase gene expression in the adult rat brain. Brain Res. Bull. 44, 351–357.

Roselli, C.E., Larkin, K., Schrunk, J.M., Stormshak, F., 2004. Sexual partner preference, hypothalamic morphology and aromatase in rams. Physiol. Behav. 83, 233–245.

Sachs, B.D., Leipheimer, R.E., 1988. Rapid effect of testosterone on striated muscle activity in rats. Neuroendocrinology 48, 453.

Sachser, N., 1987. Short-term responses of plasma norepinephrine, epinephrine, glucocorticoid and testosterone titers to social and non-social stressors in male guinea pigs of different social status. Physiol. Behav. 39, 11–20.

Saginor, M., Horton, R., 1968. Reflex release of gonadotropin and increased plasma testosterone concentration in male rabbits during copulation. Endocrinology 82, 627.

Saldanha, C.J., Tuerk, M.J., Kim, Y.H., Fernandes, A.O., Arnold, A.P., Schlinger, B.A., 2000. Distribution and regulation of telencephalic aromatase expression in the zebra finch revealed with a specific antibody. J. Comp. Neurol. 423, 619–630.

Saldanha, C.J., Schlinger, B.A., Micevych, P.E., Horvath, S., 2004. Presynaptic N-methyl-D-aspartate receptor expression is increased by estrogen in an aromatase-rich area of song bird hippocampus. J. Comp. Neurol. 469, 522–534.

Sapolsky, R.M., 1982. The endocrine stress-response and social status in the wild baboon. Horm. Behav. 16, 279–292.

Sasano, H., Harada, N., 1998. Intratumoral aromatase in human breast, endometrial and ovarian malignancies. Endocr. Rev. 19, 593–607.

Schlinger, B.A., Arnold, A.P., 1991. Brain is the major site of estrogen synthesis in a male songbird. Proc. Natl. Acad. Sci. U. S. A. 88, 4191–4194.

Schlinger, B.A., Callard, G.V., 1987. A comparison of aromatase, 5 alpha-, and 5 beta- reductase activities in the brain and pituitary of male and female quail (C. c. japonica). J. Exp. Zool. 242, 171–180.

Schlinger, B.A., Callard, G.V., 1989a. Aromatase activity in quail brain: correlation with aggressiveness. Endocrinology 124, 437–443.

Schlinger, B.A., Callard, G.V., 1989b. Localization of aromatase in synaptosomal and microsomal subfractions of quail (Coturnix coturnix japonica) brain. Neuroendocrinology 434–441.

Schlinger, B.A., Callard, G.V., 1990a. Aggressive behavior in birds: an experimental model for studies of brain-steroid interactions. Comp. Biochem. Physiol., A 97A, 307–316.

Schlinger, B.A., Callard, G.V., 1990b. Aromatization mediates aggressive behavior in quail. Gen. Comp. Endocrinol. 79, 39–53.

Schumacher, M., Balthazart, J., 1986. Testosterone-induced brain aromatase is sexually dimorphic. Brain Res. 370, 285–293.

Segars, J.H., Driggers, P.H., 2002. Estrogen action and cytoplasmic signaling cascades: Part I. Membrane-associated signaling complexes. Trends Endocrinol. Metab. 13, 349–354.

Seiwert, C.M., Adkins-Regan, E., 1998. The foam production system of the male Japanese quail: characterization of structure and function. Brain Behav. Evol. 52, 61–80.

Selye, H., 1941. Anesthetic effect of steroid hormones. Proc. Soc. Exp. Biol. Med. 46, 116–121.

Sherwin, B.B., 1985. Changes in sexual behavior as a function of plasma sex steroid levels in post-menopausal women. Maturitas 7, 225–233.

Shifren, J.L., Braunstein, G.D., Simon, J.A., Casson, P.R., Buster, J.E., Redmond, G.P., Burki, R.E., Ginsburg, E.S., Rosen, R.C., Leiblum, S.R., Caramelli, K.E., Mazer, N.A., 2000. Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. N. Engl. J. Med. 343, 682–688.

Simon, J., Braunstein, G., Nachtigall, L., Utian, W., Katz, M., Miller, S., Waldbaum, A., Bouchard, C., Derzko, C., Buch, A., Rodenberg, C., Lucas, J., Davis, S., 2005. Testosterone patch increases sexual activity and desire in surgically menopausal

women with hypoactive sexual desire disorder. J. Clin. Endocrinol. Metab. 90, 5226–5233.

Simoncini, T., Hafesi-Moghadam, A., Brazil, D.P., Ley, K., Chin, W.W., Liao, J.K., 2000. Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase. Nature 407, 538–541.

Simpson, E.R., 2004. Aromatase: biologic relevance of tissue-specific expression. Semin. Reprod. Med. 22, 11–23.

Simpson, E.R., Davis, S.R., 2001. Minireview: aromatase and the regulation of estrogen biosynthesis—Some new perspectives. Endocrinology 142, 4589–4594.

Simpson, E.R., Clyne, C., Rubin, G., Boon, W.C., Robertson, K., Britt, K., Speed, C., Jones, M., 2002. Aromatase—A brief overview. Annu. Rev. Physiol. 64, 93–127.

Sinchak, K., Shahedi, K., Dewing, P., Micevych, P., 2005. Sexual receptivity is reduced in the female mu-opioid receptor knockout mouse. NeuroReport 16, 1697–1700.

Smith, M.S., Freeman, M.E., Neill, J.D., 1975. The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. Endocrinology 96, 219–226.

Smith, R.L., Webster, D.G., Van Hartesveldt, C., Meyer, M.E., 1985. Effects of estrus, estrogen-progesterone priming, and vaginal stimulation on tonic immobility, dorsal immobility, and lordosis in the female rat. Physiol. Behav. 35, 577–581.

Snyder, S.H., Ferris, C.D., 2000. Novel neurotransmitters and their neuropsychiatric relevance. Am. J. Psychiatry 157, 1738–1751.

Soma, K.K., Sullivan, K.A., Tramontin, A.D., Saldanha, C.J., Schlinger, B.A., Wingfield, J.C., 2000. Acute and chronic effects of an aromatase inhibitor on territorial aggression in breeding and nonbreeding male song sparrow. J. Comp. Physiol., A 186, 759–769.

Soma, K.K., Alday, N.A., Hau, M., Schlinger, B.A., 2004. Dehydroepiandrosterone metabolism by 3β-hydrosteroid dehydrogenase/Δ5-Δ4 isomerase in adult zebra finch brain: sex difference and rapid effect of stress. Endocrinology 145, 1668–1677.

Song, W.-C., 2001. Biochemistry and reproductive endocrinology of estrogen sulfotransferase. Ann. N. Y. Acad. Sci. 948, 43–50.

Song, W.-C., Melner, M.H., 2000. Steroid tranformation enzymes as critical regulators of steroid action in vivo. Endocrinology 141, 1587–1589.

Steimer, T., 1988. Aromatase activity in rat brain synaptosomes. Is an enzyme associated with the neuronal cell membrane involved in mediating non-genomic effects of androgens. Eur. J. Neurosci. 9 (Suppl.).

Steimer, T., Hutchison, J.B., 1981. Androgen increases formation of behaviourally effective oestrogen in dove brain. Nature 292, 345–347.

Stoffel-Wagner, B., 2003. Neurosteroid biosynthesis in the human brain and its clinical implications. Ann. N. Y. Acad. Sci. 1007, 64–78.

Takeo, Y., 1984. Influence of continuous illumination on estrous cycle of rats: time course of changes in levels of gonadotropins and ovarian steroids until occurrence of persistent estrus. Neuroendocrinology 39, 97–104.

Thomas, T.R., Neiman, C.N., 1968. Aspects of copulatory behavior preventing atrophy in male rats' reproductive system. Endocrinology 83, 633–635.

Thomas, P., Pang, Y., Filardo, E.J., Dong, J., 2005. Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. Endocrinology 146, 624–632.

Thorneycroft, I.H., Mishell Jr., D.R., Stone, S.C., Kharma, K.M., Nakamura, R.M., 1971. The relation of serum 17-hydroxyprogesterone and estradiol-17-beta levels during the human menstrual cycle. Am. J. Obstet. Gynecol. 111, 947–951.

Timmers, R.J., Granneman, J.C., Lambert, J.G., van Oordt, P.G., 1988.

Estrogen-2-hydroxylase in the brain of the male African catfish, Clarias gariepinus. Gen. Comp. Endocrinol. 72, 190–203.

Toda, K., Saibara, T., Okada, T., Onishi, S., Shizuta, Y., 2001. A loss of aggressive behaviour and its reinstatement by oestrogen in mice lacking the aromatase gene (Cyp19). J. Endocrinol 168, 217–220.

Toran-Allerand, C.D., 2004. A plethora of estrogen receptors in the brain: where will it end? Endocrinology 145, 1069–1074.

Toran-Allerand, C.D., Guan, X., MacLusky, N.J., Horvath, T.L., Diano, S., Singh, M., Sander Connolly, E.J., Nethrapalli, I.S., Tinnikov, A.A., 2002. ER-X: a novel membrane-associated, putative estrogen receptor that is regulated during development and after ischemic brain injury. J. Neurosci. 22, 8391–8401.

Torres, J.M., Ortega, E., 2006. Steroid 5alpha-reductase isozymes in the adult female rat brain: central role of dihydrotestosterone. J. Mol. Endocrinol. 36, 239–245.

Travison, T.G., Morley, J.E., Araujo, A.B., O'Donnell A, B., McKinlay, J.B., 2006. The relationship between libido and testosterone levels in aging men. J. Clin. Endocrinol. Metab. 91, 2509–2513.

Valverde, M.A., Rojas, P., Amigo, J., Cosmelli, D., Orio, P., Bahamonde, M.I., Mann, G.E., Vergara, C., Latorre, R., 1999. Acute activation of maxi-K channels (hSlo) by estradiol binding to the b subunit. Science 285, 1929–1931.

Van Hartesveldt, C., Cottrell, G.A., Meyer, M.E., 1989. The effects of intrastriatal hormones on the dorsal immobility response in gonadectomized male and female rats. Pharmacol. Biochem. Behav. 34, 459–463.

Van Hartesveldt, C., Cottrell, G.A., Meyer, M.E., 1990. Effects of intrastriatal hormones on the dorsal immobility response in male rats. Pharmacol. Biochem. Behav. 35, 307–310.

Vasudevan, N., Kow, L.-M., Pfaff, D.W., 2001. Early membrane estrogenic effects required for full expression of slower genomic actions in a nerve cell line. Proc. Natl. Acad. Sci. 98, 12267–12271.

Vasudevan, N., Kow, L.M., Pfaff, D., 2005. Integration of steroid hormone initiated membrane action to genomic function in the brain. Steroids 70, 388–396.

Vockel, A., Pröve, E., Balthazart, J., 1990. Effects of castration and testosterone treatment on the activity of testosterone-metabolizing enzymes in the brain of male and female zebra finches. J. Neurobiol. 21, 808–825.

Wade, C.B., Dorsa, D.M., 2003. Estrogen activation of cyclic adenosine 5′-monophosphatase response element-mediated transcription requires the extracellularly regulated kinase/mitogen-activated protein kinase pathway. Endocrinology 144, 832–838.

Wade, C.B., Robinson, S., Shapiro, R.A., Dorsa, D.M., 2001. Estrogen receptor (ER)a and ERb exhibit unique pharmacologic properties when coupled to activation of the mitogen-activated protein kinase pathway. Endocrinology 142, 2336–2342.

Wagner, C.K., Morrell, J.I., 1997. Neuroanatomical distribution of aromatase mRNA in the rat brain: indications of regional regulation. J. Steroid Biochem. Mol. Biol. 61, 307–314.

Wang, L., Andersson, S., Warner, M., Gustafsson, J.A., 2001. Morphological abnormalities in the brains of estrogen receptor beta knockout mice. Proc. Natl. Acad. Sci. U. S. A. 98, 2792–2796.

Warner, M., Gustafsson, J.A., 2006. Nongenomic effects of estrogen: why all the uncertainty? Steroids 71, 91–95.

Watson, J.T., Adkins-Regan, E., 1989a. Activation of sexual behavior by implantation of testosterone propionate and estradiol benzoate into the preoptic area of the male Japanese quail (Coturnix japonica). Horm. Behav. 23, 251–268.

Watson, J.T., Adkins-Regan, E., 1989b. Testosterone implanted in the preoptic area of male Japanese quail must be aromatized to activate copulation. Horm. Behav. 23, 432–447.

Watters, J.J., campbell, J.S., Cunningham, M.J., Krebs, E.G., Dorsa, D.M., 1997. Rapid membrane effects of steroids in

neuroblastoma cells: effects of estrogen on mitogen activated protein kinase signalling cascade and c-*fos* immediate early gene transcription. Endocrinology 138, 4030–4033.

Weaver, C.E.J., Park-Chung, M., Gibbs, T.T., Farb, D.H., 1997. 17β-estradiol protects against NMDA-induced excitotoxicity by direct inhibition of NMDA receptors. Brain Res. 761, 338–341.

Wetzel, C.H.R., Hermann, B., Behl, C., Pestel, E., Rammes, G., Zieglgänsberger, W., Holsboer, F., Rupprecht, R., 1998. Functional antagonism of gonadal steroids at the 5-hydroxytryptamine type 3 receptor. Mol. Endocrinol. 12, 1441–1451.

Whitehead, S.A., Ruf, K.B., 1974. Responses of antidromicallly identified preoptic neurons in the rat to neurotransmitters and to estrogen. Brain Res. 79, 185–198.

Wildt, L., Marshall, G., Knobil, E., 1980. Experimental induction of puberty in the infantile female rhesus monkey. Science 207, 1373–1375.

Wingfield, J.C., 1985. Short-term changes in plasma levels of hormones during establishment and defense of a breeding territory in male song sparrows, *Melospiza melodia*. Horm. Behav. 19, 174–187.

Wingfield, J.C., Silverin, B., 2002. Ecophysiological studies of hormone–behavior relations in birds. In: Pfaff, D.W., Arnold, A.P., Etgen, A.M., Fahrbach, S.E., Rubin, R.T. (Eds.), Hormones, Brain and Behavior, vol. 2. Academic Press, San Diego, CA, pp. 587–648.

Wong, M., Moss, R.L., 1992. Long-term and short-term electrophysiological effects of estrogen on the synaptic properties of hippocampal CA1 neurons. J. Neurosci. 12, 3217–3225.

Wong, I.L., Morris, R.S., Lobo, R.A., 2003. Effect of finasteride on the ovulatory function of normal women. Fertil. Steril. 79, 1455–1457.

Xiao, L., Becker, J.B., 1997. Hormonal activation of the striatum and the nucleus accumbens modulates paced mating behavior in the female rat. Horm. Behav. 32, 114–124.

Yagi, K., 1973. Changes in firing rates of single preoptic and hypothalamic units following an intravenous administration of estrogen in the castrated female rat. Brain Res. 53, 343–352.

Yamaji, T., Dierschke, D.J., Bhattacharya, A.N., Knobil, E., 1972. The negative feedback control by estradiol and progesterone of LH secretion in the ovariectomized rhesus monkey. Endocrinology 90, 771–777.

Zhou, Y., Watters, J.J., Dorsa, D.M., 1996. Estrogen rapidly induces the phosphorylation of the cAMP response element binding protein in rat brain. Endocrinology 137, 2163–2166.

Zhu, B.T., Conney, A.H., 1998. Functional role of estrogen metabolism in target cells: review and perspectives. Carcinogenesis 19, 1–27.

Zhu, Y., Bond, J., Thomas, P., 2003a. Identification, classification, and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progestin receptor. Proc. Natl. Acad. Sci. 100, 2237–2242.

Zhu, Y., Rice, C.D., Pang, Y., Pace, M., Thomas, P., 2003b. Cloning, expression, and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. Proc. Natl. Acad. Sci. 100, 2231–2236.

Ziegler, T.E., Schultz-Darken, N.J., Scott, J.J., Snowdon, C.T., Ferris, C.F., 2005. Neuroendocrine response to female ovulatory odors depends upon social condition in male common marmosets, *Callithrix jacchus*. Horm. Behav. 47, 56–64.