HHS Public Access

Author manuscript

Horm Behav. Author manuscript; available in PMC 2019 March 01.

Published in final edited form as:

Horm Behav. 2018 March ; 99: 1–8. doi:10.1016/j.yhbeh.2018.01.002.

Steroid and the brain: 50 years of research, conceptual shifts and the ascent of non-classical and membrane-initiated actions

Jacques Balthazart[1], **Elena Choleris**[2], and **Luke Remage-Healey**[3]

[1]GIGA Neurosciences, University of Liege, B-4000 Liège, Belgium

[2]Department of Psychology and Neuroscience Program, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

[3]Center for Neuroendocrine Studies, University of Massachusetts Amherst, Amherst, MA 01003, USA

Abstract

This brief commentary reviews key steps in the history of endocrinology that have resulted in important conceptual shifts. Our understanding of the “Fast Effects of Steroids” has now made substantial progress, including the major concept that steroids act rapidly on a variety of physiological and behavioral responses, via mechanisms that are too fast to be fully accounted for by classical receptor-dependent regulation of gene transcription. Several so-called ‘non-classical’ mechanisms have been identified and include binding to membrane receptors and regulating non genomic signaling cascades. We survey the discovery of steroids, the initial characterization of their intracellular receptors, key progress in the understanding of the genomic effects of steroids and then the progressive discovery of the rapid non-classical and membrane-initiated actions of steroids. Foundational discoveries about brain steroid synthesis in neural processes and terminals has converged with emerging evidence for the rapid actions of steroids on brain and behavior. Had the rapid effects of steroids in the central nervous system been discovered first, these molecules would likely now be considered as a class of neurotransmitter.

Keywords

neurosteroids; history of endocrinology; genomic effects of steroids; aromatase; behavioral neuroendocrinology

1. Introduction

The notion that a diffusible message can control male sexual attributes including sexual behavior is classically associated with the experimental work of Arnold Adolf Berthold. He

Corresponding author: Jacques Balthazart: GIGA Neurosciences, University of Liege, 15 avenue Hippocrate, B-4000 Liège, Belgium, Phone: +32 4 366 59 70 ---Fax: +32 4 366 59 71 --- jbalthazart@uliege.be.

showed that the male characteristics disappear following castration but are reinstated by the graft of a testis, independently of where this testis is placed (Berthold, 1849). Later in the 19th century, Charles-Edouart Brown-Séquard injected himself with aqueous extracts of dogs and guinea pig testes and claimed that this had restored his vigor and feeling of well-being (Brown-Sequard, 1889) but effects were transient, and in retrospect presumably represented a placebo effect since testosterone is only very poorly soluble in water.

It took however nearly a century after Berthold to identify the chemical message responsible for these actions. In 1927, 20 mg of the sex steroid testosterone was isolated from about 20 kg of bulls testes and were shown to masculinize phenotypic traits in roosters, rats and pig (Gallagher and Koch, 1929). Procedures to isolate the steroid from animal tissues were then designed in the following decade by the European pharmaceutical companies Schering (Germany), Organon (The Netherlands) and Ciba (Switzerland). Organon was the first to isolate and identify the hormone and named it testosterone (David et al., 1935). Its structure was identified by Butenandt and its synthesis from cholesterol was achieved almost simultaneously by Butenandt and Hanisch and by Zubicka (Butenandt and Hanisch, 1935; Ruzicka and Wettstein, 1935) which earned them the 1939 Nobel prize in chemistry (Freeman et al., 2001).

Physiological effects of compounds from ovarian origin, to be later called estrogens, were identified by Allen and Doisy at about the same time in 1923 (Allen and Doisy, 1923). Estrone was independently isolated and purified by Allen and Doisy and by Butenandt in 1929 and estriol followed in 1930 (Parl, 2000). The most potent and best-studied estrogen, estradiol-17β was the last to be identified in 1933 (Lauritzen and Studd, 2005). It was then synthesized, chemically purified and its structure was determined by Doisy in 1935 (MacCorquodale et al., 1935). A partial synthesis from cholesterol was developed in 1940 and a full synthesis in 1948 (Lauritzen and Studd, 2005).

A colossal amount of progress has been made since these seminal discoveries of steroids. The study of hormone action has become an entire field of fundamental and applied research called Endocrinology (covering of course also actions of non-steroid hormones that are not covered here). This research has at several points reached a stage when most people could believe that all major questions had been solved and only details remained to be worked out. However new discoveries have repeatedly produced large magnitude shifts that challenge this belief and have led to major revisions of established concepts. One of the new concepts that started to emerge in the 1970's but whose real importance was only realized about two decades ago concerns the rapid, non-classical and membrane-initiated effects of steroids on brain and behavior. A special issue of *Hormones and Behavior* is currently in preparation and will provide an overview of the current knowledge in this field. Here, we briefly summarize the major conceptual shifts that have taken place in Endocrinology during the last 50 years to provide context for our current understanding of this new mode of rapid steroid action. It is of course impossible to list here all discoveries that were made during this period but we will highlight a few significant findings that surrounded and prepared what we consider as the major recent rethinking of steroid signaling.

2. The foundations of the endocrinology of steroid hormones

From the time when steroid hormones became available for experimentation, their synthesis pathways and mode of action were the subject of active research. Progress in the available biochemical methods was however needed and it is only in the 1960ies that the steroid synthesis pathways (see for review: (Feder, 1981)) and their intracellular binding sites began being uncovered in peripheral steroid-sensitive structures such as the uterus or the chicken oviduct (Jensen, 1962; Jensen and Jacobsen, 1962; Jensen et al., 1968; O'Malley et al., 1969). The detailed mechanism of action remained however unclear until more recently (see (Tsai and O'Malley, 1994)). In 1967–68, i.e., 50 years ago, a basic knowledge about steroid action was beginning to emerge. The chemical structure of steroids and a substantial part of their synthesis pathway had been identified, biochemical studies had discovered and characterized steroid receptors in peripheral steroid-sensitive structures and their presence was suspected in the central nervous system even if it remained impossible to fully characterize them due to their lower abundance.

By the end of the 1960ies, the bases of endocrinology were established and this scientific enquiry had progressed enough that it became conceivable to attack the more difficult question of the role of steroid hormones in brain functioning. Soon thereafter, a number of prominent endocrinologists interested in brain function created the International Neuroendocrine Society under the presidency of Joseph Meites (Ramirez, 2017). One could believe that only details remained to be discovered but nothing was further away from the truth and many surprises were still in store. Entire new research areas were still to be identified and explored. We briefly discuss in this review the most significant of these fundamental discoveries, focusing to a large extent on steroid action in the brain, although it is impossible to cover all of them even superficially given the diversity and large number of topics.

3. Genomic action of steroids: The last 50 years

As already mentioned, in the 1960's the detail of the interaction of sex steroids with their intracellular receptors and how the activated receptors mediate changes in transcription (enhancement or silencing) was still to be uncovered (Tsai and O'Malley, 1994) and progress is in fact still ongoing. The anatomical distribution of these receptors was first characterized by binding assays on (micro-) dissected tissue samples and subsequently by techniques providing more anatomical resolution, which allowed substantial progress especially for understanding steroid action in the brain. At approximately the same time in the late 1960's, two laboratories developed the dry mount *in vivo* autoradiographic technique that allowed the visualization of steroid binding sites in the brain. This opened the route to the identification of the steroid-sensitive circuitry that mediates the activation of reproductive behaviors (Morrell et al., 1975; Pfaff and Keiner, 1973; Pfaff, 1968; Sar and Stumpf, 1972; Stumpf, 1968; Stumpf, 1970; Stumpf and Sar, 1976).

When molecular biology techniques including DNA sequencing became routine, the first cDNA encoding the glucocorticoid receptor was cloned (Hollenberg et al., 1985; Weinberger et al., 1985) followed rapidly by cloning of the cDNA encoding other steroid receptors

including the androgen (AR) (Chang et al., 1988; Lubahn et al., 1988) and the estrogen (ER) (Green et al., 1986) receptor. This was followed by the production of specific antibodies and *in situ* hybridization probes that allowed confirming distributions identified earlier by *in vivo* autoradiography (Simerly et al., 1990). Since immunohistochemistry and *in situ* hybridization are more sensitive and more specific, these techniques identified additional populations of brain cells that express low concentrations of these receptors that were later shown to have a clear functional significance (e.g. the low density of ER present in the telencephalon that modulate cognitive processes, (Gervais et al., 2017).

Detailed analysis of the sequence of cloned steroid receptors demonstrated that they all consist of six defined domains (labeled A through F) including domains that have a conserved sequence and function (Evans, 1988; Mangelsdorf et al., 1995). Purification of receptor proteins based on the affinity for their ligand or for DNA was incredibly challenging since these proteins are expressed at extremely low levels, especially in the brain. The identification of the conserved functional domains and the development of molecular biology tools greatly facilitated this work leading to the identification of new receptors in this family including orphan receptors that had no known ligand at the time they were identified (Rousseau, 2013). This resulted namely in the cloning of a second ER that was named ERβ to distinguish it from the previously identified receptor now renamed ERα (Kuiper et al., 1996; Mosselman et al., 1996; Tremblay et al., 1997).

Huge progress was also made during the last 50 years in the understanding of how steroid receptors regulate gene expression and this work is still ongoing (Bain et al., 2014; Kumar and McEwan, 2012; Tsai and O'Malley, 1994). Early work indicated that nuclear steroid receptors require common cofactors that constitute a limiting factor when competition, or squelching, between different receptors takes place (Meyer et al., 1989). Confirmation of the existence of such cofactors was first obtained by Onate and colleagues in the O'Malley laboratory who cloned and sequenced an mRNA coding for the steroid receptor coactivator 1 (SRC-1), a protein closely associated with progestin receptors (Onate et al., 1995). This was the beginning of a new chapter in the endocrinology of steroid receptors: the identification of the steroid receptor coregulators, a class of proteins that either enhance (co-activators) or decrease (co-repressors) gene transcription mediated by steroid receptors. More than 300 members have now been identified in this family of proteins (see Nuclear Receptor Signaling Atlas at https://www.nursa.org/nursa/index.jsf) and their functional significance is still far from being elucidated (O'Malley et al., 2008; Wang et al., 2016).

Quite surprisingly it was also discovered that activation of steroid receptors could take place in the absence of steroids and play a significant functional role (Power et al., 1991). In particular, studies demonstrated that dopamine activates progestin receptors in the brain and in this way modulate female sexual receptivity (Mani et al., 1994; Mani and Blaustein, 2012). This mechanism is however not limited to the progestin receptor and, for example, also concerns ER (Schreihofer et al., 2001). Along the same lines, it became clear in the 1990's that the conventional 'reproductive' hormones like estrogens and progestins could regulate distinctly non-reproductive neural endpoints, such as basal forebrain cholinergic pathways (Gibbs, 1997), and hippocampal plasticity (Woolley, 1998). Therefore, the complexity of steroid receptor signaling and neural targets became evident as non-steroid

molecules regulated steroid receptors, and ‘reproductive’ steroid hormones shaped behavioral arousal and memory encoding.

In a remarkable convergence, the source of steroids acting in the brain has also been completely revised during these last 50 years. It was initially thought that the male brain was essentially exposed to testosterone secreted by the testes while the female brain was mostly influenced by estrogens (estradiol) and progesterone of ovarian origin. The first important shift in this concept took place when Naftolin and colleagues discovered in the early 1970’s the presence of an active aromatase enzyme transforming androgens into estrogens in the brain (Naftolin et al., 1975; Naftolin et al., 1971; Naftolin et al., 1972). Subsequent work demonstrated that many actions of testosterone in the male brain are actually mediated by the aromatized estrogenic metabolites of this androgen (Balthazart and Ball, 2013). Testosterone can actually be metabolized in a variety of other steroids that either possess androgenic effects (e.g., 5α-dihydrotestosterone) or estrogenic effects (e.g., estradiol or 5α-androstane-3β,17β-diol) or are inactive as sex steroids (e.g., 5β-dihydrotestosterone) (Balthazart, 1989; Celotti et al., 1979). Furthermore research on the control of aggression prior to gonadal recrudescence in a songbird species demonstrated that sex steroids (testosterone and estradiol) are produced in functionally active quantities in the brain by local metabolism of dehydroepiandrosterone (DHEA) produced by the adrenal glands, when gonadal sources are virtually absent (Soma et al., 2008; Soma et al., 2002). Finally, a full line of research established that all steroid-synthesizing enzymes that are needed to synthesize sex steroids from cholesterol are expressed in the brain that should thus also be considered as an endocrine organ (Akawa et al., 1990; London et al., 2006). These steroids produced in the brain have been called neurosteroids and are responsible for a number of physiological effects even if their full functional significance remains to be established (Baulieu et al., 1999; Mellon et al., 2001; Robel and Baulieu, 1994).

Traditionally, the production and regulation of steroid hormones was considered to be under control of the hypothalamo-pituitary-gonadal (HPG) axis for sex steroids and the hypothalamo-pituitary-adrenal (HPA) axis for glucocorticoids. Steroids produced in the gonads or adrenals exert a negative feedback on their production in the brain and pituitary. However the observations that steroids in the brain, in particular testosterone, are transformed into active or inactive metabolites and can be synthesized either from cholesterol or from other steroid precursors questions this traditional view. This complexity has been proposed to represent a “Balkanization” of the endocrine system in terms of localized, tissue-specific steroid synthesis, conversion, and action (Schmidt et al., 2008; Soma et al., 2008). The full consequences of this “Balkanization” still remain to be evaluated.

These are just a few of the relatively recent developments that have elucidated how steroids affect cells and specifically brain function by modulating gene transcription. More extensive reviews of the current state of the art have been published and it is clearly not the purpose of this short assay to cover this field exhaustively (see namely (Etgen and Pfaff, 2009b; McKenna, 2016)). However, the more we learn, the more we realize how little we know. Important questions remain unsolved about the metabolic pathways producing or metabolizing steroids (Miller, 2017) and, to date, only a handful of steroid co-regulators

have been purified and assigned a function. Additionally, the specific mechanisms by which activated receptors interact with the multiple proteins that constitute the transcriptional machinery to regulate DNA transcription are only understood in part.

4. Behavioral Neuroendocrinology

The availability of sex steroids in the 1930's was rapidly followed by an explosion of experimental studies assessing their role in the control of reproductive behavior. William C. Young and colleagues had shown, through the careful observation of 449 reproductive cyles in 165 females!, that sexual receptivity in female guinea pig correlates with cyclic changes in ovarian morphology (Young et al., 1935). With the use of purified steroids, they could then analyze the hormonal specificity of this phenomenon and demonstrate that activation of female receptivity requires the sequential action of estrogens and progesterone (Collins et al., 1938). Various combinations of treatments revealed that estrogens injections had to precede the injection progesterone by a couple of days to be behaviorally active. This early observation was presumably influencial in establishing the notion that steroids modulate behavior with long latencies by mechanisms that would later be characterized as genomic.

A first synthesis of the work on the endocrine controls of reproductive behavior was already published in 1948 by Frank Beach (Beach, 1948), which in many respects marks the foundation of Behavioral Endocrinology (Etgen and Pfaff, 2009a).

In the following decades, all conceptual developments in basic endocrinology led to parallel progress in our understanding of the neuroendocrine controls of behaviors. It was soon established that the behavioral effects of sex steroids are mediated by their interaction with the same receptors and similar mechanisms as their morphological or other physiological effects. A large number of experiments performed on a variety of animal species established that the effects of androgens, estrogens, progestins and glucocorticoids on behavior are blocked by the concurrent administration of antagonists that block the access of the steroids to their receptor. More recently, developments in molecular biology and genetics allowed the generation of genetically modified mice in which these receptors are either constitutively or conditionally eliminated in a time- or tissue-specific manner (Chappell et al., 1999; Dubois et al., 2016; Rissman et al., 1997). This newer approach confirmed and in some cases refined the conclusions that had been drawn from the antagonist experiments.

The brain sites where these effects are produced were identified by the stereotaxic implantation of purified steroids or of their antagonists and comparing effective sites with the results of *in vivo* autoradiographic studies describing the distribution of steroid binding sites. In selected cases it was finally demonstrated that pharmacological blockade of either the transcription of DNA into mRNA or the translation of mRNA into proteins abolishes the effects of sex steroids on reproductive behaviors. Taken together these data confirmed that sex steroids activate behaviors to a large extent by biochemical mechanisms similar to those previously identified in peripheral tissue such as the uterus or chicken oviduct (For broad overviews, see (Etgen and Pfaff, 2009b; Pfaff et al., 2002; Pfaff and Joels, 2017).

More recently, it was also demonstrated that the first steroid receptor co-activators that had been identified, namely SCR-1 or the CREB-binding protein modulate the action of sex steroids on the activation and sexual differentiation of reproductive behaviors. They are anatomically associated with sex steroid receptors and the inhibition of their expression by anti-sense technology markedly diminishes the effectiveness of the steroids (Auger et al., 2000; Charlier and Balthazart, 2005; Molenda et al., 2003; Tetel et al., 2009).

There was also an extensive research effort aimed at characterizing the significance of steroid metabolism (in particular testosterone) for the control of reproductive behaviors (Balthazart, 1989). In particular, it was demonstrated that in many species of birds and mammals, the aromatization of testosterone in the brain mediates in part or almost entirely the effects of the androgen on the sexual differentiation and activation of sexual behavior (Balthazart and Ball, 2013). However, this is not a universal phenomenon and in some species including many fishes, amphibians and reptiles and even in some mammals such as guinea pigs, rabbits and some strains of mice, male sexual behavior is efficiently activated by non-aromatizable androgens such as 5α-dihydrotestosterone (Balthazart, 1989). The same difference in endocrine specificity also applies to the sexual differentiation of behavior that is induced by estrogenic metabolites of testosterone in some species such as rats but by non aromatizable androgens in others such as guinea pigs, but this topic is beyond the scope of the present review (see (McCarthy et al., 2009b) for more information).

5. Another twist: The rapid effects of steroids on the brain

The above overview summarizes about 80 years of research on the now “classical” genomic mode of action of steroids. In the 1970’s another story started to develop in parallel, and its importance became clear in the 1990’s. Following transcriptional initiation, new messenger RNA and the protein product can be detected within 5 min and one hour respectively (Clayton, 2000). However, to produce functional responses, most proteins have to undergo post-translational modifications and translocate to their site of action (e.g., acquire enzymatic activity, be integrated in functional complexes at the membrane or synapse). Therefore genomic actions of steroids take hours and often days to develop fully (Etgen and Pfaff, 2009b; Vasudevan and Pfaff, 2008).

In the early 1970’s, a few studies began to identify faster actions of steroids in the brain that could not possibly be mediated by this type of mechanism. Initially, studies noted that excitability of hypothalamic neurons changes more rapidly as a function of the endocrine state of the animal than could be expected based on genomic mechanisms (reviewed in (Ronnekleiv and Kelly, 2009)) and in 1973, a first study showed that significant changes in the firing rate of preoptic-hypothalamic neurons take place already 16 min after a single injection of estradiol (Yagi, 1973). The first unequivocal demonstration of a direct electrophysiological effect of estradiol was obtained in 1976 by Kelly, Moss and Dudley who showed that microelectrophoresis of estradiol changes the single-neuron firing rate activity of preoptic neurons within seconds in female rats (Kelly et al., 1976). Similar studies progressively accumulated, so that in the 1990’s it had become clear that other mechanisms of steroid action required explanation. The first review papers supporting this notion of acute steroid effects began to appear even if the specific mechanisms largely remained to be

established (Levin, 1999; McEwen, 1991, 1994; Ramirez et al., 1996; Razandi et al., 1999; Schumacher, 1990). At the behavioral level, which is of central interest here, rapid effects of all classes of steroids also began to emerge in the 1980's and 1990's.

Progesterone was known to exert rapid anesthetic effects (Selye, 1941) and to activate lordosis behavior within a few hours in females rodents primed with estrogens. There was even an early report of lordosis induction within minutes in hamsters (Kent and Liberman, 1949) although this finding could apparently not be replicated (Lisciotto and DeBold, 1991). These data suggested that non-genomic mechanisms might be involved at least in part. Interactions with various intracellular signaling mechanisms were initially postulated (Beyer and Canchola, 1981) and actions at the cell-membrane were confirmed with the use of progesterone linked to Bovine Serum Albumin (BSA) that prevents the entry of the steroid into cells (DeBold and Frye, 1994; Frye et al., 1990). Many aspects of the membrane-initiated effects of progesterone have since been elucidated even if numerous questions obviously remain (Mittelman-Smith et al., 2017).

One of the first rapid behavioral effects of steroids that was firmly identified concerned corticosterone. It was shown in rough-skinned newts (*Taricha granulosa*) that stress rapidly inhibits courtship behavior and a similar inhibition is observed within a few minutes following a single injection of corticosterone (Moore and Miller, 1984). Additional experiments indicated that this rapid effect of corticosterone was largely mediated by changes in GABAergic transmission (Boyd and Moore, 1990). Specific, saturable and high affinity binding sites for corticosterone were subsequently identified in membranes purified from *Taricha* brains (Orchinik et al., 1991). This putative receptor was independent from the GABA receptor thus supporting the notion that these behavioral effects can be membrane-initiated via interaction with a membrane corticosterone receptor (Moore et al., 1995). Rapid effects of corticosteroids initiated at the neuronal cell membrane are now clearly accepted and documented in various experimental models (de Kloet et al., 2008).

In the 1980's, studies in domestic chicks also showed that testosterone modulates memory processes with latencies of 10 to 20 min (Clifton et al., 1982) (reviewed in (Andrew, 1983)). This research expanded during the last 30 years; intracellular effects of the androgen were identified (e.g., modulation of ion channels and of intracellular calcium concentrations) and interactions with the cell membrane were demonstrated even if a specific membrane androgen receptor (mAR) had not been cloned until recently (Foradori et al., 2008; Heinlein and Chang, 2002). One candidate mAR, the zinc transporter ZIP9 coupled to an inhibitory G protein, was identified in a fish species in 2004 ((Thomas et al., 2017a) for review) and shown to be expressed and functional in human prostate cancer cells (Thomas et al., 2017b). The neuroanatomical distribution of this receptor has however not yet been fully explored and it is too early to determine whether this receptor mediates all rapid effects of testosterone identified so far. Research on rapid effects of androgens on behavior has not been as active as for other classes of steroids but there is nevertheless evidence that such effects do actually exist at least in selected animal models (Lord et al., 2009; Mangiamele and Thompson, 2012; Remage-Healey and Bass, 2004, 2006) (see also (Foradori et al., 2008).

6. Rapid effects of estrogens on behavior

Interestingly, although the first rapid cellular effects of steroids concerned estradiol-induced modifications of electrophysiological properties of preoptic neurons (Kelly et al., 1976) and although currently rapid effects of steroids are probably best documented for estrogens, the rapid effects of these steroids on behavior were the last ones to be clearly identified. In 1991, Hayden-Hixon and Ferris showed that a single injection of E2 in the anterior hypothalamus slightly increases flank marking frequency in male golden hamsters but it is only in 1999 that an unambiguous effect of a single injection of E2 was observed on precopulatory behaviors (chemo-investigation and mounting) in castrated male rats after latencies of less than 35 min (Cross and Roselli, 1999). This has been followed in the following decade by a host of studies indicating that estrogens, in addition to their relatively slow genomic effects, exert more rapid effects, often shown to be membrane-initiated on various aspects of reproductive behavior in multiple vertebrate species ranging from fishes to mammals (see (Cornil et al., 2012; Remage-Healey and Bass, 2006) for review).

Estrogens have also been demonstrated to affect by similar rapid mechanisms a number of other aspects of behavior and physiology including aggression (Heimovics et al., 2015), vocalizations ((Remage-Healey and Bass, 2004, 2006)) and the central processing of auditory information (Pawlisch and Remage-Healey, 2015; Remage-Healey et al., 2010; Remage-Healey et al., 2012). A whole new field of research has also developed in the past 20 years investigating rapid effects of estrogens on memory formation and consolidation (Ervin et al., 2015; Ervin et al., 2013; Frick, 2015; Frick et al., 2017; Luine et al., 1998; Sheppard et al., 2017; Vahaba and Remage-Healey, 2015). Effects are observed as early as 15 min after treatment with estrogens and concern social as well as non-social learning paradigms (object recognition, object placement, inhibitory avoidance, Morris water maze). They are largely the result of estrogen action on the hippocampus (e.g., (Phan et al., 2015)) but other brain regions such as the medial amygdala also seem to be implicated (Lymer et al., 2018).

These rapid behavioral effects of estrogens raised the question of the mechanisms mediating these responses. Multiple intracellular signaling cascades including several protein kinases are obviously implicated (Abraham et al., 2003; Abraham and Herbison, 2005; Frick, 2015; Glidewell-Kenney et al., 2007; Guerra et al., 2004; Kow and Pfaff, 2016; Vasudevan et al., 2005) but how these cascades are rapidly activated by estrogens is not entirely clear.

Many of the rapid effects of estrogens were shown to depend on membrane receptors. High affinity membrane-binding sites for radioactive E2 were first identified on endometrial cells (Pietras and Szego, 1977) and subsequently in synaptosomal membranes prepared from the adult rat brain (Towle and Sze, 1983). Their membrane association was later confirmed using radioactive E2-BSA (Zheng and Ramirez, 1997).

Initially studies trying to characterize the mechanisms of rapid E2 action concentrated on a search for new receptors that would be specifically located at the membrane. Some rapid effects of E2 could be assigned to membrane receptors that are members of the G protein coupled transmembrane receptor (GPCR). One of them named G_q–mER could be

distinguished by its pharmacological characteristics, namely its activation by a diphenylacrylamide compound called STX. This receptor has been associated with a host of intracellular signaling cascades that appear to be specific to different brain nuclei and physiological responses. Its definitive characterization awaits cloning of the corresponding gene (Kelly and Ronnekleiv, 2002; Ronnekleiv and Kelly, 2017).

An additional line of research identified another orphan GPCR that binds estradiol with high affinity and could be initially linked to the estrogen-mediated activation of adenylyl cyclase (Filardo and Thomas, 2005). The neuroanatomical distribution of this receptor initially named GPR30 but now usually mentioned as GPER-1 (G protein estrogen receptor) has now been studied in several species by *in situ* hybridization (see (Ronnekleiv and Kelly, 2017) for review) and its implication in the control of sexual behavior activation and memory enhancement has been demonstrated (Ervin et al., 2015; Ervin et al., 2013; Gabor et al., 2015; Long et al., 2017; Lymer et al., 2017). A specific agonist (G1) and an antagonist (G15) for this receptor have been developed and have allowed major progress in the analysis of the functions of this receptor.

The existence of another membrane-associated ER enriched in caveolar-like microdomains has also be postulated to be present in postnatal but not adult cortical membranes. This putative receptor named ER-X is highly responsive the estradiol-17α as compared to estradiol-17β (E2) but it has been so far impossible to clone this receptor and confirm its existence (Toran-Allerand et al., 2002).

Most importantly, it was discovered that the two classical nuclear ER (ERα and ERβ) can also, following some post-translational modifications (e.g., a palmitoylation; (Meitzen et al., 2013)), translocate to and associate with the cell membrane where they mediate rapid effects of estrogens (Razandi et al., 1999). Many rapid effects of E2 can indeed be activated by E2-BSA or by the selective ERα and ERβ agonists (propylpyrazoletriol, PPT and diarylpropionitrile, DPN respectively) and are lost in knock-out mice where these receptors have been deleted. The functional changes in cellular physiology or behavior are proposed to be mediated by these membrane-associated ERα or ERβ interacting with metabotropic receptors 1a or 2/3 (mGluR1a, 2/3)(Boulware et al., 2005; Dewing et al., 2007)) although the exact nature of this interaction is not known.

These membrane-initiated behavioral effects of estrogens clearly interact with the genomic actions of these steroids at least in rodents (Vasudevan and Pfaff, 2008) and quail (Seredynski et al., 2013). The mechanisms mediating these interactions have been partly elucidated (Kow and Pfaff, 2004; Vasudevan et al., 2001) but many questions obviously remain. Recent work in quail has suggested that rapid membrane-initiated actions of estrogens would be mainly concerned with the activation of sexual motivation whereas slower genomic effects would be needed to activate copulatory performance (Seredynski et al., 2013) and it has been argued that this distinction motivation/performance and membrane-initiated/nuclear might be more general (Cornil et al., 2015).

Besides the fact that membrane-initiated effects of estrogens are mediated via binding to at least 4 different receptors (others are possibly waiting to be discovered), the effects are also

mediated by multiple intracellular signaling cascades. These cascades namely involve several protein kinases including Protein kinase A, Protein kinase C, MAPkinase-induced CREB phosphorylation, adenylyl cyclase and phospholipase C resulting in changes in intracellular calcium concentrations (see namely (Ronnekleiv and Kelly, 2017)). Some of these cascades ultimately affect synaptic activity (Oberlander and Woolley, 2016; Woolley, 2007), dendritic spine density (Phan et al., 2015; Sellers et al., 2015; Srivastava et al., 2010) or even gene transcription e.g., via CREB activation (Boulware et al., 2005), producing what has been called the "indirect genetic effects" (see (Mittelman-Smith et al., 2017)).

These indirect effects explain why it is more accurate to refer to 'non-classical' and membrane-initiated effects rather than non-genomic effects of steroids. These non-classical and membrane-initiated effects of estrogens are clearly not based on homogenous mechanisms and a huge amount of work remains to be done in order to identify the relevant signaling cascades and hopefully produce at least some degree of generalization. It is important to note that the 'non-classical' actions of steroids on neural circuits involve intracellular signaling cascades and transduction pathways that can be both rapid as well as independent of specific actions at membrane steroid receptors.

It has for example been shown that E2 phosphorylates Akt (protein kinase B) within an hour in the ventromedial nucleus of the hypothalamus in wild type mice. This response is lost in ERα knock out mice but restored in the $ERα^{-/-\ AA}$ mice in which a mutated ERα has been knocked-in that exerts genotropic signaling via protein-protein interactions with other transcription factors such as AP1 (activator protein-1) or SP1 specificity protein-1), but not via the classical ERE (Park et al., 2011). This therefore suggests the existence of rapid intracellular non-classical responses to E2.

This deeper layer of complexity is consistent with the fact that mechanisms mediating rapid effects of estrogens and other steroids include binding to nuclear, extranuclear and membrane receptors that activate various signaling cascades (for reviews see: (Hammes and Levin, 2007; Levin, 2009; Levin and Hammes, 2016; McDevitt et al., 2008)). Therefore, while it has been tempting to consider the rapid actions of steroids as those that occur exclusively via membrane receptor actions, the prevailing evidence suggests that the broadest definition of rapid, 'non-classical' effects of steroids includes mechanisms at the cellular membrane but also within the bounds of the cell itself.

In recent years, it has also become evident that some effects of steroids persist long after the steroid has been cleared from the circulation and the corresponding receptors are no longer occupied. These lasting effects are partly mediated by morphological changes in brain structure (neurogenesis, development of dendritic spines, synapses) but recent work also points to a prominent role of epigenetic modifications of DNA and the associated proteins (Hunter et al., 2015). These changes incidentally are also fundamentally implicated in the process of steroid-induced sexual differentiation of brain and behavior (McCarthy et al., 2017; McCarthy et al., 2009a; Nugent et al., 2015).

It should finally be noted that a substantial part of the rapid effects of steroids are triggered by steroids produced at least in part in the brain itself either because they are derived from

another steroid secreted at the periphery (e.g., estradiol produced by aromatization of testicular testosterone, sex steroids derived from metabolism of DHEA of adrenal origin) or because they are true neurosteroids produced in the brain from cholesterol. The enzymes producing these steroids in the brain are themselves subject to rapid changes in enzymatic activity (Balthazart, 2017; Soma et al., 2008) thus potentially producing transient endocrine signals that could be at the basis of the rapid membrane-initiated actions of steroids in the brain. A large number of questions remain however open at this level namely because quantifying steroids in the brain itself has been and remains quite challenging due in part to the large amount of lipids in this structure that potentially interfere with assay methods (Schumacher et al., 2015).

This brief review is of course far from being exhaustive and it would now require a multi-volume treatise to cover the field of Endocrinology or even specifically Behavioral Neuroendocrinology. As announced at the beginning of this assay, a special issue of Hormones and Behavior on ‘Fast Effects of Steroids’ will soon review the current state of knowledge concerning rapid effects of steroids on behavior. These rapid effects have now become so obvious and prominent that in retrospect it is clear that if they had been discovered first, brain steroids would be considered as neurotransmitters rather than hormones (Rudolph et al., 2016) and this special issue would appear in a journal of neurochemistry instead of a journal dedicated to neuroendocrinology.

Acknowledgments

Preparation of this manuscript was supported by grants from the NIH (RO1 MH50388 to J.B. and RO1 NS082179 to L.R-H, and from the Natural Sciences and Engineering Research Council of Canada (NSERC 400212) to E.C.

References

Abraham IM, Han SK, Todman MG, Korach KS, Herbison AE. Estrogen receptor beta mediates rapid estrogen actions on gonadotropin-releasing hormone neurons in vivo. J Neurosci. 2003; 23:5771–5777. [PubMed: 12843281]

Abraham IM, Herbison AE. Major sex differences in non-genomic estrogen actions on intracellular signaling in mouse brain in vivo. Neuroscience. 2005; 131:945–951. [PubMed: 15749347]

Akawa, Y., Young, J., Kabbadj, K., Sancho, MJ., Zucman, D., Vourc’h, C., Jung-Testa, I., Hu, ZY., Corpéchot, C., Baulieu, EE., Robel, P. Neurosteroids: biosynthesis, metabolism and function of pregnenolone and dehydroepiandrosterone in the brain. In: Motta, M., et al., editors. The endocrine functions of the brain. Raven Press; New York: 1990.

Allen E, Doisy EA. An ovarian hormone. Journal of the American Medical Association. 1923; 81(10): 819–821.

Andrew, RJ. Specific short-latency effects of oestradiol and testosterone on the distractability and memory formation in the young domestic chick. In: Balthazart, J.Pröve, E., Gilles, R., editors. Hormones and behavior in higher vertebrates. Springer-Verlag; Berlin: 1983. p. 463-473.

Auger AP, Tetel MJ, McCarthy MM. Steroid receptor coactivator-1 (SRC-1) mediates the development of sex-specific brain morphology and behavior. Proc Natl Acad Sci USA. 2000; 97:7551–7555. [PubMed: 10861018]

Bain DL, Connaghan KD, Maluf NK, Yang Q, Miura MT, De Angelis RW, Degala GD, Lambert JR. Steroid receptor-DNA interactions: toward a quantitative connection between energetics and transcriptional regulation. Nucleic acids research. 2014; 42:691–700. [PubMed: 24064251]

Balthazart, J. Steroid metabolism and the activation of social behavior. In: Balthazart, J., editor. Advances in Comparative and Environmental Physiology. 1. Vol. 3. Springer Verlag; Berlin: 1989. p. 105-159.

Balthazart J. Steroid metabolism in the brain: From bird watching to molecular biology, a personal journey. Horm Behav. 2017; 93:137–150. [PubMed: 28576650]

Balthazart, J., Ball, GF. Brain Aromatase, estrogens and behavior. Oxford University Press; New York: 2013.

Baulieu, EE., Robel, P., Schumacher, M. A new regulatory function in the nervous system. Humana Press; Totowa, NJ: 1999. Neurosteroids.

Beach, FA. Hormones and behavior. Paul B. Hoeber, Inc; New York: 1948.

Berthold AA. Transplantation der Hoden. Arch F Anat U Physiol. 1849; 16:42–46.

Beyer C, Canchola E. Facilitation of progesterone induced lordosis behavior by phosphodiesterase inhibitors in estrogen primed rats. Physiology & behavior. 1981; 27:731–733. [PubMed: 6172803]

Boulware MI, Weick JP, Becklund BR, Kuo SP, Groth RD, Mermelstein PG. Estradiol activates group I and II metabotropic glutamate receptor signaling, leading to opposing influences on cAMP response element-binding protein. J Neurosci. 2005; 25:5066–5078. [PubMed: 15901789]

Boyd SK, Moore FL. Evidence for GABA involvement in stress-induced inhibition of male amphibian sexual behavior. Horm Behav. 1990; 24:128–138. [PubMed: 2158482]

Brown-Sequard CE. The effects produced on man by subcutaneous injections of liquid obtained from the testicles of animals. Lancet. 1889; 2(3438):105–107.

Butenandt A, Hanisch G. Uber die Umwandlung des Dehydroandrosterons in Androstenol-(17)-one-(3) (Testosterone); um Weg zur Darstellung des Testosterons auf Cholesterin (Vorlauf Mitteilung) Chemische Berichte. 1935; 68(9):1859–1862.

Celotti, F., Massa, R., Martini, L. Metabolism of sex steroids in the central nervous system. In: DeGroot, LJ., editor. Endocrinology. Grune & Stratton; New York: 1979. p. 41-53.

Chang C, Kokontis J, Liao S. Molecular cloning of human and rat complementary DNA encoding androgen receptors. Science. 1988; 240:324–326. [PubMed: 3353726]

Chappell PE, Schneider JS, Kim P, Xu M, Lydon JP, O'Malley BW, Levine JE. Absence of gonadotropin surges and gonadotropin-releasing hormone self-priming in ovariectomized (OVX), estrogen (E2)-treated, progesterone receptor knockout (PRKO) mice. Endocrinology. 1999; 140:3653–3658. [PubMed: 10433223]

Charlier TD, Balthazart J. Modulation of hormonal signaling in the brain by steroid receptor coactivators. Reviews in the Neurosciences. 2005; 16:339–357. [PubMed: 16519010]

Clayton DF. The genomic action potential. Neurobiology of learning and memory. 2000; 74:185–216. [PubMed: 11031127]

Clifton PG, Andrew RJ, Gibbs ME. Limited period of action of testosterone on memory formation in the chick. J Comp Physiol Psychol. 1982; 96:212–222. [PubMed: 7068984]

Collins VJ, Boling JL, Demspey EW, Young WC. Quantitative studies of experimentally induced sexual receptivity in the spayed guinea-pig. Endocrinology. 1938:23.

Cornil CA, Ball GF, Balthazart J. Rapid control of male typical behaviors by brain-derived estrogens. Frontiers in Neuroendocrinology. 2012; 33:425–446. [PubMed: 22983088]

Cornil CA, Ball GF, Balthazart J. The dual action of estrogen hypothesis. Trends Neurosci. 2015; 38:408–416. [PubMed: 26089224]

Cross E, Roselli CE. 17beta-estradiol rapidly facilitates chemoinvestigation and mounting in castrated male rats. The American journal of physiology. 1999; 276:R1346–1350. [PubMed: 10233026]

David KG, Dingemanse E, Freud JL. Über krystallinisches mannliches Hormon aus Hoden (Testosteron) wirksamer als aus harn oder aus Cholesterin bereitetes Androsteron Hoppe-Seyler's. Z Physiol Chem. 1935; 233(5–6):281–283.

de Kloet ER, Karst H, Joels M. Corticosteroid hormones in the central stress response: quick-and-slow. Front Neuroendocrinol. 2008; 29:268–272. [PubMed: 18067954]

DeBold JF, Frye CA. Genomic and non-genomic actions of progesterone in the control of female hamster sexual behavior. Horm Behav. 1994; 28:445–453. [PubMed: 7729813]

Dewing P, Boulware MI, Sinchak K, Christensen A, Mermelstein PG, Micevych P. Membrane estrogen receptor-alpha interactions with metabotropic glutamate receptor 1a modulate female sexual receptivity in rats. J Neurosci. 2007; 27:9294–9300. [PubMed: 17728443]

Dubois SL, Wolfe A, Radovick S, Boehm U, Levine JE. Estradiol Restrains Prepubertal Gonadotropin Secretion in Female Mice via Activation of ERalpha in Kisspeptin Neurons. Endocrinology. 2016; 157:1546–1554. [PubMed: 26824364]

Ervin KS, Lymer JM, Matta R, Clipperton-Allen AE, Kavaliers M, Choleris E. Estrogen involvement in social behavior in rodents: Rapid and long-term actions. Horm Behav. 2015; 74:53–76. [PubMed: 26122289]

Ervin KS, Phan A, Gabor CS, Choleris E. Rapid oestrogenic regulation of social and nonsocial learning. Journal of neuroendocrinology. 2013; 25:1116–1132. [PubMed: 23876061]

Etgen, AM., Pfaff, DW. Historical and conceptual introduction to molecular forays intended to explain hormone/behavior relations. In: Etgen, AM., Pfaff, DW., editors. Molecular mechanisms of hormone actions on behavior. Elsevier; Amsterdam: 2009a. p. 1-4.

Etgen, AM., Pfaff, DW. Molecular mechanisms of hormone actions on behavior. Elsevier; Amsterdam: 2009b.

Evans RM. The steroid and thyroid hormone receptor super family. Science. 1988; 240:32768–32760.

Feder, HH. Essentials of Steroid Structure, Nomenclature, Reactions, Biosynthesis, and Measurements Neuroendocrinology of Reproduction. 1. Adler, NT., editor. Plenum Press; New York: 1981. p. 19-63.

Filardo EJ, Thomas P. GPR30: a seven-transmembrane-spanning estrogen receptor that triggers EGF release. Trends in endocrinology and metabolism: TEM. 2005; 16:362–367. [PubMed: 16125968]

Foradori CD, Weiser MJ, Handa RJ. Non-genomic actions of androgens. Front Neuroendocrinol. 2008; 29:169–181. [PubMed: 18093638]

Freeman ER, Bloom DA, McGuire EJ. A brief history of testosterone. The Journal of urology. 2001; 165:371–373. [PubMed: 11176375]

Frick KM. Molecular mechanisms underlying the memory-enhancing effects of estradiol. Horm Behav. 2015; 74:4–18. [PubMed: 25960081]

Frick KM, Tuscher JJ, Koss WA, Kim J, Taxier LR. Estrogenic regulation of memory consolidation: A look beyond the hippocampus, ovaries, and females. Physiology & behavior. 2017

Frye CA, Mermelstein PG, DeBold JF. Progesterone immobilized on BSA implanted in the VTA but not the hypothalamus facilitates sexual receptivity in hamsters. Society for Neuroscience. 1990; 16:0.

Gabor C, Lymer J, Phan A, Choleris E. Rapid effects of the G-protein coupled oestrogen receptor (GPER) on learning and dorsal hippocampus dendritic spines in female mice. Physiology & behavior. 2015; 149:53–60. [PubMed: 26003497]

Gallagher TF, Koch FC. The testicular hormone. J Biol Chem. 1929; 84(2):495–500.

Gervais, NJ., Brake, WG., Lacreuse, A. Ovarian Hormones and Prefrontal Cortex-Related Cognition. In: Pfaff, DW., Joels, M., editors. Hormones, Brain and Behavior. 3. Elsevier; Amsterdam: 2017. p. 439-451.

Gibbs RB. Effects of estrogen on basal forebrain cholinergic neurons vary as a function of dose and duration of treatment. Brain Res. 1997; 757:10–16. [PubMed: 9200493]

Glidewell-Kenney C, Hurley LA, Pfaff L, Weiss J, Levine JE, Jameson JL. Nonclassical estrogen receptor alpha signaling mediates negative feedback in the female mouse reproductive axis. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:8173–8177. [PubMed: 17470805]

Green S, Walter P, Kumar V, Krust A, Bornert JM, Argos P, Chambon P. Human estrogen receptor cDNA: sequence expression and homology to v-erbA. Nature. 1986; 320:134–139. [PubMed: 3754034]

Guerra B, Diaz M, Alonso R, Marin R. Plasma membrane oestrogen receptor mediates neuroprotection against beta-amyloid toxicity through activation of Raf-1/MEK/ERK cascade in septal-derived cholinergic SN56 cells. Journal of neurochemistry. 2004; 91:99–109. [PubMed: 15379891]

Hammes SR, Levin ER. Extranuclear steroid receptors: nature and actions. Endocr Rev. 2007; 28:726–741. [PubMed: 17916740]

Heimovics SA, Trainor BC, Soma KK. Rapid Effects of Estradiol on Aggression in Birds and Mice: The Fast and the Furious. Integrative and comparative biology. 2015; 55:281–293. [PubMed: 25980562]

Heinlein CA, Chang C. The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. Mol Endocrinol. 2002; 16:2181–2187. [PubMed: 12351684]

Hollenberg SM, Weinberger C, Ong ES, Cerelli G, Oro A, Lebo R, Thompson EB, Rosenfeld MG, Evans RM. Primary structure and expression of a functional human glucocorticoid receptor cDNA. Nature. 1985; 318:635–641. [PubMed: 2867473]

Hunter RG, Gagnidze K, McEwen BS, Pfaff DW. Stress and the dynamic genome: Steroids, epigenetics, and the transposome. Proceedings of the National Academy of Sciences of the United States of America. 2015; 112:6828–6833. [PubMed: 25385609]

Jensen EV. On the mechanism of estrogen action. Perspectives in biology and medicine. 1962; 6:47–59. [PubMed: 13957617]

Jensen EV, Jacobsen HI. Basic guides to the mechanism of estrogen action. Recent Prog Horm res. 1962; 18:387–414.

Jensen EV, Suzuki T, Kawasima T, Stumpf WE, Jungblut PW, De Sombre ER. A two-step mechanism for the interaction of estradiol with rat uterus. Proc Natl Acad Sci USA. 1968; 59:632–638. [PubMed: 5238991]

Kelly MJ, Moss RL, Dudley CA. Differential sensitivity of preoptic-septal neurons to microelectrophoresed estrogen during the estrous cycle. Brain research. 1976; 114:152–157. [PubMed: 986858]

Kelly, MJ., Ronnekleiv, OK. Rapid membrane effects of estrogen in the central nervous system. In: Pfaff, DW.Arnold, AP.Etgen, AM.Fahrbach, SE., Rubin, RT., editors. Hormones, Brain and Behavior. Academic press; San Diego: 2002. p. 361-380.

Kent GC Jr, Liberman MJ. Induction of psychic estrus in the hamster with progesterone administered via the lateral brain ventricle. Endocrinology. 1949; 45:29–32. [PubMed: 18152106]

Kow LM, Pfaff DW. The membrane actions of estrogens can potentiate their lordosis behavior-facilitating genomic actions. PNAS. 2004; 101:12354–12357. [PubMed: 15302933]

Kow LM, Pfaff DW. Rapid estrogen actions on ion channels: A survey in search for mechanisms. Steroids. 2016; 111:46–53. [PubMed: 26939826]

Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JÅ. Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA. 1996; 93:5925–5930. [PubMed: 8650195]

Kumar R, McEwan IJ. Allosteric Modulators of Steroid Hormone Receptors: Structural Dynamics and Gene Regulation. Endocrine Reviews. 2012; 33:271–299. [PubMed: 22433123]

Lauritzen, C., Studd, JWW. Current Management of the Menopause. CRC Press; Boca Raton, Fl: 2005.

Levin ER. Cellular Functions of the Plasma Membrane Estrogen Receptor. Trends in endocrinology and metabolism: TEM. 1999; 10:374–377. [PubMed: 10511697]

Levin ER. Plasma membrane estrogen receptors. Trends in endocrinology and metabolism: TEM. 2009; 20:477–482. [PubMed: 19783454]

Levin ER, Hammes SR. Nuclear receptors outside the nucleus: extranuclear signalling by steroid receptors. Nature reviews Molecular cell biology. 2016; 17:783–797. [PubMed: 27729652]

Lisciotto CA, DeBold JF. Intravenous administration of progesterone and the onset of receptivity in female hamsters. Physiol Behav. 1991; 49:679–683. [PubMed: 1881969]

London SE, Monks DA, Wade J, Schlinger BA. Widespread capacity for steroid synthesis in the avian brain and song system. Endocrinology. 2006; 147:5975–5987. [PubMed: 16935847]

Long N, Long B, Mana A, Le D, Nguyen L, Chokr S, Sinchak K. Tamoxifen and ICI 182,780 activate hypothalamic G protein-coupled estrogen receptor 1 to rapidly facilitate lordosis in female rats. Horm Behav. 2017; 89:98–103. [PubMed: 28063803]

Lord LD, Bond J, Thompson RR. Rapid steroid influences on visually guided sexual behavior in male goldfish. Horm Behav. 2009; 56:519–526. [PubMed: 19751737]

Lubahn DB, Joseph DR, Sullivan PM, Willard HF, French FS, Wilson EM. Cloning of human androgen receptor complementary DNA and localization to the X chromosome. Science. 1988; 240:327–330. [PubMed: 3353727]

Luine VN, Richards ST, Wu VY, Beck KD. Estradiol enhances learning and memory in a spatial memory task and effects levels of monoaminergic neurotransmitters. Horm Behav. 1998; 34:149–162. [PubMed: 9799625]

Lymer J, Robinson A, Winters BD, Choleris E. Rapid effects of dorsal hippocampal G-protein coupled estrogen receptor on learning in female mice. Psychoneuroendocrinology. 2017; 77:131–140. [PubMed: 28033587]

Lymer JM, Sheppard PAS, Kuun T, Blackman A, Jani N, Mahbub S, Choleris E. Estrogens and their receptors in the medial amygdala rapidly facilitate social recognition in female mice. Psychoneuroendocrinology. 2018 In press.

MacCorquodale DW, Thayer SA, Doisy EA. The Crystalline Ovarian Follicular Hormone. Experimental Biology and Medicine. 1935; 32(7):1182.

Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM. The nuclear receptor superfamily: the second decade. Cell. 1995; 83:835–839. [PubMed: 8521507]

Mangiamele LA, Thompson RR. Testosterone rapidly increases ejaculate volume and sperm density in competitively breeding goldfish through an estrogenic membrane receptor mechanism. Horm Behav. 2012; 62:107–112. [PubMed: 22613707]

Mani SK, Allen JMC, Clark JH, Blaustein JD, O'Malley BW. Convergent pathways for steroid hormone- and neurotransmitter-induced rat sexual behavior. Science. 1994; 265:1246–1249. [PubMed: 7915049]

Mani SK, Blaustein JD. Neural progestin receptors and female sexual behavior. Neuroendocrinology. 2012; 96:152–161. [PubMed: 22538437]

McCarthy, MM., De Vries, GJ., Forger, NG. Sexual Differentiation of the Brain: A Fresh Look at Mode, Mechanisms, and Meaning, Hormones, Brain and Behavior. 3. Elsevier-Academic Press; Amsterdam: 2017. p. 4-32.

McCarthy MM, Auger AP, Bale TL, De Vries GJ, Dunn GA, Forger NG, Murray EK, Nugent BM, Schwarz JM, Wilson ME. The epigenetics of sex differences in the brain. J Neurosci. 2009a; 29:12815–12823. [PubMed: 19828794]

McCarthy, MM., De Vries, GJ., Forger, NG. Sexual diferentiation of the brain: mode, mechanisms, and meaning. In: Pfaff, DW.Arnold, AP.Etgen, AM.Fahrbach, SE., Rubin, RT., editors. Hormones, Brain and Behavior. Academic Press; San Diego, CA: 2009b. p. 1708-1744.

McDevitt MA, Glidewell-Kenney C, Jimenez MA, Ahearn PC, Weiss J, Jameson JL, Levine JE. New insights into the classical and non-classical actions of estrogen: evidence from estrogen receptor knock-out and knock-in mice. Molecular and cellular endocrinology. 2008; 290:24–30. [PubMed: 18534740]

McEwen BS. Non-genomic and genomc effects of steroids on neural activity. Trends in Pharmacological Sciences. 1991; 12:141–147. [PubMed: 2063480]

McEwen BS. Steroid hormone actions on the brain: When is the genome involved? Horm Behav. 1994; 28:396–405. [PubMed: 7729808]

McKenna, NJ. Gonadal steroid action. In: Plant, TM., Zeleznik, AJ., editors. Physiology of Reproduction. 4. Elsevier; Amsterdam: 2016. p. 313-333.

Meitzen J, Luoma JI, Boulware MI, Hedges VL, Peterson BM, Tuomela K, Britson KA, Mermelstein PG. Palmitoylation of estrogen receptors is essential for neuronal membrane signaling. Endocrinology. 2013; 154:4293–4304. [PubMed: 24008343]

Mellon SH, Griffin LD, Compagnone NA. Biosynthesis and action of neurosteroids. Brain Res Rev. 2001; 37:3–12. [PubMed: 11744070]

Meyer ME, Gronemeyer H, Turcotte B, Bocquel MT, Tasset D, Chambon P. Steroid hormone receptors compete for factors that mediate their enhancer function. Cell. 1989; 57:433–442. [PubMed: 2720778]

Miller WL. Steroidogenesis: Unanswered Questions. Trends in endocrinology and metabolism: TEM. 2017; 28:771–793. [PubMed: 29031608]

Mittelman-Smith MA, Rudolph LM, Mohr MA, Micevych PE. Rodent Models of Non-classical Progesterone Action Regulating Ovulation. Frontiers in endocrinology. 2017; 8:165. [PubMed: 28790975]

Molenda HA, Kilts CP, Allen RL, Tetel MJ. Nuclear receptor coactivator function in reproductive physiology and behavior. Biol Reprod. 2003; 69:1449–1457. [PubMed: 12855594]

Moore FL, Miller LJ. Stress-induced inhibition of sexual behavior: corticosterone inhibits courtship behaviors of a male amphibian (Taricha granulosa). Horm Behav. 1984; 18:400–410. [PubMed: 6097527]

Moore FL, Orchinik M, Lowry C. Functional studies of corticosterone receptors in neuronal membranes. Receptor. 1995; 5:21–28. [PubMed: 7613480]

Morrell, JI., Kelley, DB., Pfaff, DW. Sex steroid binding in the brain of vertebrates. In: Knigge, KM.Scott, DE.Kobayashi, H.Miura, S., Ishii, S., editors. Brain-endocrine interactions II. Karger; Basel: 1975. p. 230-256.

Mosselman S, Polman J, Dijkema R. ER beta: identification and characterization of a novel human estrogen receptor. FEBS Lett. 1996; 392:49–53. [PubMed: 8769313]

Naftolin F, Ryan KJ, Davies IJ, Reddy VV, Flores F, Petro Z, Kuhn M, White RJ, Takaoka Y, Wolin L. The formation of estrogens by central neuroendocrine tissues. Recent progress in hormone research. 1975; 31:295–319. [PubMed: 812160]

Naftolin F, Ryan KJ, Petro Z. Aromatization of androstenedione by the diencephalon. The Journal of clinical endocrinology and metabolism. 1971; 33:368–370. [PubMed: 4935642]

Naftolin F, Ryan KJ, Petro Z. Aromatization of androstenedione by the anterior hypothalamus of adult male and female rats. Endocrinology. 1972; 90:295–298. [PubMed: 5009066]

Nugent BM, Wright CL, Shetty AC, Hodes GE, Lenz KM, Mahurkar A, Russo SJ, Devine SE, McCarthy MM. Brain feminization requires active repression of masculinization via DNA methylation. Nature neuroscience. 2015; 18:690–697. [PubMed: 25821913]

O'Malley BW, McGuire WL, Kohler PO, Korenman SG. Studies on the mechanism of steroid hormone regulation of synthesis of specific proteins. Recent progress in hormone research. 1969; 25:105–160. [PubMed: 4902947]

O'Malley BW, Qin J, Lanz RB. Cracking the coregulator codes. Curr Opin Cell Biol. 2008; 20:310–315. [PubMed: 18499426]

Oberlander JG, Woolley CS. 17beta-Estradiol Acutely Potentiates Glutamatergic Synaptic Transmission in the Hippocampus through Distinct Mechanisms in Males and Females. J Neurosci. 2016; 36:2677–2690. [PubMed: 26937008]

Onate SA, Tsai SY, Tsai MJ, O'Malley BW. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science. 1995; 270:1354–1357. [PubMed: 7481822]

Orchinik M, Murray TF, Moore FL. A corticosteroid receptor in neuronal membranes. Science. 1991; 252:1848–1851. [PubMed: 2063198]

Park CJ, Zhao Z, Glidewell-Kenney C, Lazic M, Chambon P, Krust A, Weiss J, Clegg DJ, Dunaif A, Jameson JL, Levine JE. Genetic rescue of nonclassical ERalpha signaling normalizes energy balance in obese Eralpha-null mutant mice. The Journal of clinical investigation. 2011; 121:604–612. [PubMed: 21245576]

Parl, FF. Estrogens, Estrogen Receptor and Breast Cancer. IOS Press; 2000.

Pawlisch BA, Remage-Healey L. Neuroestrogen signaling in the songbird auditory cortex propagates into a sensorimotor network via an 'interface' nucleus. Neuroscience. 2015; 284:522–535. [PubMed: 25453773]

Pfaff, D., Arnold, AP., Etgen, AM., Fahrbach, SE., Rubin, RT. Hormones, brain and behavior. Academic Press; Amsterdam: 2002.

Pfaff, D., Joels, M. Hormones, brain and behavior. 5. Elsevier; Amsterdam: 2017.

Pfaff D, Keiner M. Atlas of estradiol-concentrating cells in the central nervous system of the female rat. J Comp Neurol. 1973; 151:121–158. [PubMed: 4744471]

Pfaff DW. Autoradiographic localization of testosterone-3H in the female rat brain and estradiol-3H in the male rat brain. Experientia. 1968; 24:958–959. [PubMed: 5709053]

Phan A, Suschkov S, Molinaro L, Reynolds K, Lymer JM, Bailey CD, Kow LM, MacLusky NJ, Pfaff DW, Choleris E. Rapid increases in immature synapses parallel estrogen-induced hippocampal learning enhancements. Proceedings of the National Academy of Sciences of the United States of America. 2015; 112:16018–16023. [PubMed: 26655342]

Pietras RJ, Szego CM. Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells. Nature. 1977; 265:69–72. [PubMed: 834244]

Power RF, Mani SK, Codina J, Conneely OM, O'Malley BW. Dopaminergic and ligand-independent activation of steroid hormone receptors. Science. 1991; 254:1636–1639. [PubMed: 1749936]

Ramirez VD. The Founding Fathers of the International Neuroendocrine Society (INS). J Endocrinol. 2017; Epub ahead of print. doi: 10.1111/jne.12558

Ramirez VD, Zheng JB, Siddique KM. Membrane receptors for estrogen, progesterone, and testosterone in the rat brain: Fantasy or reality. Cell Mol Neurobiol. 1996; 16:175–198. [PubMed: 8743968]

Razandi M, Pedram A, Greene GL, Levin ER. Cell membrane and nuclear estrogen receptors (ERs) originate from a single transcript: studies of ERalpha and ERbeta expressed in Chinese hamster ovary cells. Mol Endocrinol. 1999; 13:307–319. [PubMed: 9973260]

Remage-Healey L, Bass AH. Rapid, hierarchical modulation of vocal patterning by steroid hormones. J Neurosci. 2004; 24:5892–5900. [PubMed: 15229236]

Remage-Healey L, Bass AH. A rapid neuromodulatory role for steroid hormones in the control of reproductive behavior. Brain research. 2006; 1126:27–35. [PubMed: 16854385]

Remage-Healey L, Coleman MJ, Oyama RK, Schlinger BA. Brain estrogens rapidly strengthen auditory encoding and guide song preference in a songbird. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:3852–3857. [PubMed: 20133597]

Remage-Healey L, Dong SM, Chao A, Schlinger BA. Sex-specific, rapid neuroestrogen fluctuations and neurophysiological actions in the songbird auditory forebrain. Journal of neurophysiology. 2012; 107:1621–1631. [PubMed: 22190616]

Rissman EF, Wersinger SR, Taylor JA, Lubahn DB. Estrogen receptor function as revealed by knockout studies: Neuroendocrine and behavioral aspects. Horm Behav. 1997; 31:232–243. [PubMed: 9213137]

Robel P, Baulieu EE. Neurosteroids. Biosynthesis and function. Trends Endocrinol Metab. 1994; 5:1–8. [PubMed: 18407181]

Ronnekleiv, OK., Kelly, MJ. Rapid membrane effects of estrogen in the CNS. In: Etgen, AM., Pfaff, DW., editors. Molecular mechanisms of hormone actions on behavior. Elsevier; Amsterdam: 2009. p. 7-28.

Ronnekleiv, OK., Kelly, MJ. Membrane-Initiated Effects of Estradiol in the Central Nervous System. In: Pfaff, DW., Joels, M., editors. Hormones, Brain and Behavior. Elsevier Inc; Amsterdam: 2017. p. 1-22.

Rousseau GG. Fifty years ago: the quest for steroid hormone receptors. Molecular and cellular endocrinology. 2013; 375:10–13. [PubMed: 23684885]

Rudolph LM, Cornil CA, Mittelman-Smith MA, Rainville JR, Remage-Healey L, Sinchak K, Micevych PE. Actions of Steroids: New Neurotransmitters. J Neurosci. 2016; 36:11449–11458. [PubMed: 27911748]

Ruzicka L, Wettstein A. Uber die kristallinische Herstellung des Testikelhormons, Testosteron (Androsten-3-ol-17-ol) [The crystalline production of the testicle hormone, testosterone (Androsten-3-ol-17-ol)]. Helvetica Chimica Acta. 1935; 18:1264–1275.

Sar M, Stumpf WE. Cellular localization of androgen in the brain and pituitary after injection of tritiated testosterone. Experientia. 1972; 28:1364–1366. [PubMed: 4638923]

Schmidt KL, Pradhan DS, Shah AH, Charlier TD, Chin EH, Soma KK. Neurosteroids, immunosteroids, and the Balkanization of endocrinology. General and comparative endocrinology. 2008; 157:266–274. [PubMed: 18486132]

Schreihofer DA, Resnick EM, Lin VY, Shupnik MA. Ligand-independent activation of pituitary ER: Dependence on PKA-stimulated pathways. Endocrinology. 2001; 142:3361–3368. [PubMed: 11459779]

Schumacher M. Rapid membrane effects of steroid hormones: an emerging concept in neuroendocrinology. Trends Neurosci. 1990; 13:359–362. [PubMed: 1699322]

Schumacher M, Guennoun R, Mattern C, Oudinet JP, Labombarda F, De Nicola AF, Liere P. Analytical challenges for measuring steroid responses to stress, neurodegeneration and injury in the central nervous system. Steroids. 2015; 103:42–57. [PubMed: 26301525]

Sellers KJ, Erli F, Raval P, Watson IA, Chen D, Srivastava DP. Rapid modulation of synaptogenesis and spinogenesis by 17beta-estradiol in primary cortical neurons. Frontiers in cellular neuroscience. 2015; 9:137. [PubMed: 25926772]

Selye H. The anesthetic effect of steroid hormones. Proc Soc Exptl Biol Med. 1941; 46:116–121.

Seredynski AL, Balthazart J, Christophe VJ, Ball GF, Cornil CA. Neuroestrogens rapidly regulate sexual motivation but not performance. Journal of Neuroscience. 2013; 33:164–174. [PubMed: 23283331]

Sheppard PAS, Koss WA, Frick KM, Choleris E. Rapid actions of estrogens and their receptors on memory acquisition and consolidation in females. Journal of neuroendocrinology. 2017

Simerly RB, Chang C, Muramatsu M, Swanson LW. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. The Journal of comparative neurology. 1990; 294:76–95. [PubMed: 2324335]

Soma KK, Scotti MA, Newman AE, Charlier TD, Demas GE. Novel mechanisms for neuroendocrine regulation of aggression. Front Neuroendocrinol. 2008; 29:476–489. [PubMed: 18280561]

Soma KK, Wissman AM, Brenowitz EA, Wingfield JC. Dehydroepiandrosterone (DHEA) increases territorial song and the size of an associated brain region in a male songbird. Horm Behav. 2002; 41:203–212. [PubMed: 11855905]

Srivastava DP, Woolfrey KM, Liu F, Brandon NJ, Penzes P. Estrogen receptor ss activity modulates synaptic signaling and structure. J Neurosci. 2010; 30:13454–13460. [PubMed: 20926671]

Stumpf WE. Estradiol-concentrating neurons: topography in the hypothalamus by dry-mount autoradiography. Science. 1968; 162:1001–1003. [PubMed: 5698834]

Stumpf WE. Estrogen-neurons and estrogen-neuron systems in the periventricular brain. Am J Anat. 1970; 129:207–200. [PubMed: 4394394]

Stumpf WE, Sar M. Steroid hormone target sites in the brain: the differential distribution of estrogin, progestin, androgen and glucocorticosteroid. J Steroid Biochem. 1976; 7:1163–1170. [PubMed: 1025363]

Tetel MJ, Auger AP, Charlier TD. Who's in charge? Nuclear receptor coactivator and corepressor function in brain and behavior. Front Neuroendocrinol. 2009; 30:328–342. [PubMed: 19401208]

Thomas P, Converse A, Berg HA. ZIP9, a novel membrane androgen receptor and zinc transporter protein. General and comparative endocrinology. 2017a

Thomas P, Pang Y, Dong J. Membrane androgen receptor characteristics of human ZIP9 (SLC39A) zinc transporter in prostate cancer cells: Androgen-specific activation and involvement of an inhibitory G protein in zinc and MAP kinase signaling. Molecular and cellular endocrinology. 2017b; 447:23–34. [PubMed: 28219737]

Toran-Allerand CD, Guan XP, MacLusky NJ, Horvath TL, Diano S, Singh M, Connolly ES Jr, Nethrapalli IS, Tinnikov AA. ER-X: A novel, plasma membrane-associated, putative estrogen receptor that is regulated during development and after ischemic brain injury. J Neurosci. 2002; 22:8391–8401. [PubMed: 12351713]

Towle AC, Sze PY. Steroid binding to synaptic plasma membrane: differential binding of glucocorticoids and gonadal steroids. J Steroid Biochem. 1983; 18:135–143. [PubMed: 6843116]

Tremblay GB, Tremblay A, Copeland NG, Gilbert DJ, Jenkins NA, Labrie F, Giguere V. Cloning, chromosomal localization, and functional analysis of the murine estrogen receptor beta. Mol Endocrinol. 1997; 11:353–365. [PubMed: 9058381]

Tsai MJ, O'Malley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem. 1994; 63:451–486. [PubMed: 7979245]

Vahaba DM, Remage-Healey L. Brain estrogen production and the encoding of recent experience. Current opinion in behavioral sciences. 2015; 6:148–153. [PubMed: 27453921]

Vasudevan N, Kow LM, Pfaff DW. Early membrane estrogenic effects required for full expression of slower genomic actions in a nerve cell line. PNAS. 2001; 98:12267–12271. [PubMed: 11572951]

Vasudevan N, Kow LM, Pfaff D. Integration of steroid hormone initiated membrane action to genomic function in the brain. Steroids. 2005; 70:388–396. [PubMed: 15862822]

Vasudevan N, Pfaff DW. Non-genomic actions of estrogens and their interaction with genomic actions in the brain. Front Neuroendocrinol. 2008; 29:238–257. [PubMed: 18083219]

Wang L, Lonard DM, O'Malley BW. The Role of Steroid Receptor Coactivators in Hormone Dependent Cancers and Their Potential as Therapeutic Targets. Hormones & cancer. 2016; 7:229–235. [PubMed: 27125199]

Weinberger C, Hollenberg SM, Rosenfeld MG, Evans RM. Domain structure of human glucocorticoid receptor and its relationship to the v-erb-A oncogene product. Nature. 1985; 318:670–672. [PubMed: 3841189]

Woolley CS. Estrogen-mediated structural and functional synaptic plasticity in the female rat hippocampus. Horm Behav. 1998; 34:140–148. [PubMed: 9799624]

Woolley CS. Acute effects of estrogen on neuronal physiology. Annu Rev Pharmacol Toxicol. 2007; 47:657–680. [PubMed: 16918306]

Yagi K. Changes in firing rates of single preoptic and hypothalamic units following an intravenous administration of estrogen in the castrated female rat. Brain research. 1973; 53:343–352. [PubMed: 4706033]

Young WC, Dempsey EW, Myers HI. Cyclic reproductive behavior in the female guinea pig. J Comp Psychol. 1935:19.

Zheng J, Ramirez VD. Demonstration of membrane estrogen binding proteins in rat brain by ligand blotting using a 17beta-estradiol-[125I]bovine serum albumin conjugate. The Journal of steroid biochemistry and molecular biology. 1997; 62:327–336. [PubMed: 9408087]

Highlights

- Steroids affect brain by binding to nuclear receptors that modify transcription
- Steroids acting in the brain originate in the periphery or are produced locally
- Steroids also exert fast effects on brain/behavior that can be membrane-initiated
- Rapid neural effects concern estrogens, androgens, progestins and corticosteroids
- Membrane receptors have been identified for most classes of steroids