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Brain estrogen signaling and acute modulation of acoustic communication behaviors: a working hypothesis

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Summary

Although estrogens are widely considered circulating 'sex steroid hormones' typically associated with female reproduction, recent evidence suggests that estrogens can act as local modulators of brain circuits in both males and females. Functional implications of this newly-characterized estrogen signaling system have begun to emerge. This essay summarizes evidence in support of the hypothesis that the rapid production of estrogens in brain circuits can drive acute changes in both the production and perception of acoustic communication behaviors. These studies reveal two fundamental neurobiological concepts: 1) estrogens can be produced locally in brain circuits independent of levels in nearby circuits and in the circulation, and 2) estrogens can have very rapid effects within these brain circuits to modulate social vocalizations, acoustic processing, and sensorimotor integration. This research relies on a vertebrate-wide span of investigations, including vocalizing fishes, amphibians and birds, emphasizing the importance of comparative model systems in understanding principles of neurobiology.

Keywords

Nongenomic; Estrogen; Signaling; Behavior; Cortex

Introduction

Like most sensorimotor behaviors, the neural pathways for both vocalization and audition (here grouped as 'acoustic communication behaviors') ultimately pass through the hindbrain/spinal cord. Specifically, despite widely different mechanisms of sound production across the vertebrate radiation (larynx, syrinx, swimbladder, etc.) the production of social vocalizations depends upon hindbrain-spinal circuits that are organized according to a conserved developmental/molecular 'blueprint' across all major vertebrate lineages [1-3]. Similarly, ascending auditory pathways that project from peripheral hair cells through brainstem, thalamic and telencephalic structures rely on a conserved basic functional architecture in frogs, fishes, birds, as well as both eutherian and marsupial mammals [4-7]. Therefore the evolution of acoustic communication in birds, mammals, fishes, and other vertebrates appears to have relied on similar toolkits for molecular and neural connectivity [1].

How can the conserved neural wiring diagram for the production and perception of acoustic signals account for the wide variation in vertebrate acoustic behaviors seen in nature? One mechanism for both species- and individual-level behavioral variation is the actions of neuromodulators, which can alter the moment-by-moment functional connectivity within neural circuits. For example, the nonapeptides (e.g,. arginine vasotocin and vasopressin) regulate the neural and behavioral patterning of vocalizations in birds, mammals and teleost fishes [8-10]. Here, I summarize recent research that has uncovered a parallel neuromodulatory system – local signaling by estrogens within brain circuits – that can also

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rapidly modulate both the production and perception of acoustic communication signals in vertebrates. Collectively, this evidence shows there is strong support for acute estrogen-dependent modulation of acoustic communication circuits and behavior. As this signaling system comes into focus, these findings can guide investigations regarding how estrogens exert rapid and local actions on neural circuits more broadly (i.e., pathways not involved in acoustic communication per se). Following the evaluation of the hypothesis below, I then present several key pieces of the puzzle that currently await more detailed investigation before this estrogen-dependent neuromodulatory system can be fully understood.

A working hypothesis linking brain estrogen signaling and behavior

The canonical mode of the actions of estrogens is via intracellular receptors that mediate long-term changes in gene expression. These changes typically manifest within hours to in some cases weeks after initiation. It is now clear that estrogens can also act extremely rapidly (i.e., within seconds to minutes) on cellular functions throughout the body, including the central nervous system [11,12]. These nonclassical actions are of particular interest to neurobiologists and neuroendocrinologists because this rapid timescale is consistent with that of momentary changes in neural activity and behavior. A parallel line of evidence demonstrates that the brain itself can produce its' own local supply of estrogens, on demand and locally at the synaptic connections between neurons [13-15]. The working hypothesis presented here evaluates whether these two recent observations are fundamentally linked in certain brain circuits, and whether this synthesis provides mechanistic explanations for rapid changes in behavior.

The historical backdrop to the central hypothesis of this essay consists of four main themes, drawn from a literature focused broadly on neuroendocrinology/neurobiology (and not communication circuits per se). First was the identification of the rapid actions of steroids (including estrogens) on the excitability of neurons using both in vitro and in vivo preparations [16-19]. Second was the observation that rapid changes in the social environment (including stimuli such as acoustic communication signals and social challenges) were accompanied by rapid changes in the levels of circulating steroid hormones, such as plasma testosterone [20-25]. Third was the discovery that plasma androgens could be converted into estrogens via the enzyme aromatase within specific brain regions [26,27]. Last was the observation that relatively fast changes in brain aromatase activity were coupled to changes in sociosexual behaviors [28-30]. A forthcoming volume published by Oxford University Press on brain aromatase provides further exploration and context of the above foundational concepts that cannot be addressed in depth in this essay [31].

Because acoustic communication behaviors are largely accessible and quantifiable in the laboratory, combining the study of rapid estrogen signaling with acoustic communication behaviors became an attractive area of study. The above foundational observations led to the hypothesis that the rapid production and action of estrogens in brain circuits drives acute, minute-by-minute changes in acoustic signal production and perception. Below, I present the evidence in favor of this hypothesis, first for the rapid actions of estrogens on acoustic signal production, followed then by evidence for the rapid actions of estrogens on acoustic perception and sensorimotor integration. I then describe recent studies characterizing a precise control mechanism for estrogen synthesis in brain circuits that could provide the necessary spatial and temporal resolution for this newly-identified mode of estrogen signaling. This essay relies mainly on studies in amphibians, birds and teleost fishes, and therefore emphasizes the continued importance of a comparative approach to neurobiology and neuroendocrinology.

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Estrogens rapidly influence vocal motor output

Estrogen receptors and the estrogen-synthesis enzyme aromatase are expressed in the neural pathways involved in acoustic signal production and perception in a wide range of vertebrates, including teleost fishes [32,33], frogs [34,35], and birds [36-38]. Indeed, estrogens can alter the activity of neurons in communication circuits via long-term mechanisms [39], and estrogens can change vocalizations in rodents [40,41] and human beings [42,43]. However, only recently have we come to understand that rapid estrogen signaling can occur within acoustic communication circuits to guide moment-by-moment changes in sensorimotor behaviors.

The neuromuscular junction of amphibians has historically provided a wealth of knowledge about how neuronal synapses operate and are modulated [e.g., 44]. Studies focused on the larynx of Xenopus demonstrated that estradiol can have acute effects on synaptic strength at the neuromuscular junction governing vocalizations [45]. Using laryngeal stimulation to evoke neuromuscular potentials, the authors of this study showed that the transmission of vocal motor commands was altered by estradiol within 60 min, consistent with a relatively rapid action of this steroid on vocalizations. To date, this is the only demonstration of an acute effect of any steroid on the peripheral control of acoustic communication behaviors.

Experiments over the past decade have uncovered evidence that estrogens can have rapid, neuromodulatory effects on the patterning of vocalizations by acting within the central nervous system itself. These studies focused on the plainfin midshipman, a teleost fish that uses vocalizations for mate attraction and territoriality [6,46]. A neural circuit in the midshipman hindbrain patterns both the frequency and duration of midshipman vocalizations [47], and this circuit is enriched with the expression of aromatase enzyme [48,49]. In vivo electrophysiology recordings of the activity of this hindbrain circuit showed that estrogens can modulate the duration of vocal motor output signals within 5 min of injection [50]. These fast effects of estradiol on vocal patterning were transient and were observed in the hindbrain of both males and females [51], consistent with the local and high levels of aromatase and estrogen-receptor expression surrounding the hindbrain circuit [48,49]. Therefore, these studies showed that estrogens can have acute actions on neural circuits that pattern vertebrate vocalizations.

Because the midshipman brain contains widespread aromatase expression, one hypothesis consistent with these findings is that estrogens could be produced locally within brain circuits, and that these events could occur within a very fast timescale in support of rapid changes in communication behaviors. Therefore, the studies above led to a new direction of investigations into whether neural circuits involved in acoustic communication could drive estrogen-dependent modulation by synthesizing estrogens themselves, locally and acutely. This idea has since received experimental support from multiple labs that have examined local estrogen production and action in the brain of songbirds.

Measurement of acute estrogen fluctuations in avian brain

In theory, brain circuits can generate their own supply of estrogens – rapidly, on-demand, precisely targeted, and independent of the circulation – to guide rapid changes in behavior. Support for this idea has come from studies of birds, in which behavioral stimuli such as copulation [52] and social challenges [53] have been associated with rapid changes in steroidogenic enzyme activity in brain tissues. Thus, enzymatic proteins that synthesize steroids in the brain exhibit rapid changes in their activity during changes in the social environment.

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As a complement to enzyme activity assays, brain estrogen content itself has been examined using combined liquid- and solid-phase extraction procedures. In the zebra finch, brain estrogen levels are elevated relative to levels in the circulation [54,55], supporting the view that the brain provides the predominant source of circulating estrogens in male zebra finches [56]. The zebra finch therefore presents a model system in which the role of steroid 'microenvironments' could be measured and manipulated in the laboratory to understand how estrogens locally and acutely regulate brain function and behavior. Until recently, existing methods have not enabled the spatial or temporal resolution to follow changing levels of estrogens in brain nuclei. To address this question a method for measuring fluctuating estrogens in discrete brain circuits – in vivo steroid microdialysis coupled to highly-sensitive assays – was recently developed [57]. This technology has since provided evidence that estrogens fluctuate in the brain on acute time scales in awake, behaving animals, as described below.

Initial in vivo microdialysis experiments [57] were focused on the caudomedial nidopallium (NCM) of the zebra finch, which is enriched in expression of aromatase [58,59]. These methods were first validated through in vitro studies and via independent confirmation using unequivocal detection with gas chromatography coupled to mass spectrometry. Subsequent in vivo microdialysis experiments were conducted with probes directed at the NCM of awake males and samples were collected in 30-min time bins. These experiments showed that local levels of estradiol increased when males were interacting with females and hearing experimental playback of songs [57]. Similar observations were made in females, emphasizing a shared, sex-independent auditory role for rapid elevations in NCM estrogens, since females do not sing in this species [60]. In both males and females, circulating levels or levels within an adjacent auditory region were unchanged during similar social manipulations, collectively emphasizing the region-specificity of the events in NCM. Taken together, these experiments indicated that brain circuits can respond to social and/or auditory stimuli with acute elevations in local concentrations of estrogens. The consequences of rapid changes in estrogens within brain circuits for the activity of nearby neurons as well as the control of sensorimotor behaviors then became a primary research focus.

Estrogens can rapidly influence auditory processing

The NCM of songbirds has been identified as an auditory processing region analogous to mammalian secondary auditory cortex based on experiments using electrophysiological and immediate-early gene approaches [61-66]. Therefore, the observation that NCM estradiol levels were elevated in response to auditory stimuli in awake, behaving animals led to the hypothesis that acute estrogen signaling could regulate either the firing properties of NCM neurons, auditory-dependent behaviors, or both. One approach coupled reverse-microdialysis ('retrodialysis') of estrogens or the estrogen-synthesis inhibitor fadrozole (FAD) with extracellular recordings of neuronal activity in the NCM of males, in vivo [67]. These experiments revealed that rapid elevations in local estrogens caused rapid increases in the auditory-evoked activity of NCM neurons, and caused the firing activity of NCM neurons to switch to a mode of burst-firing (i.e., less tonic and greater phasic spiking activity). Conversely, inhibiting estrogen production via retrodialysis of FAD caused a rapid suppression of burst firing in NCM [67]. Largely convergent findings (published prior to as well as concurrent with the above-mentioned study) were generated by an independent laboratory using awake-restrained extracellular recordings in the zebra finch NCM. These observations showed that estrogens and estrogen blockers each exerted acute effects on NCM auditory encoding in zebra finches [68,69]. This same research group further showed that estrogens can rapidly suppress inhibitory synaptic currents in NCM neurons using whole-cell patch recordings, providing a candidate mechanism for the rapid actions of estrogens on auditory processing in NCM [68]. In sum, independently-replicated findings

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together strongly indicate that estrogens can exert rapid actions on the auditory-response patterning of neurons in NCM.

The functional consequences for rapid estrogen signaling in NCM were recently evaluated in two independent studies. Each of these studies in awake, behaving animals took advantage of an established literature on the reliable preferences that zebra finches express for familiar vs. unfamiliar songs in laboratory playback experiments [70,71]. In one study, FAD was retrodialyzed for 30 min into the left NCM of adult male zebra finches, and the behavioral expression of song preferences (ordinarily ~75% biased in favor of familiar songs) was reduced to 50:50 chance [67]. Furthermore, when FAD was washed out with normal aCSF over 30 min, the song preference of individuals for a familiar song was restored to control levels (~75%). Similar findings for song preference were observed in an independent lab and published at approximately the same time [69]. This study showed that song preferences were similarly disrupted when estrogen receptors or aromatase blockers were infused via intracranial injections into NCM [69]. These authors also showed that blocking estrogen signaling disrupted innate behavioral preferences that were specific for complex song stimuli, while preferences for simple calls were unaffected. In summary, convergent studies from independent research labs have shown that acute estrogen signaling within the NCM can enhance auditory processing as well as behavioral song preferences in adult zebra finches.

Estrogens can rapidly influence sensorimotor integration

The discovery of rapid actions of estrogens on neural activity and behavioral responses in the NCM of zebra finches raised the question whether these actions were entirely local within NCM (e.g., to enhance the efficiency of NCM song coding and neural discrimination [e.g., 69]) or also further disseminated through the interconnected network of forebrain nuclei (Fig. 1). Recent evidence demonstrates that estrogen signaling events in the auditory NCM are in fact communicated to downstream sensorimotor regions [72]. A downstream nucleus that receives indirect auditory input from NCM (see Fig. 1) is the sensorimotor HVC. The critical role of HVC in sensorimotor integration has been identified through lesion and recording experiments, in which HVC neurons have been found essential for both the perception and the production of complex vocalizations [73-75]. In particular, neurons within HVC are active both when a male hears his own song as well as when he is singing. Individual auditory-motor ‘mirror’ neurons were identified in HVC that are activated in both auditory and motor contexts with exquisite syllable-level precision [76]. The inherent neuronal ‘selectivity’ of enhanced responses to the birds' own vocalizations in HVC (see Fig. 1) therefore likely relates to its central role in sensorimotor integration.

As shown in Figure 1, when estrogens are delivered to NCM via retrodialysis, the responses of single neurons in HVC to the bird's own vocalizations are enhanced. This is in contrast to the responses of these same single HVC neurons to other vocal stimuli (such as conspecific vocalizations (CON)), which are not altered by estradiol retrodialysis into NCM. Therefore, estrogens acting in NCM cause the selectivity of neurons in downstream HVC to be even further enhanced above baseline for sensorimotor-relevant stimuli. These transsynaptic effects in NCM are region-specific, since there is no effect of identical rapid estrogen delivery into the adjacent CMM (right panel of Fig. 1). Importantly, when local aromatase activity is blocked via retrodialysis of the inhibitor fadrozole into NCM, the HVC selectivity for the bird's own vocalizations are rapidly suppressed, confirming the endogenous nature of rapid estrogen signaling in NCM. These findings therefore show that rapid estrogen signaling events are not locally restricted to one brain region and can be transmitted to downstream brain circuits. Specifically, the neural representation of incoming auditory stimuli is rapidly altered by estrogen signaling in NCM and this information is transmitted

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indirectly into the sensorimotor HVC [72]. A likely scenario is that this auditory-sensorimotor transformation by estrogens enhances either the perception or production of complex behaviors, or both, occurring in HVC and its downstream targets. It is also likely that this modulation depends upon established mechanisms for shifting HVC response properties and selectivity, and chiefly among these is catecholamine input to HVC afferents [77]. Regardless of mechanism, it is now evident that rapid estrogen signaling can lead to enhanced neural representations of complex stimuli that are distributed across a neural network, with potential impacts for cognitive function.

These findings resonate with studies in canaries, in which the HVC selectivity for the bird's own vocalizations were enhanced during the long-day (breeding) season relative to short-days [78]. Though not explicitly measured in that study, their finding suggests a role for seasonal changes in plasma- or brain-derived steroids such as estrogens in modulating HVC neuronal selectivity in a more protracted, seasonal timescale. In a similar fashion, seasonal changes in estrogen availability at the level of HVC can also transsynaptically regulate downstream motor targets of HVC, specifically causing elevated firing rates in the premotor RA [79]. Taken together, this recent body of work has shown that estrogens can act transsynaptically at multiple timescales to alter auditory processing, sensorimotor integration, and premotor firing patterns in the songbird forebrain. These actions at multiple neural loci can provide proximate clues for the way that estrogens alter behavioral song preferences as well as song motor output. They also lead to the prediction that rapid estrogen signaling can modulate ongoing song motor output(s), such as song stereotypy [for similar seasonal effects on stereotypy, see: 79]. Together, these recent findings give insight into how and why estrogen signaling events within the CNS are transmitted within and between brain circuits in support of behaviors (such as song) that depend on distributed neural representations.

A proposed mechanism for rapid estrogen signaling in neurons

An important consideration regarding rapid estrogen signaling within neural circuits (including but not limited to acoustic communication circuits) is the extent to which the bioavailability of estrogens within these circuits fluctuates with sufficient spatiotemporal precision to enable rapid neuro-behavioral actions to occur. Aromatase expression in the brain is most commonly localized to neuronal cell bodies [80], however, in some vertebrate brain regions the aromatase enzyme is also expressed along axonal processes and is even found at presynaptic terminals [81-83]. In principle, this alternative ‘synaptocrine’ source of aromatase activity provides a means for highly localized and rapid delivery of estrogens to precise synaptic targets, much in the way of classically-defined neuromodulators [14,15].

As a model system for ‘synaptocrine’ signaling, the zebra finch brain contains a substantial fraction of aromatase expressed [58,81] and active [82,84] in presynaptic terminals. A recent series of studies identified a key control mechanism for the rapid estradiol changes at the presynaptic terminal in zebra finches. First, local estradiol levels were shown to be rapidly regulated by excitatory events in the NCM, using a combined in vivo retrodialysis/microdialysis approach [57,85]. In particular, increased local excitation within NCM (either with retrodialysis of glutamate or elevated K+ concentrations) each induced an acute downregulation of estradiol concentrations within NCM in vivo, which then recovered during washout conditions. These actions were somewhat specific to glutamatergic inputs, because similar treatments with NMDA or GABA produced no significant changes in local estradiol concentrations. Second, a similar approach was used to deliver the specific presynaptic voltage-gated calcium channel blocker omega conotoxin to NCM and simultaneously measure local changes in estradiol levels. In the presence of omega conotoxin, the rapid excitatory-induced downregulation of NCM estradiol levels were

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blocked. The authors of this study concluded that a primary component of rapid estrogen fluctuations occurs directly at the level of presynaptic terminals, which were dependent upon calcium channel opening [85]. More recently, the biochemical activity of the aromatase enzyme was shown to be specifically regulated within presynaptic terminals (synaptosomes) by acute calcium-dependent phosphorylation events [86].

Together, these studies indicate that momentary neuronal excitation within the songbird forebrain drives depolarization-sensitive phosphorylation of the aromatase protein within presynaptic boutons. This mechanism can therefore theoretically provide acute and highly localized fluctuations in estradiol levels at precise synaptic targets. This model is summarized schematically in Figure 2. The extent to which this precise control mechanism accounts for the rapid regulation of auditory processing or vocalizations (as presented above) remains to be determined. This model also opens up several unexplored avenues for further research [see also 14,15]. First, the nature of calcium-dependent phosphorylation of the aromatase enzyme is unclear at the level of synaptic terminals. In quail and human preparations, phosphorylating conditions induce rapid downregulation of aromatase activity, but the search for critical amino acid residues that enable this process has proven difficult [87]. Second, the extent to which the aromatase protein can be controlled at presynaptic terminals independently from the regulation of the abundant aromatase in cell bodies is unclear. In theory, alternative splice variants of the aromatase protein [e.g., 88,89] could be localized to somal vs. synaptic cellular compartments and may confer differential sensitivity to rapid calcium-dependent phosphorylation. Perhaps most importantly, studies to date consistently show that excitatory-induced, calcium-dependent phosphorylation of aromatase in synaptic terminals accounts for a rapid downregulation of aromatase/estradiol levels, whereas the behavioral studies above rely on an as yet undefined control mechanism for rapid increases in estradiol levels within acoustic circuits. The molecular control of aromatase to enable rapid increases in local estradiol levels, especially within synaptic terminals, is now an active area of research.

Conclusions and outlook

This essay presents evidence supporting the hypothesis that estrogens produced in the brain modulate vocal patterning, auditory processing, and sensorimotor integration via local and acute actions in identified brain circuits. These initial conclusions are drawn mainly from studies of vocalizing fish and birds, but may be broadly applicable to the wide variety of vertebrates that use vocalizations for social communication purposes. Interestingly, the elevated expression of aromatase and estrogen receptors in the song learning/auditory processing regions of the songbird brain have not been found in the equivalent brain regions in bird species that do not learn vocalizations, such as quail, pigeons, etc. [e.g., 37,90,91]. Moreover, aromatase expression is elevated in human temporal cortex [13,92,93], which is the primary locus for speech sensorimotor processing. Therefore, the evolution of complex vocal signaling and perhaps even vocal learning itself may be intimately linked to the innovative use of estrogen signaling in the cortex.

These initial observations open new research directions regarding acute estrogen production and action in brain circuits for acoustic communication. First, the specific receptor mechanisms and downstream cell signaling pathways are poorly understood for the modulation of acoustic communication circuits, although the availability of specific receptor ligands has led to a wealth of new information in other neural systems regarding the rapid actions of estrogens in recent years [94-98]. Second, in addition to emphasizing the central actions of estrogens on acoutic communication within brain circuits, it will be important to characterize how estrogens may coordinate peripheral mechanisms for both perception and production of acoustic communication signals. The expression of estrogen receptors in both

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vocal organs [99-102] and auditory end-organs [38,48,103,104] appears to be a conserved feature among vertebrates and can guide these investigations. It is intriguing to consider that the evolutionary innovation that enabled brain circuits to locally synthesize estrogens could have been linked to the common ancestral origins of acoustic communication circuits among vertebrates [3]. Continuing to explore these questions from a comparative perspective will provide a rich understanding of neuromodulatory mechanisms that regulate vertebrate communication behavior.

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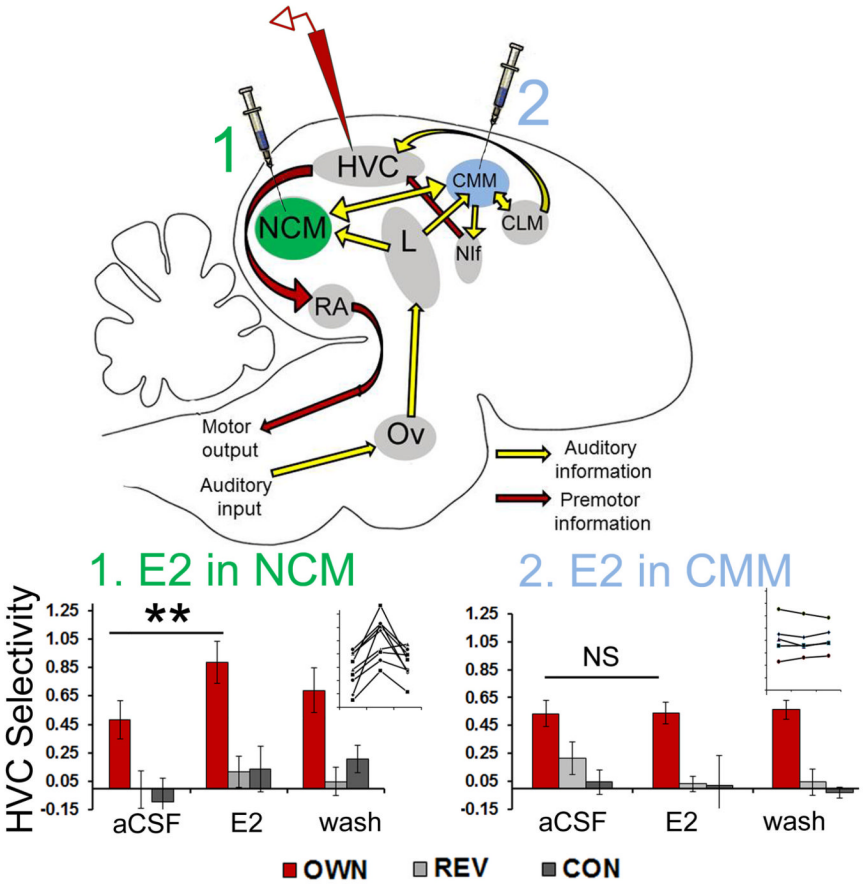

Figure 1.

Rapid actions of estradiol (E2) in NCM on auditory processing and stimulus selectivity in a downstream nucleus HVC. Top, a schematic of the zebra finch brain in the sagittal plane, showing the primary auditory network (light yellow arrows) and the song motor pathway (dark red arrows). The caudomedial nidopallium (NCM) is particularly enriched with expression of aromatase, the protein that catalyzes the local synthesis of estrogens. Ov, ovoidalis; L, primary thalamorecipient Field L; HVC (proper name); CMM, caudomedial mesopallium; CLM, caudolateral mesopallium; NIf, nucleus interface; RA, robust nucleus of the arcopallium. Bottom, a selective neural representation in HVC for playback of the bird's own vocalizations ('OWN') is enhanced by rapid delivery of estrogens (E2) to NCM, but not to nearby CMM. Bar graphs show mean ± s.e. for HVC responses to stimulation with OWN (bird's own song), REV (reverse OWN), or CON (conspecific song), each standardized as a ratio of responses to white noise (a measure of stimulus selectivity). The HVC selectivity for OWN is significantly elevated during E2 delivery to NCM (**$p < 0.01$ for paired Wilcoxon test vs. aCSF) but not to CMM (NS = nonsignificant). Insets show individual data for HVC OWN selectivity during aCSF, E2, and wash manipulations in NCM (n = 9) and CMM (n = 5). Adapted from Remage-Healey and Joshi, In press, J Neurosci, (ref 72).

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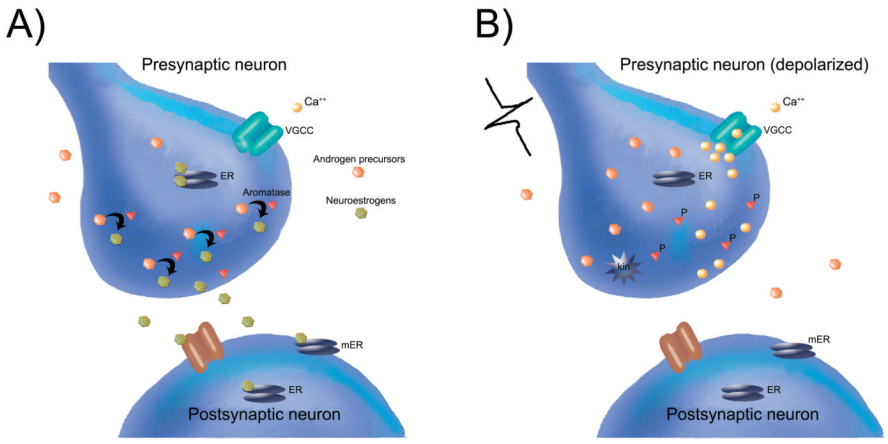

Figure 2.

Synaptocrine Signalling:Testosterone acts as a precursor (orange hexagons) diffusing from the extracellular space to be available as a substrate for the enzyme aromatase (red triangles) located within the presynaptic bouton. Estrogens such as 17-beta-estradiol (green hexagons) synthesized within the presynaptic bouton are then available to bind presynaptic estrogen receptors (ERs; grey ovals) and modulate presynaptic physiology. Synaptocrine estrogens may also diffuse across the synaptic cleft to interact with postsynaptic ion channel receptors (brown channel), and/or postsynaptic ERs (grey ovals, cytosolic) or membrane ERs (mER; grey ovals, membrane-bound). Synaptocrine estrogens could therefore modulate postsynaptic neurotransmitter receptors, cell signaling pathways, and/or have genomic effects on the postsynaptic cell. Because the activity of the aromatase enzyme is highest in a dephosphorylated state, the synaptocrine actions of estradiol are most likely to occur when the cell membrane is at rest (i.e., not depolarized) and when voltage-gated calcium channels (VGCCs) are closed. In contrast, (right panel), depolarization of the presynaptic neuron leads to calcium influx into the presynaptic bouton. This can activate a calcium-dependent kinase (kin) to phosphorylate presynaptic aromatase (P) and thereby cause a rapid reduction in the synthesis of synaptocrine estrogens, while simultaneously potentially increasing the local concentration of androgens. Adapted from Remage-Healey et al., 2011, Frontiers in Endocrinology, (ref 14).