Chapter 4
Modulation of Peripheral and Central Auditory Processing by Estrogens in Birds

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Abstract A growing body of literature points to the importance of estrogens in the development, maintenance, modulation, and protection of vertebrate audition. Birds, with their long history in endocrinological research, and their reliance on vocal communication for reproductive and social interactions, have proven to be particularly fruitful for such studies. This chapter summarizes current knowledge about the role of estrogens in avian auditory function, with a special emphasis on songbirds. Abundant evidence supports the notion that both peripheral and brain-derived estrogens modulate sound-evoked activity throughout the songbird auditory neuraxis. Estrogens influence audition at multiple timescales, ranging from seasonal variation to acute minute-by-minute variation. Moreover, estrogen actions occur in both males and females, indicating that estrogens are principal regulators of auditory function. Collectively, these findings highlight the need for new avenues of inquiry. Future areas of investigation include the intracellular mechanisms triggered by non-classical estrogenic pathways and a deeper exploration of the effects of estrogens on sensory encoding and decoding.

Keywords ABR • Aromatase • Electrophysiology • Estradiol • Field L • Hearing • Hormone • Microdialysis • NCM • Neurosteroid • Plasticity • Seasonal • Spike timing

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4.1 Introduction

Human auditory function is sensitive to a variety of environmental and hormonal influences. Historically, hormonal effects on hearing have been particularly evident in women, who demonstrate changes in audiometric thresholds, otoacoustic emissions, and tinnitus across the menstrual cycle and during pregnancy (for reviews see Al-Mana et al. 2008; Caras 2013). Therefore, the associations between changes in circulating levels of steroid hormones (e.g., estrogens) and changes in auditory function and dysfunction have become increasingly important to both basic and clinical researchers (see also Frisina and Frisina, Chap. 8 in this volume). Due to practical considerations, studies of hormonal influences on auditory processing in animal models have made great strides in helping to understand these phenomena in human beings.

For more than half a century, songbirds have been a central focus of endocrinological investigation. Originally studied in the context of diurnal photoperiods and gonadal activation (Farner et al. 1953), attention later shifted to hormonal effects on behavior, particularly song production (Marler et al. 1988) and its underlying neural substrate (Nottebohm 1981; Brenowitz et al. 1991). In the years since, studies have firmly established that testosterone and its androgenic metabolites play an important role in regulating vocal motor control (Park et al. 2005; Meitzen et al. 2009). More recently, corresponding estrogenic effects have been documented in the avian auditory system. These latter effects will be the focus of the current chapter.

Estrogens play an important role in regulating female reproductive behavior. In birds, elevated estrogen levels are closely tied to breeding activity, such as nest building, mating, and egg laying (Wingfield and Farner 1978a, b). One mechanism by which estrogens exert their effects is by modulating the physiological response to social signals, particularly male advertisements. For example, the exposure of female zebra finches (*Taeniopygia guttata*) to a live male is associated with increases in systemic estradiol levels (as measured from repeated fecal samples) and shorter latencies to egg laying (Tchernichovski et al. 1998). Work by Nottebohm and Nottebohm (1971) suggested that the avian auditory system is directly involved in this process: For deaf female ring doves (*Streptopelia risoria*), there is a longer latency between opposite-sex pairing and ovulation compared to normal-hearing counterparts. Similarly, acoustic stimulation alone is sufficient for generating a reproductive response. In socially isolated female ring doves, for instance, exposure to sounds of a nearby breeding colony facilitates follicular growth (Lehrman and Friedman 1969). In fact, the acoustic details of the social stimulus directly correlate with the physiological and behavioral outcome in songbirds. In canaries (*Serinus canarius*), exposure to playback of larger conspecific song repertoires leads females to gather significantly more nesting material and lay significantly more eggs than exposure to smaller repertoires, even if the total sound duration is held constant (Kroodsma 1976). In a later study, Bentley et al. (2000) found that heterospecific (zebra finch) song playback is also sufficient to stimulate follicular growth and egg laying in canaries but is not as effective as conspecific song. Thus, auditory signals, particularly those with ethological relevance, stimulate estrogen synthesis and reproductive activity in female birds.

4.2 The Brain as a Source and Target of Estrogens

Estrogens have powerful effects on reproductive physiology and behavior. Estrogen production is widespread throughout the body, as estrogens can be synthesized in peripheral organs, including the gonads, adrenals, and adipose tissue (Martel et al. 1994; MacKenzie et al. 2008). As in many other vertebrates, it is now clear that the avian brain itself, and auditory circuits in particular, can be both the source and the target of estrogenic actions.

The study of the neurobiology and behavior of birds has led to a number of important insights into the role of estrogen synthesis in the brain. In the avian brain the distribution of aromatase, the enzyme that synthesizes estrogens from androgens like testosterone, is abundant in regions that are conserved across vertebrates, including the hypothalamus and preoptic area (Roselli and Resko 1987; Balthazart et al. 1990). The local conversion of testosterone into estradiol by neurons in the hypothalamic/preoptic region is essential for male sexual behavior in many bird species, notably the Japanese quail (*Coturnix japonica*) (Balthazart et al. 2006; Cornil and Charlier 2010). While the expression of aromatase in the hypothalamic/preoptic region is highly conserved across the avian lineage, the expression of aromatase in the telencephalon (and auditory circuits specifically) is particularly pronounced in songbirds as compared to non-songbirds (Saldanha et al. 2013). In some songbird species, such as the zebra finch, the complete suite of specialized enzymes that catalyze the conversion of estrogens from the original cholesterol precursor have been localized to the telencephalon (London et al. 2006). Therefore, the de novo synthesis of estrogens from cholesterol is possible in some forebrain regions in songbirds. In the case of many male songbirds, there is essentially no capacity for estrogen synthesis in peripheral organs, and estrogens that are measured in the plasma of males originate from the abundant production of estrogens in the central nervous system (Schlinger and Arnold 1992).

A further important consideration when examining estrogen synthesis in the brain is the cellular compartment in which aromatase is found in neurons. Aromatase protein is expressed in the neuronal soma (cell body), but it is also found along fibers extending away from the soma, consistent with expression in putative axonal processes and terminal puncta (Saldanha et al. 2000; Peterson et al. 2005). Immunoelectron microscopic imaging has revealed that aromatase is found in presynaptic terminals in the songbird telencephalon, in addition to rodent, quail, and primate brains (Naftolin et al. 1996; Peterson et al. 2005). Biochemical analysis following differential centrifugation has also confirmed that enzymatic aromatase activity is present—and in some auditory regions enriched—in telencephalic synaptosomes, the neuronal compartment composed of presynaptic and postsynaptic terminals (Rohmann et al. 2007; Remage-Healey et al. 2009). Further biochemical and in vivo pharmacological experiments have indicated that the pool of presynaptic aromatase may be independently controlled vis-à-vis the somal pool of aromatase in the auditory forebrain (Remage-Healey et al. 2011a; Cornil et al. 2012). Taken together, the evidence for estrogen synthesis in the auditory circuits of the songbird brain is consistent with a neuromodulatory mechanism that is temporally and spatially precise, as befits a classical neuromodulator (Saldanha et al. 2011; Remage-Healey 2012).

4.3 Overview of the Avian Auditory System

4.3.1 Organization of the Songbird Auditory Pathway

The organization of the avian auditory pathway (Figs. 4.1 and 4.2) is similar to that of the mammalian system (Reiner et al. 2005; Köppl 2011). Mechanotransduction occurs in the hair cells of the basilar papilla (homologous to the mammalian cochlea), resulting in the generation of action potentials in the innervating cochlear ganglion afferents. This activity is transmitted via the eighth cranial nerve to the avian cochlear nuclei: nucleus magnocellularis (NM) and nucleus angularis (NA) (Boord and Rasmussen 1963). From there, the signal diverges into parallel brainstem pathways, each involving multiple synapses, before converging again in the midbrain nucleus mesencephalicus pars dorsalis (MLd) (Conlee and Parks 1986; Wild et al. 2010). The signal is then transmitted up to the auditory thalamic nucleus ovoidalis (Ov) (Karten 1967). The primary forebrain target of Ov is the avian analogue of the mammalian auditory cortex, Field L. A heterogeneous nucleus, Field L is actually a complex of four subregions (L1, L2a, L2b, and L3), which are distinct based on cellular morphology and connectivity, but are all interconnected (Fortune and Margoliash 1992; Vates et al. 1996). L2a receives the vast majority of thalamic auditory input, though L2b is also a target. Like many other auditory nuclei, Field L is tonotopically organized, with low frequencies represented dorsally and caudally, and higher frequencies represented ventrally and rostrally (Zaretsky and Konishi 1976; Wild et al. 1993). The intrinsic electrical and response properties of each Field L cell type are still unknown, though differences in tuning, and perhaps stimulus selectivity, have been demonstrated among the different subregions (Bonke et al. 1979; Kim and Doupe 2011).

Many of the subregions of Field L make specific, reciprocal connections with secondary auditory regions, including the caudomedial nidopallium (NCM), which is discussed at length later in this chapter, and the caudal mesopallium (CM). CM, in turn, connects to the sensorimotor region HVC (used as a proper name), which both responds to song playback, and is responsible for generating vocal output.

4.3.2 Estrogen Receptor and Aromatase Expression in the Songbird Auditory System

The songbird auditory system is directly sensitive to estrogenic modulation, as evidenced by the presence of estrogen receptors (ERs) in the auditory pathway (Fig. 4.1). In male and female zebra finches, for example, immunohistochemical analyses have revealed ERα expression in hair cells, support cells, and cochlear ganglion cell bodies (Noirot et al. 2009). In the forebrain, ERs localize to both NCM (both α and β subtypes) and HVC (α subtype only) (Bernard et al. 1999; Metzdorf et al. 1999). Currently, the precise function of these classical receptors in avian

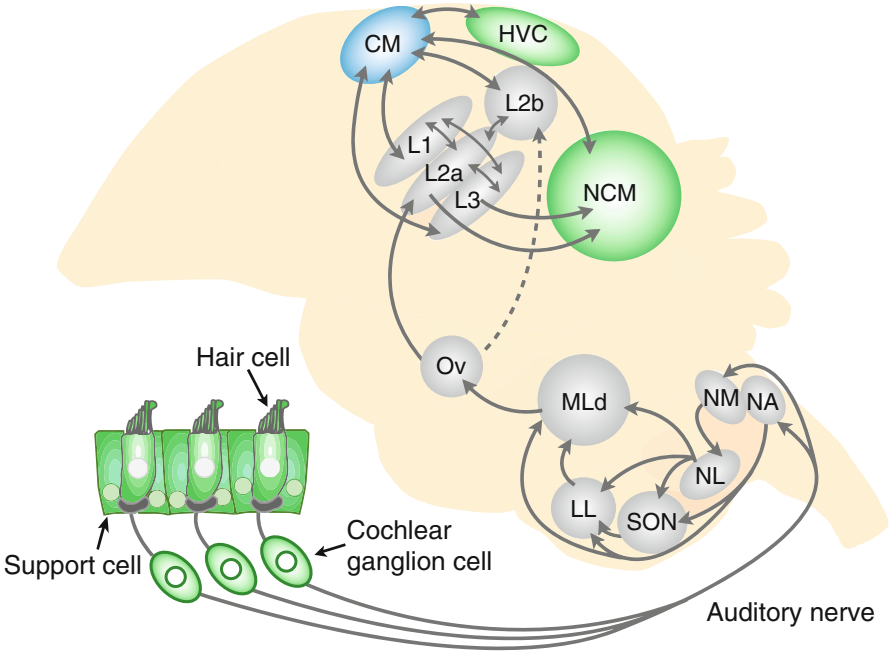

Fig. 4.1 Estrogen sensitivity in the songbird auditory pathway. Known (*green*) and putative (*cyan*) sites of estrogen receptor expression. In the periphery, classical estrogen receptor (ER) subtype expression has been reported in hair cells, support cells, and cochlear ganglion cell bodies. In the forebrain, HVC and NCM both express classical ERs, as well as the more recently discovered GPER1 (G protein-coupled estrogen receptor 1). GPER1 may be expressed in CM as well. The *dashed line* connecting Ov and L2b indicates that this projection is weaker than the one from Ov to L2a, and originates in a restricted, medial portion of Ov. *CM* caudal mesopallium, *HVC* used as a proper name, *LL* lateral lemniscus, *MLd* mesencephalicus pars dorsalis, *NA* nucleus angularis, *NCM* caudomedial nidopallium, *NL* nucleus laminaris, *NM* nucleus magnocellularis, *Ov* nucleus ovoidalis, *SON* superior olivary nucleus. *L1*, *L2a*, *L2b* and *L3* refer to individual subunits of the Field L complex. Adapted from Caras (2013)

auditory processing is unclear. One likely possibility is that they are involved in mediating seasonal changes in auditory function (see Sect. 4.4). Evidence for this idea arises from the finding that neurons in the HVC of male canaries express higher levels of ERα mRNA during the fall non-breeding season compared to the spring breeding period (Fusani et al. 2000). A separate study also revealed the presence of G protein-coupled estrogen receptor 1 (GPER1, formally referred to as GPR30) in zebra finch NCM, HVC, and possibly CM (Acharya and Veney 2011). GPER1 is a membrane-bound receptor that supports non-genomic intracellular signaling (Barton 2012). Though its relative abundance in these regions is not well-characterized (compared to the classical ER subtypes), its presence in NCM and HVC suggests that GPER1 may play an important role in rapid estrogenic signaling (see Sect. 4.5 below).

As noted in Sect. 4.2, cells in the auditory pathway not only express ERs but also synthesize estradiol (Fig. 4.2). In the periphery, immunoreactivity for aromatase, the

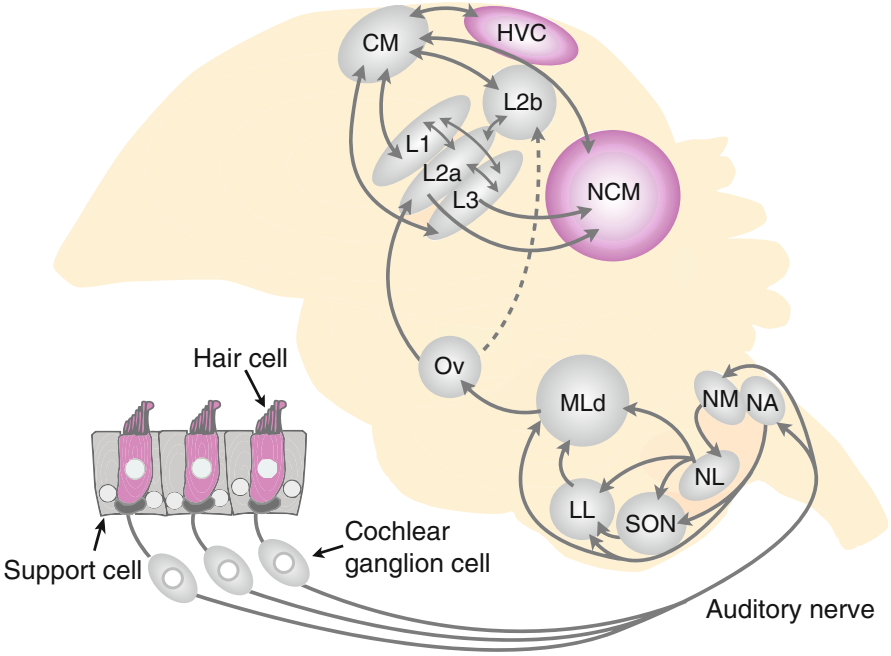

Fig. 4.2 Estrogen production in the songbird auditory pathway, illustrating known sites of estrogen synthesis in the auditory pathway (*magenta*). Aromatase immunoreactivity has been reported in songbird hair cells, as well as HVC and NCM. Abbreviations and plot conventions as in Fig. 4.1. Adapted from Caras (2013)

enzyme responsible for converting testosterone into estradiol, has been found in songbird hair cells (Noirot et al. 2009). Furthermore, in canary NCM, aromatase mRNA expression levels are higher during the breeding season than the non-breeding season (Fusani et al. 2000). Thus, estrogen-producing and estrogen-sensitive circuits overlap in both the peripheral and central nervous system and, in some species, fluctuate with reproductive condition. Moreover, aromatase is expressed in both soma and presynaptic terminals, and in some areas, such as HVC and NCM, the terminal expression is particularly abundant (Saldanha et al. 2000; Peterson et al. 2005). These findings are consistent with a 'synaptocrine' model of estrogen delivery (Saldanha et al. 2011) in which estrogens can be targeted rapidly to precise individual synaptic targets on demand (Fig. 4.3).

4.4 Seasonal Plasticity of Auditory Function

A common approach to investigate the hormonal regulation of auditory processing takes advantage of a naturally occurring, behaviorally relevant phenomenon: adult seasonal plasticity. Seasonal plasticity occurs in response to changes in photoperiod, temperature, or rainfall that trigger the gonads to produce androgenic and/or estrogenic

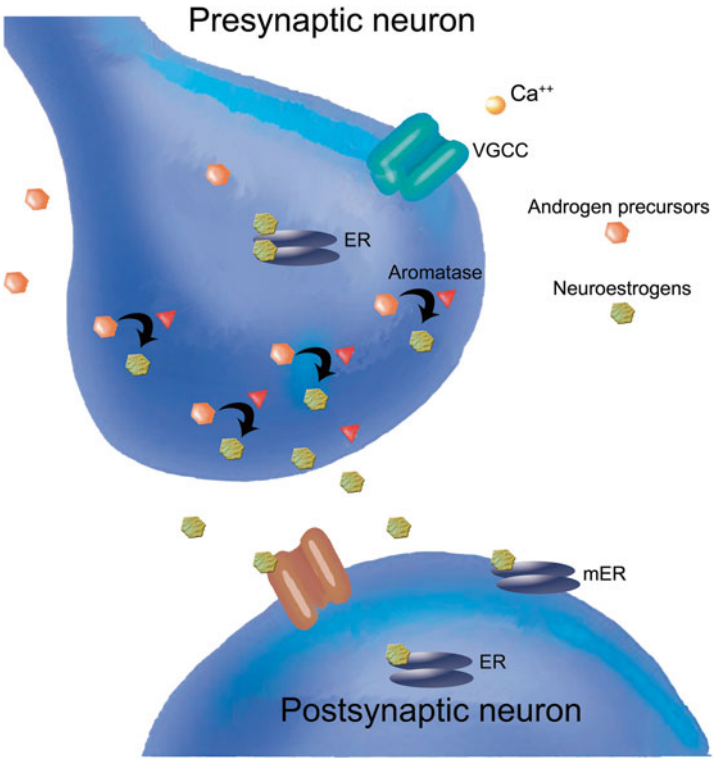

Fig. 4.3 The 'synaptocrine' model of estrogen delivery. Androgen precursors (*orange hexagons*) from the periphery or derived within the brain itself are available as substrates for the enzyme aromatase (*red triangles*) located in presynaptic terminals. The locally generated estrogens (*green hexagons*) are then available to bind to estrogen receptors (ERs; *gray ovals*) and alter the physiology of presynaptic terminals, such as neurotransmitter gating. Synaptocrine-secreted estrogens may also diffuse into the synaptic cleft and interact with post-synaptic elements, including ion channels (*brown channel*), intracellular ERs and/or membrane ERs (mER). *VGCC* voltage-gated calcium channel. Adapted from Remage-Healey et al. (2011b)

steroids (Brenowitz 2004). These hormonal shifts alter the morphology and physiology of the neural circuitry that supports vocal communication and auditory processing (e.g. Bass 2008; Sisneros 2009; also see Forlano, Maruska, Sisneros, and Bass, Chap. 2; Wilczynski and Burmeister, Chap. 3). The following sections discuss several studies that have used seasonally breeding songbirds to explore how estrogens modulate sensory function throughout the avian auditory pathway.

4.4.1 Seasonal Plasticity of Peripheral Auditory Physiology

An initial study by Lucas et al. (2002) explored the effect of season on the avian auditory system. The authors collected wild birds of several species and divided them into two groups according to the time of year: winter (October through January)

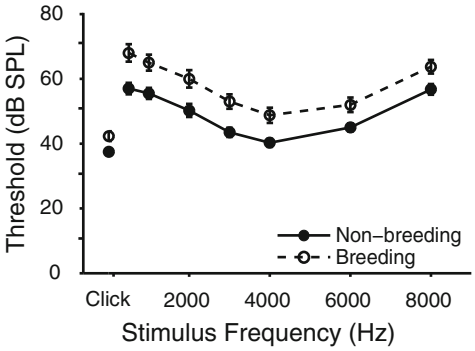

Fig. 4.4 White-crowned sparrows housed under breeding (high-estrogen) condition have elevated auditory brainstem response thresholds than those housed under non-breeding (low estrogen) condition across all stimulus frequencies. Thresholds to clicks (in dB peak equivalent SPL) are shown at the left most position of the graph. Each point represents the average and SEM of 20 animals, except for clicks, where $n = 21$ for the breeding condition group. Adapted from Caras et al. (2010)

and spring (February through April). Within a few days of capture, they recorded the auditory brainstem response (ABR), a multi-wave neural response that occurs within 10–15 ms after the onset of an auditory stimulus (Hall 2007). The authors reported species-specific effects of season. In the downy woodpecker (*Picoides pubescens*) and white-breasted nuthatch (*Sitta carolinensis*), for instance, ABR peak latencies were longer and peak amplitudes were lower during the spring compared to the winter. In the Carolina chickadee (*Poecile carolinensis*) and house sparrow (*Passer domesticus*), on the other hand, the reverse was true: ABR amplitudes were large in the spring and small in the winter. Two follow-up studies reported similar findings (Lucas et al. 2007; Henry and Lucas 2009). These results suggested that in some species, peripheral auditory function is sensitive to seasonal environmental changes, though the lack of sex-specific comparisons and hormonal measurements prevented further interpretation of the data.

Two later papers provided strong evidence that peripheral function is, in fact, sensitive to estrogenic state. First, in 2010, Caras and colleagues recorded ABRs from a songbird species with well-characterized seasonal fluctuations in sex steroid levels: Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). Birds were caught in the wild and exposed to one of two photoperiod and hormonal manipulations designed to mimic natural breeding or non-breeding conditions in the laboratory. The authors found that female birds housed under breeding-like conditions demonstrated elevated ABR thresholds and prolonged ABR peak latencies compared to their non-breeding counterparts (Fig. 4.4). To determine where in the auditory pathway this effect emerged, distortion product otoacoustic emissions (DPOAEs) were recorded and compared across breeding and non-breeding conditions. DPOAEs are low-amplitude sounds generated by the active non-linear properties of the inner ear (Kemp 1978, 2002). The authors found that DPOAE amplitudes were unaffected by reproductive state, indicating that the elevation in ABR thresholds under breeding condition were not the result of inadequate inner

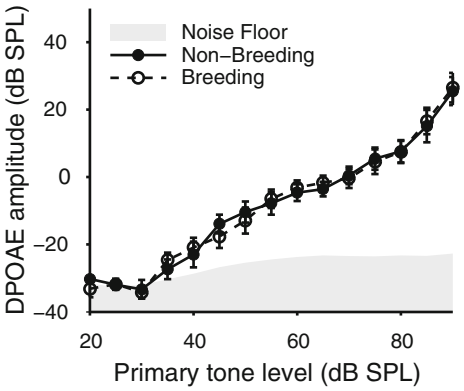

Fig. 4.5 Breeding (high-estrogen) condition does not affect distortion product otoacoustic emission (DPOAE) amplitude in white-crowned sparrows. DPOAEs were elicited by the presentation of two simultaneous pure tones. DPOAE amplitudes increased as the level of the primary tone (6.09 kHz) increased, but no difference was observed between breeding and non-breeding condition. The level of the secondary tone (7 kHz) was always 10 dB lower than the primary. Each point represents the mean and SEM of 11 birds (except $n=8$ breeding condition birds at 20 and 25 dB SPL). Adapted from Caras et al. (2010)

ear amplification (Fig. 4.5). Plasma samples taken from the birds immediately before ABR and DPOAE recording confirmed that estradiol levels significantly differed between the two groups, with higher concentrations found in the breeding condition females. A short while later, Gall et al. (2013) captured female house sparrows, took blood samples to measure systemic hormone levels, and recorded ABRs generated by tone bursts in spectrally notched white noise (to assess peripheral frequency resolution) and tone burst pairs (to assess peripheral temporal resolution). The authors found that during the breeding season (February–April), when estradiol levels were elevated, frequency selectivity was enhanced and temporal resolution was poorer than in the non-breeding season (October–November), when estradiol levels were basal. Taken together, these findings suggest that estrogens play an important role in modulating peripheral auditory function, but the precise nature of the regulation depends on the songbird species.

4.4.2 Seasonal Plasticity of Central Auditory Physiology

An important question that arose from the previous studies was whether the estrogenic effects that are present in the periphery are transmitted faithfully to central regions more likely to be involved in perceptual processing. Caras et al. (2012) explored this issue by bringing wild-caught female white-crowned sparrows into breeding or non-breeding condition in the laboratory, as described above, and recorded the extracellular responses of single units in Field L to pure tones and white-crowned sparrow song exemplars. The authors found robust, cell-selective effects of

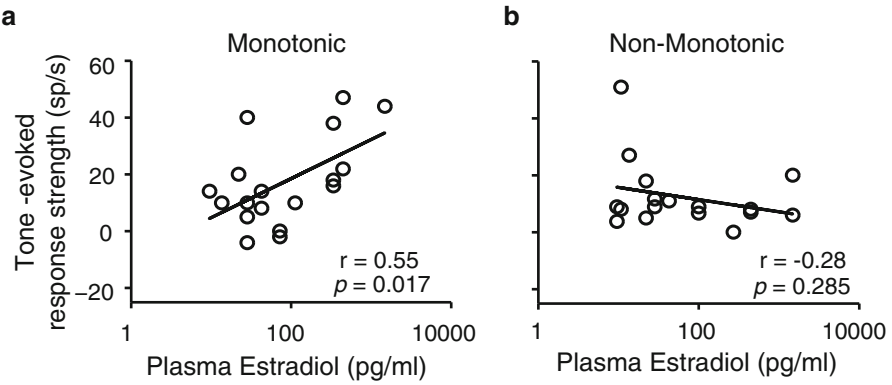

Fig. 4.6 Plasma estradiol concentration predicts monotonic neuron response properties in Field L of white-crowned sparrows. (**a**) *Each circle* represents the response strength (i.e., the evoked firing rate—the spontaneous firing rate) of a single neuron. Responses were elicited by 50 dB SPL pure tones at each neuron's characteristic frequency. Response strengths are plotted as a function of the level of plasma estradiol measured in the subject from which the neuron was recorded. All cells plotted in this panel were classified as "monotonic" because their tone-evoked firing rates increased monotonically with increasing sound levels. A significant positive correlation was found, such that the response strengths of individual monotonic neurons increase as the concentration of circulating estradiol increases. (**b**) Data from cells that were classified as "non-monotonic" because their tone-evoked firing rates were maximum at middle or low sound levels and were suppressed at higher levels. The response strengths of these cells were not correlated with the concentration of plasma estradiol. Adapted from Caras et al. (2012)

breeding condition on neuronal physiology. Specifically, breeding condition increased spontaneous firing rates, maximum firing rates, and sound-evoked response strengths in cells with monotonic pure-tone rate-level profiles. This enhanced activation led to lower pure-tone thresholds and larger dynamic ranges for white-crowned sparrow song. Furthermore, the firing rates and sound-evoked response strengths of individual monotonic neurons were significantly correlated with the concentration of circulating estradiol (Fig. 4.6a). In contrast, cells with non-monotonic rate-level functions were largely unaffected by breeding condition, despite the fact that their cellular distribution overlapped anatomically with that of the monotonic cells (Fig. 4.6b). One potential role for non-monotonic neurons in auditory coding involves the maintenance of stable sound encoding across a wide range of signal intensities (Sadagopan and Wang 2008; Watkins and Barbour 2011). Thus, the authors hypothesized that during the breeding season, estradiol might selectively strengthen monotonic neuron responses to enhance song detection, while the relative stability of the non-monotonic cells may enable stable song encoding throughout the year.

An additional study by the same group used a similar approach to explore the effect of estradiol on the encoding of sound level in wild-caught female white-crowned sparrows (Caras et al. 2015). Specifically, a pattern classifier was applied to song-evoked single-unit responses recorded from Field L and CM of birds exposed to breeding or non-breeding-like conditions in the laboratory. The classifier revealed

that cells clustered into one of three functional groups based on their ability to utilize spike counts and/or spike timing during the coding process, and the relative proportions of these cell types varied with reproductive condition. During the non-breeding season, for instance, cells capable of encoding sound level using both spike counts and precise spike times (i.e., "bi-coding" cells) comprised the majority (53 %) of the cellular population. Cells capable of using only precise spike timing (i.e., "temporal cells") made up 38 % of the population, and cells that utilized only spike counts (i.e., "count cells") were a small minority. In contrast, during the breeding season, the cellular distribution shifted, such that the proportion of temporal cells increased to nearly 70 %, and the relative numbers of both bi-coding and count cells decreased accordingly. Furthermore, the authors once again noted a cell-selective effect of estradiol on neuronal response properties. In bi-coding cells (but not temporal or count cells), the spike timing-based encoding of song amplitude was enhanced under breeding condition. In addition, in both bi-coding and temporal cells, estradiol reduced the width of the temporal window at which optimal intensity discrimination could be achieved, from ~63 to ~14 ms, amounting to a fourfold-to-fivefold increase in temporal resolution under breeding condition (Fig. 4.7).

Collectively, these findings suggest that in the white-crowned sparrow, elevated levels of circulating estradiol associated with the breeding season lead to a reduction in peripheral auditory sensitivity (Caras et al. 2010) that is compensated for by enhanced sound detection and discrimination in select cell populations of the auditory forebrain (Caras et al. 2012, 2015). Whether these observations reflect a general pattern of modulation in seasonally breeding songbirds remains to be determined.

4.4.3 Seasonal Plasticity of Immediate Early Gene Expression

Neurons within NCM and CM exhibit robust auditory responses to playback stimuli in a variety of songbird species (Müller and Leppelsack 1985; Terleph et al. 2007). A map of immediate early gene activation in both NCM and CM was first identified by Mello and colleagues (Mello et al. 1992; Mello and Clayton 1994). The staining intensity of the EGR1 protein (also known as ZENK) is thought to reflect activity-dependent membrane depolarizations in neurons and is robustly upregulated in the songbird NCM and CM in auditory contexts (Mello et al. 1998; Woolley and Doupe 2008). The strong auditory activation of these secondary auditory cortical regions makes them prime candidates to understand how estrogens modulate audition.

Female white-throated sparrows (*Zonotrichia albicollis*) breed seasonally, and their auditory processing demands change as they enter the breeding season. In a series of studies, Maney and colleagues found that estradiol can shape the immediate early gene response to sounds in NCM, CM, and MLd of females (see Maney, Chap. 5). Specifically, females maintained on a short-day photoperiod to eliminate the gonadal source of estrogens (due to photoperiod-dependent gonadal regression) were treated with 7-day estradiol implants to mimic the season-dependent rise in gonadal estrogen secretion. Compared to control birds, the ZENK induction to song

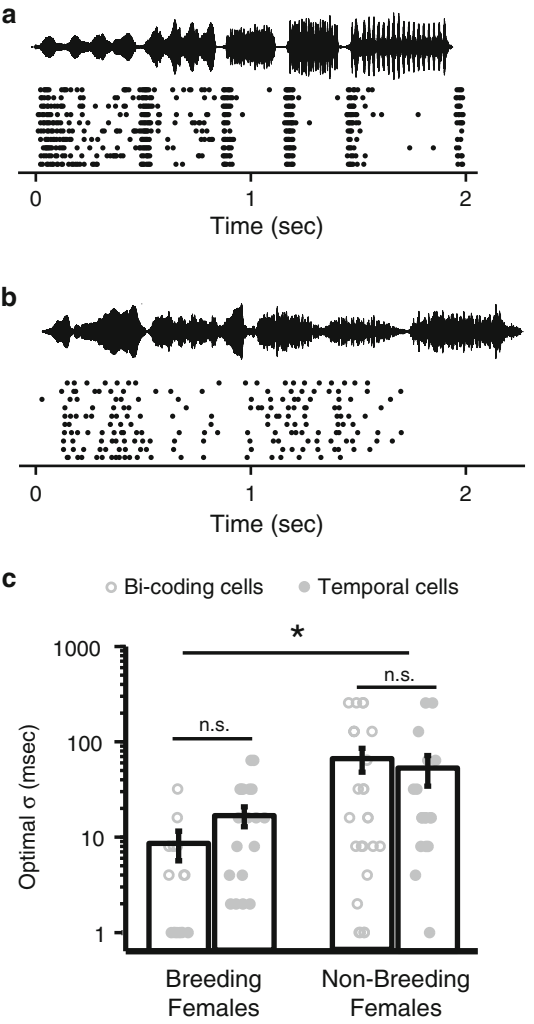

Fig. 4.7 Breeding (high estrogen) condition increases the temporal resolution required for optimal spike timing-based intensity discrimination in female white-crowned sparrows. (**a**) Raster plot from a bi-coding neuron recorded in a breeding female (see Sect. 4.4.2 for details). Responses were elicited by a conspecific song exemplar presented at 90 dB SPL. The amplitude envelope of the song is shown at the top of the panel. This neuron had an optimal temporal window (σ) of 4 ms. (**b**) Raster plot from a bi-coding neuron recorded in a non-breeding female. This cell had an optimal σ of 64 ms. (**c**) Both bi-coding (*open circles*) and temporal cells (*filled circles*) demonstrated smaller temporal windows under breeding condition, indicating an increase in temporal resolution. Bars indicate mean; error bars indicate ±SEM; $*p<0.05$; *n.s.* not significant. Adapted from Caras et al. (2015)

stimuli was significantly elevated over that to tone stimuli in NCM, CM, and MLd (Maney et al. 2006). Follow-up studies showed that the estradiol-mediated upregulation of selectivity for song stimuli was sub-region dependent in NCM, indicating that NCM contains specific domains that are especially responsive to estrogen treatment (Sanford et al. 2010).

The neurochemical domains within NCM have become increasingly important in understanding its function (Maney and Pinaud 2011). One important aspect has been the non-uniform distribution of fibers that express biogenic amines, such as catecholamines and serotonin. The actions of estrogens within NCM, therefore, are likely dependent on interactions and co-modulation of these subdomains of NCM by biogenic amines. Further studies conducted by Maney and colleagues showed that the effects of elevated circulating estrogens were associated with an upregulation of serotonergic signaling in NCM and CM of female white-throated sparrows (Matragrano et al. 2012). In NCM, estradiol implants led to an elevation in the density of fibers expressing the serotonin transporter, while in CM, estradiol caused an increase in the tissue concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid. Together, this body of work showed that auditory neurons in the songbird forebrain are sensitive to the longer term (days-to-weeks) actions of estrogens and that these actions may depend on interactions with classical neuromodulators to shape the salience or valence of auditory cues. Because these implant studies were intended to mimic a seasonal increase in estrogens, they indicate that auditory immediate early gene induction is likely sensitive to photoperiod. Indeed, in male black-capped chickadees (*Poecile atricapillus*), the selectivity of the ZENK response to conspecific and heterospecific song varied seasonally (Phillmore et al. 2011), suggestive of an interaction with steroids or other seasonal factors.

4.5 Acute Estrogenic Effects in the Central Auditory Pathway

ZENK upregulation in neurons within NCM can be driven by estradiol itself without accompanying acoustic playback of song or other stimuli. This upregulation has been observed in white-throated sparrows (Sanford et al. 2010) as well as zebra finches (Tremere et al. 2009). The magnitude of the ZENK response to exogenous estradiol treatment was similar to that observed for song activation. This finding raised the possibility that local estrogen signaling in NCM in response to auditory playback may provide an intermediary neurochemical mechanism leading to the upregulation of ZENK in NCM neurons. For clarification about this possibility, studies using in vivo microdialysis to measure fluctuations in brain estrogen are presented below.

4.5.1 Microdialysis Approaches

The means to measure and manipulate estrogen fluctuations in the auditory forebrain was developed specifically for songbirds using an in vivo microdialysis approach (Remage-Healey et al. 2008). Microdialysis has been used for decades to detect fluctuations in neuromodulators like dopamine (Schultz 2007). It relies on the

implantation of a miniaturized probe directed at a brain region of interest, such as NCM. The probe is inserted through a pre-implanted cannula and it has a porous membrane that allows extracellular neurochemicals below a size threshold to passively diffuse into a slow-flowing solution of artificial cerebrospinal fluid (aCSF). The solution is continuously flowing and dialysate fractions on a minute-by-minute timescale are collected for analysis.

The analysis of steroids like estrogens by using microdialysis is relatively new as compared to traditional analytes like dopamine and other neurotransmitters. Partly this is due to the chemical structure of steroids, since their lipid solubility makes aqueous diffusion and quantification more difficult. Equally, a mature understanding of the capacity of neurons to locally synthesize neurosteroids has lagged behind the wealth of knowledge regarding the secretion of neurotransmitters and neuropeptides. Remage-Healey et al. (2008) validated in vivo microdialysis for estrogens in the zebra finch brain using a combination of analytical approaches. This method enabled a temporal resolution of ≥30 min bins due to assay detection limits. Nevertheless, several findings using this technology have helped clarify the relationships between auditory processing and neuroestrogen fluctuations in the songbird auditory forebrain.

First, the acute responses of neuroestrogens in NCM of males were specific for song as compared to other stimuli, as was identified by Maney and colleagues for song-specific, immediate early gene responses (Maney et al. 2006; Sanford et al. 2010). Specifically, as shown in Fig. 4.8, neuroestradiol levels were elevated during 30 min of playback of intermittent male song relative to baseline, while no detectable change occurred in response to playback of duration-matched white noise (Remage-Healey et al. 2008). This rapid elevation in local estradiol levels occurred specifically in NCM and not in other brain regions or in the circulating plasma. This

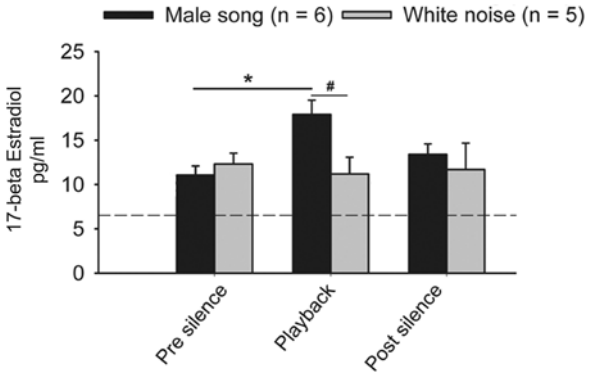

Fig. 4.8 Neuroestradiol levels fluctuate in auditory forebrain. Exposure to playback of male song for 30 min increases estradiol in zebra finch NCM, relative to a pre-treatment silence condition. A similar treatment with white noise does not change local neuroestradiol levels in NCM, consistent with a selectivity for socially relevant sounds. *Dashed line* indicates background estradiol concentrations for aCSF (artificial cerebrospinal fluid) alone as reported by ELISA. $^{*}p<0.05$ for within subject, $^{\#}p<0.05$ for between subject. Adapted from Remage-Healey et al. (2008)

pattern was also observed in NCM of females in response to song but not white noise (Remage-Healey et al. 2012), indicating that neuroestrogen fluctuations are a general (and not sex-specific) neuromodulatory property of NCM. Therefore, in adult zebra finches there is an acute, local elevation of estradiol within NCM of awake, behaving males and females when they are hearing song. In juvenile males and females, by contrast, estradiol was actively suppressed in NCM when they heard song for the first time (i.e., during tutoring), and levels were elevated only during the 60-min period after the song playback was over (Chao et al. 2014). Together, these studies show that neuroestrogens can fluctuate in the auditory forebrain of zebra finches during auditory stimulation, yet with different temporal patterns depending on developmental stage.

The acute fluctuations in neuroestradiol in response to auditory stimuli are an indication that some rapid control mechanism must account for the neuromodulator-like changes in local concentrations of estrogens in the brain. The most established control mechanism for brain estrogen fluctuations is via rapid alterations of the activity of the aromatase enzyme (Balthazart et al. 2006). Unlike conventional neurotransmitters, the lipophilic steroids are generally thought not to be packaged in vesicles; therefore, the 'release' of steroids is controlled at the level of the enzymatic synthesis activity (Balthazart et al. 2006; Remage-Healey 2014). The consensus control mechanism for the aromatase enzyme is via rapid calcium-dependent phosphorylation, which can induce a rapid downregulation of the enzymatic activity in vitro (Cornil and Charlier 2010; Cornil et al. 2012). Similarly, in NCM, in vivo microdialysis experiments have shown that local estradiol levels are sensitive to blockade of presynaptic voltage-gated calcium channels (Remage-Healey et al. 2011a) and glutamate excitation (Remage-Healey et al. 2008). Interestingly, neither retrodialysis with norepinephrine nor γ-aminobutyric acid (GABA) had any detectable effects on local estradiol levels (Ikeda et al. 2015; Remage-Healey et al. 2008). Therefore, while the exact control mechanism is still unclear, it appears that auditory forebrain neuroestrogen levels are dynamic in spatially and temporally precise ways, most likely via excitation-dependent and calcium-dependent events within presynaptic terminals (see Sect. 4.2).

4.5.2 Acute Effects of Estrogens on Central Auditory Physiology

The accumulated evidence for rapid neuroestrogen fluctuations in the songbird auditory forebrain as presented above begs the question: What does this mean for auditory function? In addition to the evidence presented for immediate early genes (Sect. 4.5.1), several studies have documented more acute effects (seconds to minutes) of neuroestrogens on the activity patterns and coding properties of neurons in NCM using electrophysiology. Within minutes, exogenous application of estradiol to NCM via pressure microinjection caused an upregulation of auditory responsiveness (Tremere et al. 2009; Tremere and Pinaud 2011). Similarly, reverse

microdialysis of estradiol induced the same qualitative effect on NCM responsiveness (Remage-Healey et al. 2010, 2012; Remage-Healey and Joshi 2012). More specifically, there were four primary observed effects.

First, estradiol caused a switch from a tonic firing pattern to a burst-mode firing pattern for NCM neurons, which is significant because bursts carry more information about auditory stimuli than isolated tonic action potentials in NCM (Remage-Healey et al. 2010). Second, the auditory-evoked firing rate of NCM neurons was significantly elevated in the presence of estradiol when compared to pre-treatment control conditions or a control treatment in the contralateral hemisphere (Remage-Healey et al. 2010, 2012). Third, the estradiol-mediated enhancement of auditory-evoked activity in NCM was carried downstream into other interconnected song regions, including the sensorimotor nucleus HVC (Fig. 4.9), to enhance the selectivity for sensorimotor-relevant stimuli (Remage-Healey and Joshi 2012; Pawlisch and Remage-Healey 2015). Fourth, the acute actions of estradiol (i.e., within 30 min) in NCM were mimicked by reverse microdialysis of a biotin-conjugated estradiol that restricts diffusion across neuronal membranes (Remage-Healey et al. 2012) and were not mimicked by agonists for the classical nuclear estrogen receptors (Remage-Healey et al. 2013). This evidence is consistent with a non-classical mode of action for estrogens in NCM to modulate auditory neurons, likely to be via a membrane-bound receptor. A potential candidate receptor is the formerly orphaned G protein-coupled receptor, GPER1, which is expressed in NCM (Acharya and Veney 2011).

A series of electrophysiology experiments further confirmed that *endogenous* neuroestrogens are important for NCM neuronal activity patterns. Using the steroidal aromatase inhibitor 1,4,6-androstatriene-3,17-dione as well as the broad-spectrum anti-estrogen tamoxifen, Tremere et al. (2009) showed that inhibiting estrogenic signaling in NCM diminished local auditory-evoked firing rates. Similarly, Remage-Healey et al. (2010) showed that the non-steroidal, specific aromatase inhibitor fadrozole suppressed the auditory-evoked bursting of NCM neurons in males and caused a suppression of auditory-evoked activity in NCM of females (Remage-Healey et al. 2012). Furthermore, the suppression of local NCM aromatase activity had downstream consequences on the auditory stimulus selectivity in HVC (Remage-Healey and Joshi 2012), as well as in an interface nucleus between NCM and HVC (Pawlisch and Remage-Healey 2015). Finally, combining reverse microdialysis with behavioral testing yielded insight into the functional significance of local neuroestrogen synthesis in NCM. When fadrozole was delivered via reverse microdialysis during a song preference task (zebra finches naturally spend the majority of their time near a speaker playing a familiar song versus a novel song), the preference for familiar songs was significantly disrupted, since the males reduced their time spent next to the familiar-song speaker from ~75 % under control conditions to ~50 % during fadrozole treatment (Remage-Healey et al. 2010). At present, it is unclear whether this behavioral disruption is due to a deficit in motivation, performance, discrimination, perception, or a combination of these cognitive processes, but it is clear that local estrogen synthesis is important to guide behavioral song preferences in this species.

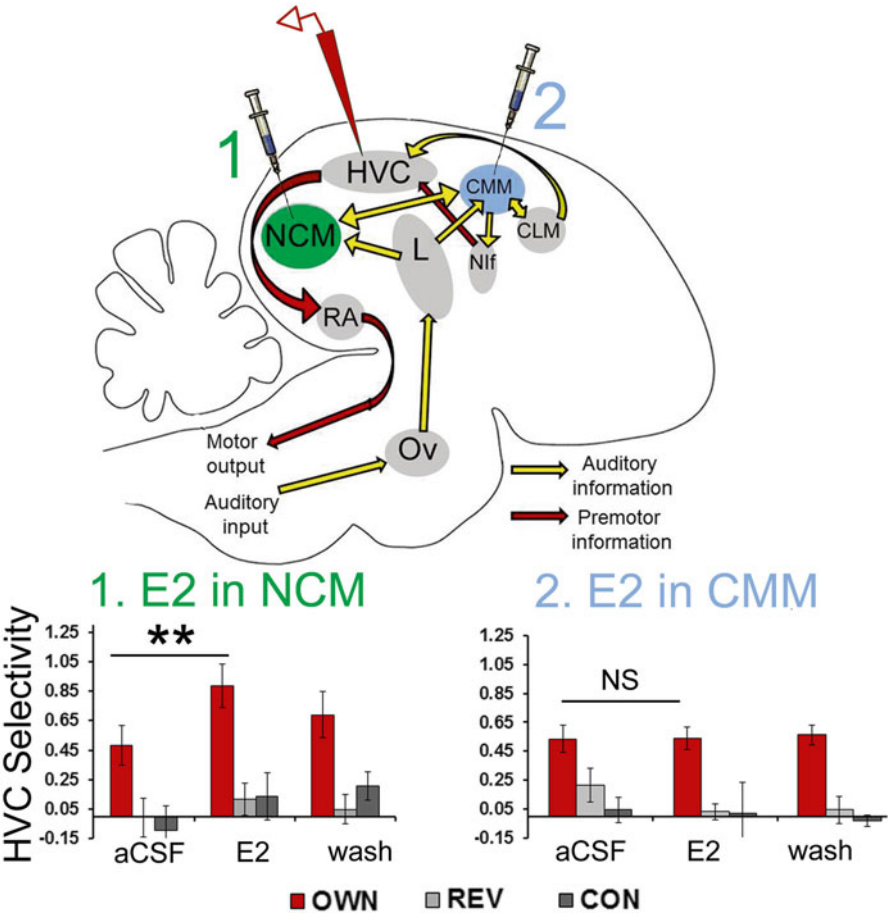

Fig. 4.9 Rapid modulation of NCM (caudomedial nidopallium) by estradiol (E2) also influences downstream stimulus selectivity in the HVC of male zebra finches. *Top*, a schematic of the zebra finch song circuitry from the sagittal view, with the primary auditory network (*yellow arrows*) and the song motor pathway (*red arrows*). Abbreviations as elsewhere in this chapter, with the exception that the caudal mesopallium (CM) is divided between a lateral aspect (CLM) and a medial aspect (CMM) that are interconnected. *Bottom*, a selective neural representation of the bird's own song (OWN) in HVC is enhanced rapidly following delivery of estradiol to NCM (*left*). Identical treatment in the nearby CMM does not mimic this effect (*right*). Other stimuli include reverse OWN (REV), conspecific song (CON), and all stimuli are standardized with a d' score as a ratio of responsiveness to white noise presentation; *aCSF* artificial cerebrospinal fluid; ** $p < 0.01$; *NS* not significant. Adapted from Remage-Healey and Joshi (2012)

4.6 Summary

This chapter considers the role of estrogens in auditory function in birds. Songbirds rely on auditory communication for most major life events, such as territorial defense and mate choice. It is now clear that estrogens regulate neural processing throughout

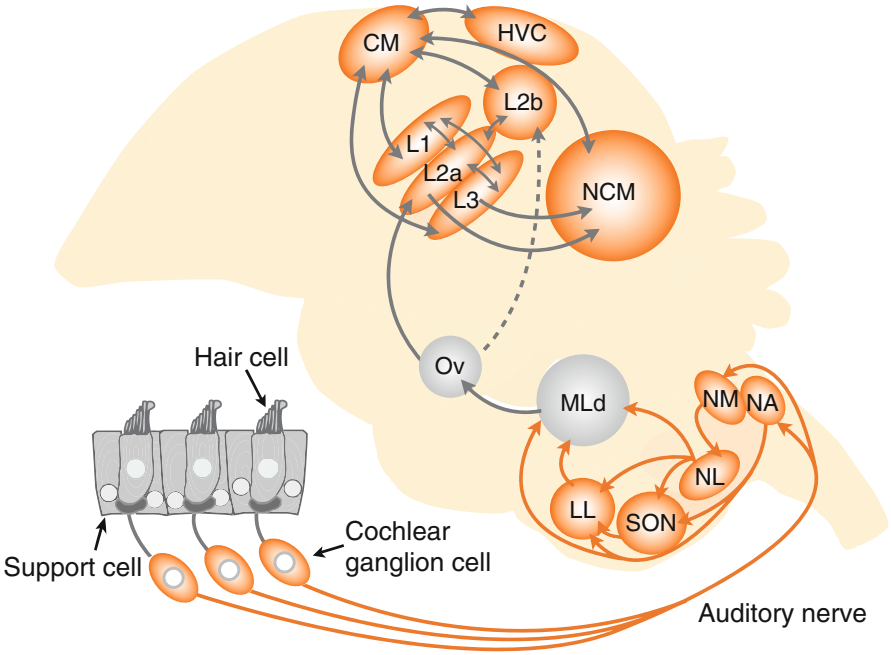

Fig. 4.10 In contrast to the relatively restricted expression of estrogen receptors (Fig. 4.1) and aromatase (Fig. 4.2) in the songbird auditory pathway, estrogenic effects on sound-evoked physiology have been found in nearly every site along the auditory neuraxis (*orange*). Note that effects in the brainstem are supported by ABR findings, but single unit recordings are needed to establish the precise effects within each individual nucleus. Abbreviations and plot conventions as in Fig. 4.1. Adapted from Caras (2013)

the songbird auditory pathway (Fig. 4.10). Both gonadal and local brain-derived estrogens can modulate songbird hearing, and both can have immediate and long-lasting effects. Moreover, many of these estrogenic effects are observed in both males and females, highlighting a fundamental mechanism for auditory modulation that is not strictly tied to ovarian reproductive function. The implications and new directions of this work include the following five themes. First, it is not yet known whether acute (rapid) estrogenic effects occur outside of the songbird NCM, including other important auditory regions such as Field L or CM. Similarly, the seasonal dependence of acute estrogenic effects in NCM are also unknown, in contrast to the evidence presented here for key auditory and motor regions. Third, the source material highlighted in this chapter has focused on either the sensory periphery or the primary thalamo-recipient forebrain region Field L and its downstream targets. The relevant intervening auditory structures in the midbrain and/or thalamus may also exhibit estrogenic sensitivity at either seasonal or rapid timescales, similar to estrogenic effects in other vertebrates, such as anurans (Arch and Narins 2009; Wilczynski and Burmeister, Chap. 3). Fourth, the comparative aspects of this line of research are relevant to understanding its broader significance and applicability, and

the advent of a highly clarified avian molecular phylogeny (Zhang et al. 2014) can help guide future comparative studies of estrogen modulation of songbird audition across species. Lastly, the actions of estrogens on the coding properties of, and immediate early gene cascades within, auditory neurons are consistent with non-classical mechanisms. Further clarification of these mechanisms in songbirds is needed.

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