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NEUROESTROGEN SIGNALING IN THE SONGBIRD AUDITORY CORTEX PROPAGATES INTO A SENSORIMOTOR NETWORK VIA AN 'INTERFACE' NUCLEUS

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Abstract—Neuromodulators rapidly alter activity of neural circuits and can therefore shape higher order functions, such as sensorimotor integration. Increasing evidence suggests that brain-derived estrogens, such as 17-β-estradiol, can act rapidly to modulate sensory processing. However, less is known about how rapid estrogen signaling can impact downstream circuits. Past studies have demonstrated that estradiol levels increase within the songbird auditory cortex (the caudomedial nidopallium, NCM) during social interactions. Local estradiol signaling enhances the auditory-evoked firing rate of neurons in NCM to a variety of stimuli, while also enhancing the selectivity of auditory-evoked responses of neurons in a downstream sensorimotor brain region, HVC (proper name). Since these two brain regions are not directly connected, we employed dual extracellular recordings in HVC and the upstream nucleus interfacialis of the nidopallium (NIf) during manipulations of estradiol within NCM to better understand the pathway by which estradiol signaling propagates to downstream circuits. NIf has direct input into HVC, passing auditory information into the vocal motor output pathway, and is a possible source of the neural selectivity within HVC. Here, during acute estradiol administration in NCM, NIf neurons showed increases in baseline firing rates and auditory-evoked firing rates to all stimuli. Furthermore, when estradiol synthesis was blocked in NCM, we observed simultaneous decreases in the selectivity of NIf and HVC neurons. These effects were not due to direct estradiol actions because NIf has little to no capability for local estrogen synthesis or estrogen receptors, and these effects were specific to NIf because other neurons immediately surrounding NIf did not show these changes. Our results demonstrate that transsynaptic, rapid fluctuations in neuroestrogens are transmitted into NIf and subsequently HVC, both regions important for sensorimotor integration. Overall, these findings support the hypothesis that acute neurosteroid actions can propagate within and between neural circuits to modulate their functional connectivity.

Key words: estrogen, songbird, aromatase, nucleus interfacialis, HVC, nidopallium.

INTRODUCTION

Neuromodulators quickly alter the activity of neural circuits (Bargmann, 2012). For example, neuromodulators, such as norepinephrine and acetylcholine, have been implicated in state-dependent changes in activity, altering sensory processing, motor output, and sensorimotor integration during changes in wakefulness and attention (Wenk, 1997; Berridge and Waterhouse, 2003; Aston-Jones and Cohen, 2005). Recently, estradiol has been implicated as a neuromodulator in sensory circuits in addition to its primary role as a reproductive hormone (Balthazart and Ball, 2006; Cherian et al., 2014). However, the mechanism by which rapid estrogen signaling within sensory processing brain regions is transmitted to other brain regions is unclear.

Like classic neuromodulators, estradiol can rapidly (secs to mins) modulate neural activity (Balthazart and Ball, 2006; Woolley, 2007; Roepke et al., 2011; Meitzen et al., 2012). Rapid, local changes in estradiol occur within brain regions that express the enzyme aromatase, which converts testosterone into estradiol. Aromatase-positive neurons are present in a variety of brain regions in vertebrates, including the human temporal cortex (Cornil et al., 2006; Forlano et al., 2006; Azcoitia et al., 2011; Cohen and Wade, 2011). As in humans, songbirds have populations of aromatase-positive neurons in some pallial regions, including the caudomedial nidopallium (NCM), a higher order sensory processing brain region (Saldanha et al., 2000; Fusani and Gahr, 2006). Microdialysis within the NCM of male and female songbirds has demonstrated that estradiol increases when songbirds hear songs and during social interactions (Remage-Healey et al., 2008, 2012). Acute infusions of fadrozole (FAD), which blocks aromatase and suppresses estradiol, disrupt both auditory processing and song preference behaviors (Tremere et al., 2009; Remage-Healey et al., 2010; Tremere and Pinaud, 2011). However, how neural circuits and pathways are modulated by neuroestrogens to support auditory processing and preference behaviors is still relatively unclear.

Because of their discrete, well-characterized pathways involved in auditory processing and vocal motor output and the known connections between these pathways (Fig. 1), songbirds have become an excellent model for

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Abbreviations: aCSF, Artificial cerebrospinal fluid; ANOVAs, analyses of variance; BOS, bird's own song; CON, conspecific; FAD, fadrozole; MANOVAs, multivariate analyses of variance; NCM, caudomedial nidopallium; NIf, nidopallium; REV, reverse; RS, response strength; WN, white noise.

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asking questions regarding how neuromodulators may be involved in sensory processing. Like the auditory system in mammals, there are thalamo-cortical projections from nucleus ovoidalis to a primary cortical region, the Field L complex (Vates et al., 1996; Theunissen et al., 2008). Parts of the Field L complex project to the NCM and the caudal mesopallium, which are distinct, but reciprocally connected secondary cortical regions (Vates et al., 1996; Gentner, 2008). NCM indirectly connects to the vocal motor pathway through the nucleus interfacialis of the nidopallium (NIf). NIf receives projections from the caudal mesopallium as well as other input from secondary thalamic projections, which are thought to relay information regarding breathing during singing (Bauer et al., 2008; Akutagawa and Konishi, 2010; Lewandowski et al., 2013). NIf provides auditory information directly to HVC (proper name), which is a key nucleus within the vocal motor pathway (Nottebohm et al., 1976; Fortune and Margoliash, 1995; Bottjer et al., 2000). In addition to auditory-evoked activity, NIf and HVC show singing-related (motor) activity (Yu and Margoliash, 1996), indicating that they each have key roles in sensorimotor integration.

Tract-tracing studies, delineating the connections between auditory processing and vocal motor pathways, in concert with electrophysiological studies testing the connectivity of these pathways have together begun to shed light on how neuromodulation can alter functional connectivity of the songbird brain. While not the only projection to HVC, NIf has been shown to be the primary source of auditory input into HVC, since inactivation of NIf can greatly reduce auditory-evoked electrophysiological activity HVC (Coleman and Mooney, 2004; Cardin and Schmidt, 2004a; Lewandowski et al., 2013). Furthermore, inactivation of upstream auditory processing regions can reduce auditory-evoked electrophysiological activity in both NIf and HVC (Bauer et al., 2008). Although it has been shown that norepinephrine and acetylcholine act directly in HVC (Dave et al., 1998; Shea and Margoliash, 2003; Shea et al., 2010), upstream regions, such as NIf, are also responsive to varying behavioral states and modulators (Cardin and Schmidt, 2004b). Therefore, neuromodulators in upstream brain regions, including NCM and NIf, are likely key to changes in the activity of HVC neurons.

Aromatase-containing neurons are present within NCM, which is upstream of NIf and HVC, and very few aromatase-containing cells are found in HVC and NIf (Saldanha et al., 2000; Fusani and Gahr, 2006). Changes in estradiol within NCM enhance electrophysiological responses to many types of auditory stimuli within NCM, including a bird's own song (BOS), other male zebra finch's songs, and even white noise (WN) (Tremere et al., 2009; Remage-Healey and Joshi, 2012). While it is thought that selective neural responses to BOS gradually emerge along the input pathways into HVC (Janata and Margoliash, 1999; Bauer et al., 2008), local increases in estradiol within NCM result in enhanced neural selectivity downstream in HVC (Remage-Healey and Joshi, 2012). One of the goals of the current study was to iden-

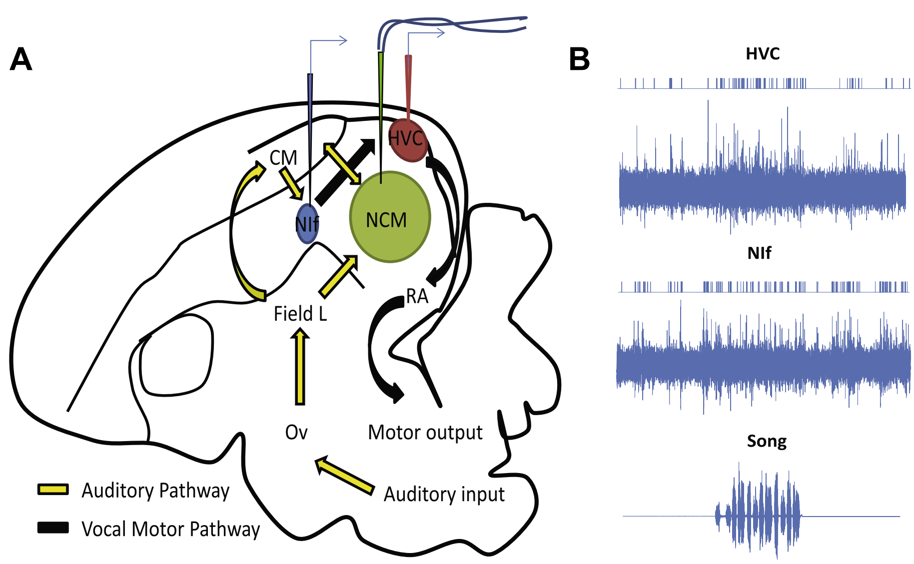

Fig. 1. (A) Schematic of a sagittal view of the auditory and vocal motor pathways of the male zebra finch brain, showing the placements of a retrodialysis probe and electrodes during retrodialysis and dual electrophysiological recording. The green triangle depicts the presence of a retrodialysis probe within the caudomedial nidopallium (NCM), which is a higher order auditory region that contains aromatase-positive neurons. The blue triangle represents the presence of an extracellular electrode into the nucleus interfacialis of the nidopallium (NIf), a region connecting auditory and vocal motor regions, and the red triangle represents the presence of an extracellular electrode in HVC (proper name), a vocal motor region critical for singing behavior in male songbirds. Other abbreviations: the nucleus ovoidalis (Ov), Field L complex (Field L), caudal mesopallium (CM), and robust nucleus of the arcopallium (RA). (B) Exemplar of simultaneous electrophysiological activity in NIf and HVC. Supra-threshold activity is shown in raster form above the multi-unit electrophysiological activity in HVC and NIf. Supra-threshold activity was used to measure firing rate, *Z* score, and *d′* REV (for more detail see the Experimental procedures). Song, as shown as a waveform, elicits auditory-evoked activity above baseline activity simultaneously in NIf and HVC. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)