BRAIN RESEARCH 1171 (2007) 93–103

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Estradiol modulates brainstem catecholaminergic cell groups and projections to the auditory forebrain in a female songbird

Meredith M. LeBlanc[a], *Christopher T. Goode*[a], *Elizabeth A. MacDougall-Shackleton*[b], *Donna L. Maney*[a,*]

[a]*Department of Psychology, 532 Kilgo Circle, Emory University, Atlanta, GA 30322, USA*
[b]*Department of Biology, University of Western Ontario, London, Ontario, Canada*

ARTICLE INFO

Article history:
Accepted 19 June 2007
Available online 27 July 2007

Keywords:
Estrogen
Auditory
Norepinephrine
Dopamine
Song
Mate choice

ABSTRACT

In songbirds, hearing conspecific song induces robust expression of the immediate early gene *zenk* in the auditory forebrain. This genomic response to song is well characterized in males and females of many species, and is highly selective for behaviorally relevant song. In white-throated sparrows, the selectivity of the *zenk* response requires breeding levels of estradiol; we previously showed that in non-breeding females with low levels of plasma estradiol, the *zenk* response to hearing song is no different than the response to hearing frequency-matched tones. Here, we investigated the role of brainstem catecholaminergic cells groups, which project to the forebrain, in estradiol-dependent selectivity. First, we hypothesized that estradiol treatment affects catecholaminergic innervation of the auditory forebrain as well as its possible sources in the brainstem. Immunohistochemical staining of tyrosine hydroxylase revealed that estradiol treatment significantly increased the density of catecholaminergic innervation of the auditory forebrain as well as the number of catecholaminergic cells in the locus coeruleus (A6) and the ventral tegmental area (A10), both of which are known to contain estrogen receptors in songbirds. Second, we hypothesized that during song perception, catecholaminergic cell groups of the brainstem actively participate in auditory selectivity via estrogen-dependent changes in activity. We found that hearing songs did not induce the expression of *zenk*, a putative marker of activity, within catecholaminergic neurons in any of the cell groups quantified. Together, our results suggest that estradiol induces changes in brainstem catecholaminergic cell groups that may play a neuromodulatory role in behavioral and auditory selectivity.

1. Introduction

Lengthening days in the early spring mark the beginning of a chain of events that affect hormones and behavior in many species. Even a small change in photoperiod can trigger shifts in the hormonal state of songbirds such as white-throated sparrows (*Zonotrichia albicollis*), causing their gonads to grow (Wolfson, 1958; Shank, 1959). The resulting marked change in gonadal steroids during this time supports the development of seasonally appropriate reproductive behavior. For example, in female songbirds, behavioral responses to male song change dramatically when estradiol (E2) levels rise. During the breeding season, females of many species respond to audio recordings of male song with a stereotyped behavior known as

* *Corresponding author*. Fax: +1 404 727 0372.
E-mail address: dmaney@emory.edu (D.L. Maney).

0006-8993/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2007.06.086

copulation solicitation display (CSD); during the non-breeding season, however, females do not perform CSDs even when presented with song that in the breeding season would be highly stimulatory (Kern and King, 1972; Moore, 1983). This robust change in behavioral responses to sociosexual auditory stimuli suggests that E2 may act within the auditory system to affect the processing of auditory signals.

Song-induced auditory responses in the brain are often studied by quantifying the expression of the immediate early gene *zenk* (*zif-268*, *egr-1*, *ngfi-a*, and *krox24*; Mello et al., 1992) or its protein product (ZENK; Mello and Ribeiro, 1998). This "genomic response" to song is well-characterized in males and females of many species, including zebra finches (*Taeniopygia guttata*; Bailey et al., 2002; Mello and Clayton, 1994; Mello et al., 1992; Stripling et al., 2001), canaries (*Serinus canaria*; Leitner et al., 2005; Ribeiro et al., 1998; Terleph et al., 2006), and European starlings (*Sturnus vulgaris*; Gentner et al., 2001; Sockman et al., 2002). The magnitude of this response in the caudomedial nidopallium (NCM), a region of the auditory forebrain, is greater in response to song than to synthetic tones, and greater to conspecific than heterospecific song (Mello and Clayton, 1994; Stripling et al., 2001). Social factors known to affect the magnitude of behavioral responses, such as song complexity, dialect, or familiarity to the listener, also affect the genomic response (Gentner et al., 2001; Leitner et al., 2005; Maney et al., 2003; Sockman et al., 2002; Terpstra et al., 2006). Thus, the magnitude of this response relates to the behavioral relevance of the stimulus.

Just as E2 appears to alter the behavioral relevance of song, it also affects song-induced genomic responses in the auditory forebrain. We previously showed that the selectivity of the genomic response in NCM requires breeding levels of E2. In non-breeding female white-throated sparrows with low levels of plasma E2, the genomic response to hearing songs is not distinguishable from the response to hearing frequency-matched tones (Maney et al., 2006). The plastic nature of auditory selectivity suggests that E2 modulates auditory pathways and processing centers to promote recognition of and attention to conspecific song during the breeding season.

Increased auditory selectivity could be related to one or more cognitive processes that depend on catecholamines (CAs). CAs, particularly norepinephrine from the locus coeruleus, are widely known to shape the response properties of sensory networks to alter selectivity (see Hurley et al., 2004 for review) and may play a role in selective attention (see Aston-Jones and Cohen, 2005, for review). In songbirds, CA projections to the forebrain have been hypothesized to affect the auditory processing of as well as behavioral responses to behaviorally relevant social signals such as song (e.g., Appeltants et al., 2002a,b; 2005; Bharati and Goodson, 2006; Cardin and Schmidt, 2004; Maney and Ball, 2003; Riters and Pawlisch, 2007). In female canaries, noradrenergic denervation of the forebrain causes a reduction in CSD behavior along with an apparent deficit in selective attention to sexually stimulating song (Appeltants et al., 2002b). If CA fibers and their sources are sensitive to gonadal steroids, they may mediate seasonal changes in selective attention and auditory responses to these signals. A large literature indicates that in mammals, CA neurons are in fact targets of E2. E2 treatment increases mRNA for tyrosine hydroxylase (TH) and dopamine beta-hydroxylase (DBH), rate-limiting enzymes in the synthesis of CAs, in brainstem CA cell groups (Pau et al., 2000; Serova et al., 2002, 2004). E2 may also affect developing CA cells; E2 treatment promotes the expression of TH mRNA as well as neurite branching in cultured embryonic midbrain cells (Ivanova and Beyer, 2003; Kuppers et al., 2000). Kritzer and Kohama (1998, 1999) reported that in rhesus monkeys, TH and DBH immunoreactivity (IR) in the forebrain is depleted by ovariectomy and restored by ovarian hormone replacement. In songbirds, forebrain CA turnover as well as adrenergic receptor density are modulated seasonally by gonadal steroids (Barclay and Harding, 1990; Riters et al., 2002). CAs therefore represent an excellent candidate system for mediating seasonal changes in auditory and behavioral responses to sociosexual stimuli.

In the present study, we looked for evidence that the E2-induced plasticity in the auditory forebrain described by Maney et al. (2006) is mediated by CAs. Our first hypothesis was that E2 alters the CA innervation of the auditory forebrain as well as the possible sources of this innervation in the brainstem. To test this hypothesis, we measured the effects of E2 treatment on the density of TH-IR innervation of NCM as well as the number of TH-IR cells in brainstem CA cell groups (Fig. 1). Although the exact origin of CA fibers in NCM (Fig. 2) is currently unknown, tract tracing studies have demonstrated that CA cell groups A6, A9, A10, and A11 project to areas of the canary forebrain involved in song learning and production (Appeltants et al., 2000, 2002a). Thus, it is plausible that CA innervation of NCM originates in one or more of these regions. We predicted E2-dependent changes in TH-IR both in NCM and in these CA brainstem cell groups.

Our second hypothesis was that these CA cells directly regulate forebrain selectivity by altering their activity, and therefore CA synaptic activity in the forebrain, during song perception. To test this hypothesis, we quantified transcription activity, a putative measure of depolarization activity (see Mello et al., 2004), by counting the number of TH-IR cells that were immunopositive for ZENK protein in each CA cell group of the brainstem after the birds listened to song or tone stimuli. We predicted that the *zenk* response in CA cells would parallel and therefore possibly contribute toward E2-dependent selectivity in NCM (Maney et al., 2006).

2. Results

2.1. Behavioral analysis

Only E2-treated birds who heard songs performed CSDs during the stimulus presentation, which confirmed that the implants raised plasma E2 levels and that the tones were not interpreted as songs (Maney et al., 2006). Within this group ($n=6$), there were no significant correlations between CSD behavior and any of the neural variables we quantified, demonstrating that the variation in these variables was not completely explained by variation in CSD behavior.

2.2. Effects of E2 treatment on TH immunoreactivity

A MANOVA revealed a significant effect of treatment on TH immunoreactivity in our regions of interest ($F_{1,21}=3.042$,