BRAIN RESEARCH 1527 (2013) 29–39

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/brainres

Research Report

Curvilinear relationships between mu-opioid receptor labeling and undirected song in male European starlings (*Sturnus vulgaris*)

*Cynthia A. Kelm-Nelson**, *Lauren V. Riters*

Department of Zoology, University of Wisconsin-Madison, 428 Birge Hall, 430 Lincoln Drive, Madison, WI 53706, USA

ARTICLE INFO

Article history:
Accepted 6 June 2013
Available online 15 June 2013

Keywords:
Opioids
Vocal communication
Reward
Birdsong
Motivation
Mu-opioid receptor

ABSTRACT

Female-directed communication in male songbirds has been reasonably well studied; yet, relatively little is known about communication in other social contexts. Songbirds also produce song that is not clearly directed towards another individual (undirected song) when alone or in flocks. Although the precise functions of undirected song may differ across species, this type of song is considered important for flock maintenance, song learning or practice. Past studies show that undirected song is tightly coupled to analgesia and positive affective state, which are both mediated by opioid activity. Furthermore, labeling for the opioid met-enkephalin in the medial preoptic nucleus (POM) correlates positively with undirected song production. We propose that undirected song is facilitated and maintained by opioid receptor activity in the POM and other brain regions involved in affective state, analgesia, and social behavior. To provide insight into this hypothesis, we used immunohistochemistry to examine relationships between undirected song and mu-opioid receptors in male starlings. Polynomial regression analyses revealed significant inverted-U shaped relationships between measures of undirected song and mu-opioid receptor labeling in the POM, medial bed nucleus of the stria terminalis (BSTm), and periaqueductal gray (PAG). These results suggest that low rates of undirected song may stimulate and/or be maintained by mu-opioid receptor activity; however, it may be that sustained levels of mu-opioid receptor activity associated with high rates of undirected song cause mu-opioid receptor down-regulation. The results indicate that mu-opioid receptor activity in POM, BSTm, and PAG may underlie previous links identified between undirected song, analgesia, and affective state.

1. Introduction

Across the animal kingdom, vocal communication is critical for successful social interactions. Although female-directed courtship vocalizations produced by males have been relatively well-studied, less is known about communication in other social contexts. In some songbirds, song is observed at high rates in large affiliative flocks (Eens, 1997; Riters et al., 2000; Zann, 1996). Although facilitated by social contact (Jesse and Riebel, 2012), this type of song does not (at least to a human

---

**Corresponding author*. Fax: +1 608 265 6320.
E-mail addresses: cakelm@wisc.edu (C.A. Kelm-Nelson), LVRiters@wisc.edu (L.V. Riters).

0006-8993/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2013.06.010

observer) appear to be directed towards a specific individual (Dunn and Zann, 1996a; Hessler and Doupe, 1999; Jarvis et al., 1998; Zann, 1996). In studies of zebra finches, song that is not clearly directed towards conspecifics is referred to as “undirected”, and we will use the term here. Although the precise function of undirected song may differ across species, this type of song is proposed to play a role in flock maintenance (e.g., starlings (Hausberger et al., 1995)), song learning or practice (e.g., zebra finches and starlings (Eens, 1997; Kao et al., 2005)), and may be used by females for future mating decisions (e.g., zebra finches (Dunn and Zann, 1996b; Holveck and Riebel, 2007,2010)).

Multiple lines of evidence link opioids to undirected birdsong. First, positive correlations were identified between labeling for the opioid protein met-enkephalin (mENK) in the medial preoptic nucleus (POM) and undirected song produced by male European starlings (with a similar trend identified for the ventral tegmental area (VTA); $p=0.06$) (Riters et al., 2005). Opioids in the medial preoptic area induce both analgesia and reward/positive affect, at least in rats (Ãgmo and Gomez, 1991; Tseng et al., 1980; Tseng and Wang, 1992), and recent data link undirected song to both opioid-mediated analgesia and positive affect. Specifically, analgesia measures correlated positively with male starling undirected song rates (Kelm-Nelson et al., 2012), and both male starlings and zebra finches singing high rates of undirected song developed a conditioned place preference for a chamber previously paired with undirected singing behavior (Riters and Stevenson, 2012). These results link undirected song to a positive (or at least a less negative) affective state, which we hypothesize may, in part, be mediated by opioids (Riters, 2012).

The present study was designed to further examine links between opioids and undirected song in male starlings. During fall and winter months (i.e., the non-breeding season) when testosterone concentrations are low, male starlings do not sing to attract mates or defend nesting territories (Riters et al., 2000). However, they sing at high rates as part of affiliative overwintering flocks (Eens, 1997; Riters et al., 2000). Song in these flocks is proposed to function primarily to maintain flock cohesion (Hausberger et al., 1995) and may facilitate song learning in these open ended learners (Chaiken et al., 1994). Although the functions and mechanisms underlying this form of song may differ from that described in zebra finches (Heimovics and Riters, 2007), song in these flocks is not (at least to a human observer) clearly directed towards another individual. We thus consider non-breeding season starling song to be a form of undirected, affiliative communication.

In the present study, we used immunohistochemistry to examine links between undirected song in male starlings with low testosterone singing in flocks and mu-opioid receptor labeling. If previously reported links between undirected song, analgesia and reward are mediated by opioids, then we predict that undirected song production will be linked to mu-opioid receptor labeling in regions in which opioids have been found to induce analgesia and/or reward, which include the POM, the ventral tegmental area (VTA), periaqueductal gray (PAG), bed nucleus of the stria terminalis (BSTm), lateral septum (SL) and periventricular nucleus (PVN) (analgesia: (Altier and Stewart, 1997; Hashimoto et al., 1991; Tseng et al., 1980; Tseng and Wang, 1992; Yaksh et al., 1976) and reward: (Ãgmo and Gomez, 1991; Bozarth and Wise, 1981; Jackson, 2009; Le Merrer et al., 2009; Olmstead and Franklin, 1997; Phillips and LePiane, 1982).

2. Results

2.1. Behavior and testosterone concentrations

Eighteen males were used for the behavioral analysis. Male testes were regressed and testosterone concentrations were in the range typical of the non-breeding season. T concentrations were below detectable levels for all but four males (for males with detectable levels $n=4$, mean=144.85 pg/mL, SD=207.65). For reference, the average T measure for breeding males is approximately 2930.00 pg/mL (Dawson, 1983). Furthermore, individuals did not show behaviors indicative of birds during the breeding season (i.e., they did not collect nest material, wing wave, or displace other males from nesting sites). Taken together, these results indicate that the hormone and photoperiod states were similar to wild birds in the *non*-breeding season when male starlings sing high rates of undirected but not female-directed song.

2.2. Area covered by mu-opioid receptor labeling and total song

We ran linear regression analyses to examine relationships between the area for each nucleus covered by mu-opioid receptor immunolabeling (mean total pixel area) and total song; (untransformed data range for total song, lower limit=0, upper limit=67; median=18.5). In all cases linear regression results were not significant and the data did not fit a linear model ($p>0.50$; Table 1). Therefore, we ran non-linear, higher-order curve polynomial regression analyses. Specifically, in separate polynomial regression analyses the mean total pixel area for a brain region was entered as the dependent variable and the total song was entered as the

Table 1 – Non-significant linear regression results of mu-opioid receptor label (pixel area) and total song.

Brain region	$F_{(df)}$	R^2	Adj. R^2	Standard error of the estimate	p-value
POM	$0.0000139_{(1,\ 15)}$	0.0000009	−0.07	0.770	0.99
PAG	$0.05_{(1,\ 14)}$	0.001	−0.08	23381.100	0.90
BSTm	$0.16_{(1,\ 13)}$	0.01	−0.06	0.625	0.69
VTA	$0.44_{(1,\ 13)}$	0.03	−0.04	9515.550	0.52
PVN	$0.30_{(1,\ 16)}$	0.02	−0.04	1.349	0.59
SL	$0.31_{(1,\ 14)}$	0.02	−0.05	2735.632	0.59