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Research report

Prodynorphin and kappa opioid receptor mRNA expression in the brain relates to social status and behavior in male European starlings

Lauren V. Riters*, Melissa A. Cordes[1], Sharon A. Stevenson

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

HIGHLIGHTS

- Dominant male starlings had lower OPRK1 mRNA in VTA and mPOA than subordinates.
- OPRK1 in VTA related negatively to sexual/agonistic behaviors and gonad volume.
- PDYN in mPOA related negatively to nesting behaviors and positively to gonad volume.
- Area X PDYN related positively to singing; OPRK1 related positively to gonad volume.
- Kappa receptors/dynorphin relate to individual differences in sociosexual behaviors.

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ABSTRACT

Numerous animal species display behavioral changes in response to changes in social status or territory possession. For example, in male European starlings only males that acquire nesting sites display high rates of sexual and agonistic behavior. Past studies show that mu and delta opioid receptors regulate behaviors associated with social ascension or defeat. Opioids also act at kappa receptors, with dynorphin binding with the highest affinity; however, the role of these opioids in social behavior has not been well studied. We observed flocks of male starlings during the breeding season and ran quantitative real-time polymerase chain reaction (qPCR) to measure expression of kappa opioid receptors (OPRK1) and prodynorphin (PDYN) in brain regions involved in social behavior and motivation (ventral tegmental area [VTA], medial preoptic nucleus [mPOA]) and vocal behavior (Area X). Males with nesting territories displayed more sexual/agonistic behavior than males without nesting territories. They also had lower OPRK1 expression in VTA and mPOA. OPRK1 expression in VTA correlated negatively with sexual/agonistic behaviors, consistent with past studies showing kappa receptors in VTA to inhibit sociosexual behaviors. PDYN in mPOA correlated negatively with a measure of nesting behavior that may also reflect sexual motivation. PDYN in Area X related positively to song. Distinct patterns of OPRK1 and PDYN expression in VTA, mPOA, and Area X related to gonad volume, suggesting that breeding condition may modify (or be modified by) OPRK1 and PDYN expression. Studies are now needed to further characterize the role of OPRK1 and PDYN in status-appropriate social behaviors.

1. Introduction

Many species dramatically alter vocal, sexual, and agonistic behaviors in response to changes in social status or territory acquisition (e.g. [1–6]); however, the neural mechanisms underlying these status-appropriate changes are not well characterized. Opioid neuropeptides and receptors are distributed widely in the brain and are well-studied for roles in addiction, learning and memory, feeding, analgesia, and reward (e.g. [7]). Endogenous opioid systems also regulate social behaviors, including behaviors that change in association with social ascension or defeat (e.g., sexual and agonistic behaviors), suggesting opioids as candidate modulators of status-appropriate behavior.

Most research on the role of opioids in social and sexual behavior is on mu and delta opioid receptors and their high-affinity ligands, which include beta-endorphin and enkephalin opioids.

Abbreviations: mPOA, medial preoptic nucleus; OPRK1, kappa opioid receptor; PDYN, prodynorphin; qPCR, quantitative real-time polymerase chain reaction; VTA, ventral tegmental area.

* Corresponding author.
E-mail address: LVRiters@wisc.edu (L.V. Riters).
[1] Present address: Department of Biological Sciences, Bethel University, 3900 Bethel Drive, Saint Paul, MN 55112, USA.

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Multiple studies indicate that these receptors and ligands act region-specifically to reward and reinforce sociosexual behaviors (e.g. [8–12]). Although less studied, opioids also act at kappa receptors, with the opioid dynorphin binding with the highest affinity to these receptors [13,14]. In contrast to mu and delta receptors and their ligands, dynorphin and the stimulation of kappa receptors induces dysphoria and place aversion (e.g. [15–18]). The kappa/dynorphin system generally inhibits sexual [19,20], prosocial [18,21], and agonistic behaviors [22], including vocal behaviors associated with positive social contact in rats [23]. This system intensifies submissive behavior and stress after social defeat in mice [22,24] and is less sensitive in mice that repeatedly win social interactions [25,26]. This suggests that the kappa/dynorphin system may be important for adjusting behavior to match changes in social status.

Male European starlings, *Sturnus vulgaris*, are seasonally breeding songbirds that provide an excellent study species in which to examine the neural bases of status-appropriate behavior. At the onset of the breeding season in spring, testosterone concentrations rise and males compete for nesting territories. Males that fail to acquire a nesting territory tend to avoid other males and appear to ignore females. In contrast, males that win a territory sing high rates of courtship song to females, gather green nesting material, and more often displace other males [4,6,27–30]. Because males are competing over a limited resource that only some are able to successfully defend, we consider males that successfully acquire and defend nest boxes to socially dominate males that fail to do so, as in prior studies of starlings (e.g. [29]).

Multiple studies implicate brain regions that are centrally involved in motivation in the regulation of status-appropriate behaviors. These areas include the ventral tegmental area (VTA) and the medial preoptic nucleus (mPOA) [31–37]. The mPOA is larger, and both the mPOA and VTA express more cFOS in male starlings with nesting territories compared to those without nesting territories [6,38]. Mu opioid receptors and enkephalin measures are also higher in males without compared to those with nest sites [28,39]; however the kappa/dynorphin system has not been examined previously.

In male songbirds, including starlings, dopamine, dopamine metabolites and activity in dopaminergic neurons in VTA (that project to the striatum) relate positively to production of sexually-motivated song [40–45]. In rats, kappa receptors on dopamine neurons in VTA inhibit neuronal firing [46], induce aversion [17,47–49], and block female-directed sexual behaviors [20]. Kappa receptor mRNA levels in VTA are also reduced in male mice that repeatedly win agonistic interactions [26]. These studies lead to the prediction that the kappa/dynorphin system will be down-regulated in the VTA in male starlings that win nesting sites and display high rates of sexually-motivated behavior to females. We made a similar prediction for mPOA based on past work that shows a central role for the mPOA in female-directed song in songbirds [50–52] and that kappa receptor stimulation in the mPOA inhibits male sexual behavior in rats [20]. Finally, in songbirds Area X is a striatal brain region that receives dense projections from VTA as well as the substantia nigra [53–57]. Area X regulates vocal learning and the adjustment of song structure to match specific social contexts [58–62]. Given the function of this region and the fact that it is rich in kappa opioid receptors [63], it is possible that the kappa/dynorphin system in Area X may play a role in adjusting song attributes to match social status.

If the kappa/dynorphin opioid system modifies behavior to match social status, this may be reflected in mRNA expression levels. To test this prediction, we observed flocks of male starlings in outdoor aviaries during the breeding season, collected brains and ran quantitative real-time polymerase chain reaction (qPCR) to measure expression of kappa opioid receptors (OPRK1) and prodynorphin (PDYN) in VTA, mPOA and Area X in males with and without nesting sites.

2. Material and methods

2.1. Animals and housing

Twenty male starlings were used in this study. All birds were trapped in winter 2009–2010 on a local farm in Madison, WI using baited fly-in traps. Each male was banded with stainless steel identification bands and a unique combination of plastic color bands for individual identification. Birds were housed indoors in single sex cages (91 cm × 47 cm × 47 cm; 5 birds/cage) in the University of Wisconsin Department of Zoology animal facility. Birds were exposed to artificial photoperiods of 18 h light (L):6 h dark (D) for 6 weeks, followed by 6 weeks of 8L:16D to induce photosensitivity, a condition in which exposure to a long spring photoperiod will stimulate testosterone production and male sexual behaviors [64]. All procedures and protocols followed the guidelines of the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* and a protocol approved by the University of Wisconsin Institutional Animal Care and Use Committee.

2.2. Behavioral observations

Photosensitive males were placed into outdoor aviaries (2.13 m × 2.4 m × 1.98 m) and exposed to a natural spring photoperiod (approximately 13L:11D). Aviaries contained 4 nest boxes, perches, nesting material, baths, food and water. Initially twenty-five male starlings were randomly placed into the aviaries (5 birds/aviary) and allowed to habituate for 7-days; however, over the course of the study 5 birds were removed for various reasons (e.g., concerns about injury from agonistic interactions) leaving 20 birds. After the habituation period, male behaviors were observed in response to a female stimulus bird for 20 min on 4 consecutive days. Prior to each observation period a single observer placed a handful of green nesting material and a stimulus female into the aviary. Stimulus females consisted of 4 photosensitive females that were housed in standard cages indoors on a photoperiod mimicking the natural outdoor photoperiod. A different stimulus female was used on each test day. During each 20 min observation period, the observer noted the number of times a male 1) approached another male [landed within approx. 4 cm] followed by that male's departure (displacements), 2) waved his wings (courtship behavior), 3) gathered nest material, 4) looked into a nest box, and 5) produced a full song (song rate). The observer also used a stop watch to record the time each male spent singing in secs. Finally, the observer recorded bouts of eating and drinking, with bouts of behavior separated by at least 2 s. Each male was categorized as a nest box owner or non-owner, with owners identified as males that spent a majority time near a nest box opening or were observed entering and exiting the box. Males that acquired nest boxes displaced other males significantly more often than males without nest boxes (comparison of median displacement behavior in males with and without nest boxes; $t_{1,16} = 9.83$, $p = 0.0064$), confirming that these males were socially dominant. After each observation period, the stimulus female was returned to her home cage. The median behavior for each male across the 4 test days was used for analyses.

2.3. Quantitative real-time polymerase chain reaction preparation and analyses

After the last behavioral observation (on day 4), males were rapidly decapitated, trunk blood was collected, and brains were