

Photoperiodic Condition Is Associated with Region-Specific Expression of *GNRH1* mRNA in the Preoptic Area of the Male Starling (*Sturnus vulgaris*)¹

Tyler J. Stevenson,^{2,3} Daniel J. Bernard,⁴ and Gregory F. Ball³

Department of Psychological and Brain Sciences,³ The Johns Hopkins University, Baltimore, Maryland
Department of Pharmacology and Therapeutics,⁴ McGill University, Montreal, Quebec, Canada

ABSTRACT

Many seasonally breeding avian species exhibit marked changes in hypothalamic content of gonadotropin-releasing hormone 1 (GNRH1) protein that are reflective of breeding condition. We recently cloned the *GNRH1* cDNA in European starlings and demonstrated that changes in *GNRH1* mRNA levels occur with a time course similar to what has been observed with GNRH1 protein. However, we did not previously resolve whether these differences were attributable to changes in the number of cells expressing the gene. Herein, we investigated photoperiod-induced changes in the number and distribution of *GNRH1* mRNA-expressing cells in the preoptic area of male starlings. *GNRH1* mRNA-expressing cell number was significantly greater in breeding birds than in nonbreeding birds. Starlings maintained in short nonstimulatory day length (i.e., prebreeding) showed intermediate cell numbers. Detailed analysis of the rostrocaudal and mediolateral distribution revealed that breeding birds had greater numbers of cells expressing *GNRH1* mRNA in the medial intermediate, medio-caudal, and lateral intermediate preoptic area compared with prebreeding and nonbreeding birds. These data demonstrate that photoperiodic changes in reproductive state in starlings are associated with region-specific alterations in the number of cells expressing the *GNRH1* gene. It remains to be determined whether these changes reflect quantitative differences in gene expression among an otherwise stable population of cells or a phenotypic switch in which cells gain or lose the ability to make *GNRH1* mRNA in response to environmental cues.

gonadotropin-releasing hormone, neuroendocrinology, seasonal reproduction

INTRODUCTION

Gonadotropin-releasing hormone 1 (GNRH1) is essential for reproduction in vertebrates [1]. GNRH1 cell populations have been reported in specific brain regions in different bird orders, including galliformes (i.e., chickens and turkey [2–6]) and passeriformes (i.e., songbirds [7–16]). Immunocytochemical studies [2, 4, 5, 9, 17–19] demonstrated that GNRH1 cells are primarily distributed bilaterally along the midline in the

preoptic area (POA), extending caudodorsally to the lateral septum. GNRH1 immunoreactive fibers are present throughout the preoptic septal areas and project ventrocaudally into the median eminence [2, 4, 16, 17]. Immunoreactive fibers also project directly to two circumventricular nuclei: the organum vasculosum of the lamina terminalis (OVL) and the subseptal organ (SSO) [6]. The secretion of GNRH1 from the median eminence stimulates the release of gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) from the pituitary gland [20, 21], ultimately controlling gonadal function.

In seasonally breeding birds, hypothalamic GNRH1 exhibits profound changes in synthesis and secretion that regulate annual changes in reproduction [22–25]. The length of the breeding period varies across species, and in some birds the breeding season is asymmetric with changes in photoperiod [26, 27]. In these cases, annual changes in day length give rise to three physiological states: photosensitive (i.e., prebreeding), photostimulated (i.e., breeding), and photorefractory (i.e., nonbreeding) [23, 27]. Photosensitive birds have the capacity to respond to an increase in photoperiod with a dramatic growth in the gonads and an increase in gonadotropins and sex steroid hormones [23]. We refer to them as “prebreeding” birds in this article. Photostimulated birds have large gonads and marked secretion of reproductive hormones characteristic of “breeding” birds, as we refer to them herein. Finally, photorefractory birds have experienced long day length, which leads to the regression of the reproductive axis and a “nonbreeding” condition. These birds can only be restored to a photosensitive state characteristic of the prebreeding condition if they experience short day length [23].

The European starling (*Sturnus vulgaris*) has been a valuable species for investigating the neural mechanisms controlling seasonal breeding in birds. In short photoperiods contiguous with the winter solstice, brain GNRH1 content is low, and the gonads are in a regressed state [9, 10]. Vernal increases in photoperiod stimulate dramatic increases in *GNRH1* mRNA [28] and protein expression [12, 14–16, 29–32] in the POA. The increase in GNRH1 peptide secretion results in gonadal recrudescence in breeding birds. In many species, prolonged exposure to long day length results in a decline in hypothalamic GNRH1 protein content as measured via radioimmunoassay [13, 33, 34] or immunocytochemistry [9, 10, 15, 34]. The termination of photorefractoriness occurs after exposure to short day length and is characterized by a slight increase in GNRH1 protein content [33, 34]. Indeed, there is significant variation in *GNRH1* gene expression across reproductive states; however, the degree of neuroanatomical specificity that occurs in association with these overall changes in *GNRH1* mRNA expression is as yet unknown. Recently, data have accumulated indicating that the POA in birds is topographically organized based on functional criteria related to the control of reproductive behavior [35]. Whether variation in the GNRH1 system induced by photoperiodic control is

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²Correspondence: Tyler J. Stevenson, Department of Psychological and Brain Sciences, The Johns Hopkins University, Baltimore, MD 21218. FAX: 410 516 4478; e-mail: tsteve13@jhu.edu

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restricted or particularly enhanced in specialized neuroanatomical subregions has not been determined, to our knowledge.

The distribution of *GNRH1* mRNA has been described in few avian species [36–38]. We recently cloned *GNRH1* cDNA in two songbird species: zebra finches (*Taeniopygia guttata*) and starlings [28]. However, we did not determine whether the variation in *GNRH1* expression is the result of changes in the number of cells expressing the mRNA, as is observed with *GNRH1* protein-expressing cells [19]. Herein, we describe the topographical distribution of *GNRH1* mRNA-expressing cells in the POA of male starlings from three different reproductive states. These data provide novel insight into how changes in the reproductive condition are associated with region-specific variation in *GNRH1* mRNA expression in the POA. These findings thus make a significant contribution to our understanding of the neural pathways that mediate the effects of environmental stimuli on the hypothalamo-pituitary-gonadal axis.

MATERIALS AND METHODS

Animals

All birds were treated and handled in accord with institutional, state, and federal animal care guidelines and permits. European starlings were captured using a drop-down V-trap in the early spring of 2008 in Conneautville, PA (41° 45 min north latitude and 80° 22 min west longitude). After capture, starlings were transported to The Johns Hopkins University and were group housed under the natural photoperiod. Food and water were provided ad libitum for the duration of the experiment. The birds and procedures used in the present study are thoroughly described in a previous publication [28]. In brief, a total of 20 male starlings in three reproductive states were used. All birds were caught in late winter and held on short day length (11L:13D) before beginning the experiment. Prebreeding birds ($n = 7$) were exposed to short day length (11L:13D) for the entire duration of the experiment, breeding birds ($n = 6$) were exposed to long day length (16L:8D) for 4 wk, and nonbreeding birds ($n = 7$) were exposed to long day length for 9 wk [28]. All birds were killed by rapid decapitation, and the treatment groups were organized so as to conclude the treatment phase on the same calendar day. Brains were quickly extracted, frozen on dry ice, and placed at -80°C before sectioning for in situ hybridization.

Assessment of Reproductive State

Reproductive condition was determined via testis volume measured as $V = 4/3\pi a^2 b$, where a is half the width, and b is half the length. Blood samples were collected at killing via puncturing of the alar (wing) vein with a 25-gauge needle, and 300–500 μl of blood was collected into heparinized tubes. The blood samples were transferred into microfuge tubes and centrifuged at 8900 rpm for 10 min. The plasma was removed and stored in Eppendorf vials at -20° until assayed for testosterone. The serum was analyzed in duplicate (50 μl) using a commercially available ^{125}I Coat-A-Count kit for total testosterone (Siemens Medical Solutions Diagnostics, Los Angeles, CA). The antiserum is highly specific for testosterone (i.e., 100 pg/ml) and shows negligible cross-reactivity with other steroids, including dihydrotestosterone (<3.5%), 17β -estradiol (<0.01%), and corticosterone (<0.01%). The intraassay coefficient of variation averaged 12%.

In Situ Hybridization

Brains were sectioned on a cryostat at 20- μm thickness and mounted directly onto Superfrost Plus slides (Fisher Scientific, Santa Clara, CA) and stored at -20°C until in situ hybridization. Sections were collected beginning with the tractus septomesencephalicus (TSM) split and continued every fourth section (80 μm apart) until the caudal aspect of the anterior commissure. This sampling method differs from previous immunocytochemistry procedures that consist of thicker tissue sections and prevent precise comparisons of total cell counts using the two approaches. A plasmid-containing starling *GNRH1* cDNA (GenBank accession number FJ178434) corresponding to the decapeptide *GNRH1*-associated peptide and the 3' untranslated region (approximately 350 base pair) was linearized with *SacI* (sense; Promega, Madison, WI) or *NcoI* (antisense; Promega) and purified using GENE-CLEAN (Qbiogene, Vista, CA). ^{35}S -labeled cRNA probes (PerkinElmer Life and Analytical Sciences, Boston, MA) were transcribed by SP6 (antisense) or T7 (sense) RNA

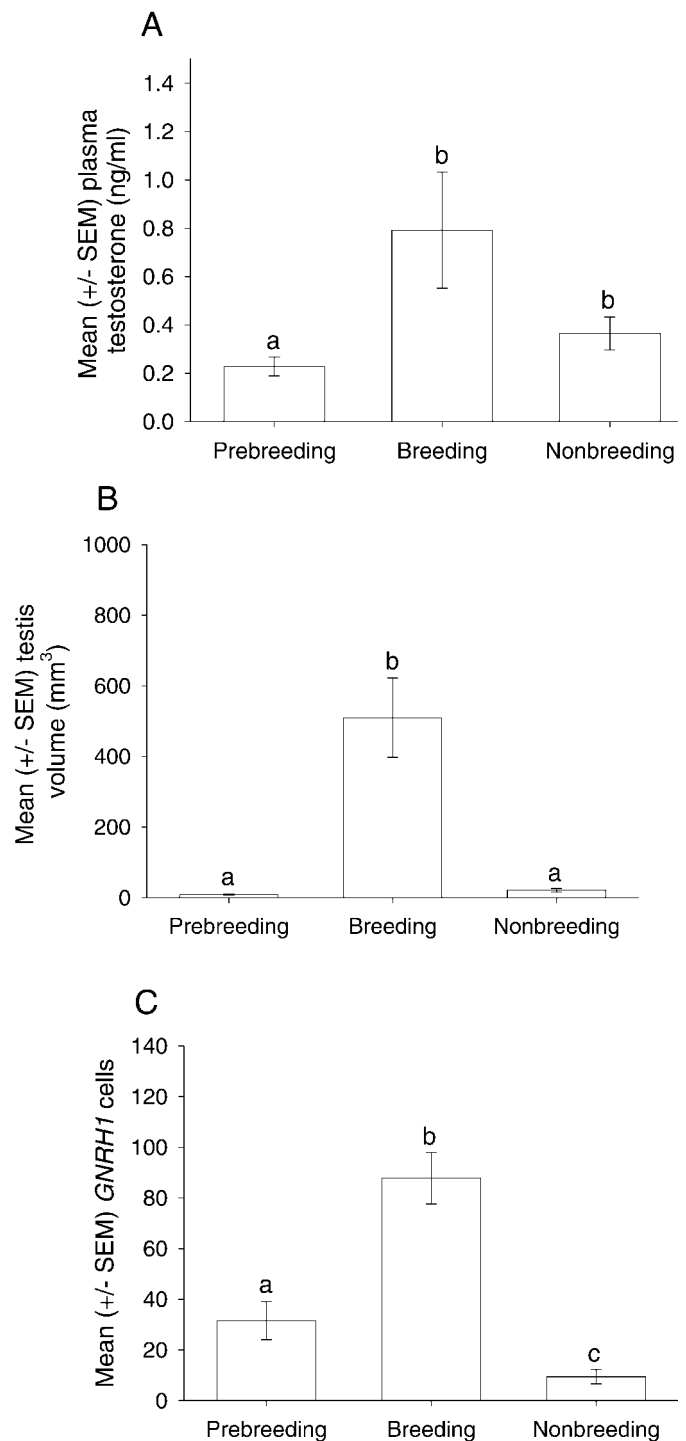
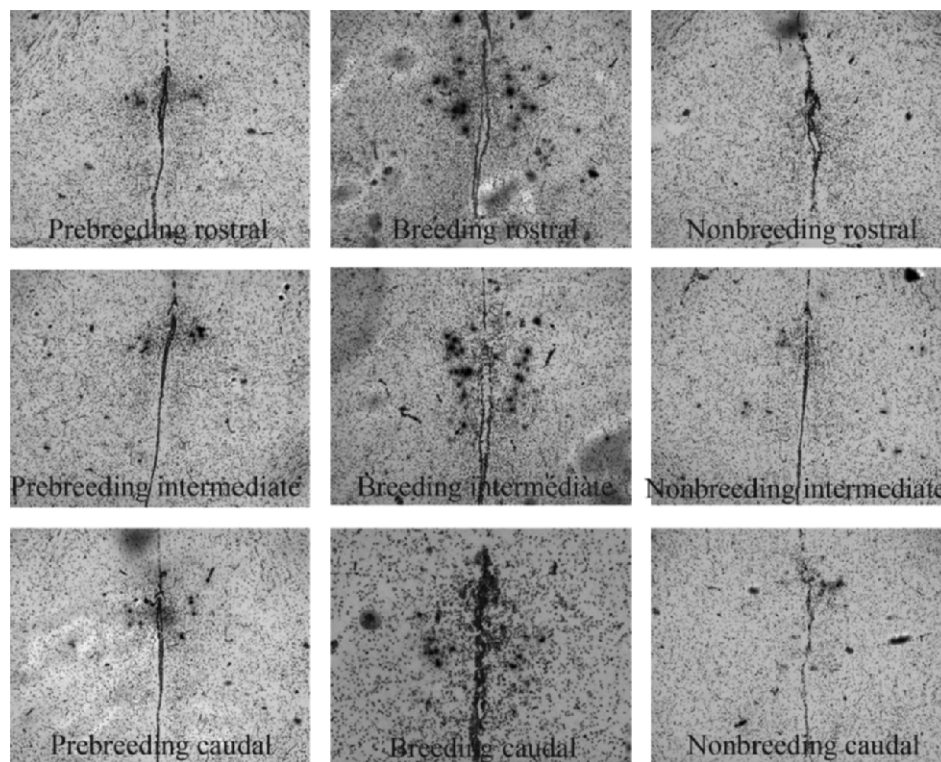


FIG. 1. Reproductive state and comparison of *GNRH1* mRNA-expressing cells in male prebreeding, breeding, and nonbreeding starlings. **A**) Mean \pm SEM testosterone concentration. **B**) Mean \pm SEM testicular volume. **C**) Mean \pm SEM number of *GNRH1* cells in the POA across treatment groups. Lowercase letters represent significant differences; see Results for details.

polymerases using a MAXIScript kit (Ambion, Austin, TX). Unincorporated nucleotides were separated with NucAway spin columns (Ambion). Probe quality was verified by gel electrophoresis, and probe-specific activity was determined using a scintillation counter.

GNRH1 mRNA expression in slide-mounted sections was analyzed by in situ hybridization in two separate batches, and treatment groups were counterbalanced across batches. Slides were thawed and fixed for 10 min in 4% formaldehyde (diluted from 37% formaldehyde ampules; Ted Pella,

FIG. 2. Representation of *GNRH1* mRNA-expressing cells from coronal sections of the POA in prebreeding (left), breeding (center), and nonbreeding (right) starlings from rostral (top), intermediate (middle), and caudal (bottom) regions of the POA. Arrows indicate examples of positively labeled cells; bar = 200 μ m.



Redding, CA) in 1 \times PBS (Ambion). Slides were rinsed in 4 \times saline sodium citrate (SSC; Ambion) and then in 0.1 M triethanolamine (TEA; Sigma-Aldrich, St. Louis, MO). Tissue charge was neutralized using 0.5% acetic anhydride (Sigma-Aldrich) in 0.1 M TEA for 10 min. Slides were transferred to 2 \times SSC for 5 min and then dehydrated in 50%, 75%, 95%, and 100% ethanol (5 min each). The slides were rehydrated in radiolabeled riboprobe solution (2.5×10^5 cpm/ml diluted in 1 \times hybridization buffer [Sigma-Aldrich] with 0.01 M dithiothreitol [Sigma-Aldrich]), sealed with cover glasses, and hybridized overnight in a mineral oil bath at 60 $^\circ$ C. After hybridization, oil was removed by two chloroform rinses and two 2 \times SSC rinses. Slides were then washed at 60 $^\circ$ C to remove nonspecific probe binding as follows: 20 min in 2 \times SSC and 50% formamide (Sigma-Aldrich), followed by two washes for 20 min each in 0.1 \times SSC. Slides were then dehydrated in increasing ethanol concentrations.

Emulsion

Slides were dipped in Kodak NTB-2 Autoradiography Emulsion (Carestream Health Systems, New Haven, CT). Slides were then dried for 15–20 min before placement in light-tight slide racks. The slide racks were then wrapped loosely in black paper sealed with black electrical tape and stored at 4 $^\circ$ C for 6 days. The slides were developed by rinsing twice in xylene for 5 min, followed by two rinses in 100% ethanol for 5 min, and then placed in Kodak D-19 Developer (Fisher Scientific) at 16 $^\circ$ C for 5 min. Slides were rinsed in a water bath and then transferred to a Kodak Fixer (Fisher Scientific) bath for 6 min. The slides were rinsed again in water before counterstaining with 1% cresyl violet for 5 min, rinsed in 0.1 M PBS, and then serially dehydrated in ethanol and placed in xylene for 10 min. Slides were coverslipped using Permount (Fisher Scientific).

Quantification of *GNRH1* Cells

The distribution and number of *GNRH1* mRNA-expressing cells were determined at 10 \times magnification using a brightfield light microscope (Zeiss Axioskop; Carl Zeiss, Thornwood, NY). Cells were evaluated throughout the brain and manually counted in the POA beginning at the TSM split to the caudal extent of the anterior commissure. *GNRH1* mRNA cells were classified using two criteria: 1) there was a clear distribution of silver grains over cresyl-stained cell bodies, and 2) the silver grain area exceeded 50 μ m² [14]. The second criterion was selected as an initial reference to provide the basis to eliminate false-positive cells due to artifacts. We utilized a sampling procedure to provide an estimate of *GNRH1* cells by using methods developed by West [39] and subsequently applied to birds in other avian investigations [40].

Briefly, the focal plane was adjusted to view the number of *GNRH1*-expressing cells throughout the tissue section. Because we measured cells from every fourth section, we eliminated the possibility of counting the same cell twice. This sampling interval may produce an underestimate of the total cell number; however, because the same methods were used to analyze all birds in the study, we are confident that the comparison between groups provides an accurate assessment of relative cell number and distribution. Measurements for the three-dimensional analysis for each *GNRH1* cell were obtained using a charge-coupled device camera connected to a Macintosh computer (Apple Inc., Cupertino, CA). The brain images were digitized, and distance was determined using Openlab 5.0.2 (Improvision Inc., Waltham, MA). The POA can be subdivided into functional subregions based on lesion and immediate-early gene experiments conducted in relation to POA regulation of reproductive and courtship behaviors [35, 41]. For example, POA lesions in male European starlings result in decreased singing, but the degree of these effects is selective depending on the specific subregion lesioned (i.e., rostral, intermediate, or caudal POA) [41]. Guided by these findings indicating that there are functional subdivisions within the POA, we devised a method for analyzing the regional effects of photoperiod on the number of *GNRH1* cells in the POA. The z-axis was calculated based on the rostrocaudal orientation determined from the plane of section beginning at the posterior aspect of the anterior commissure and continuing rostral to the TSM split. For statistical analysis, the rostrocaudal plane was divided into three subdivisions consisting of rostral, intermediate, and caudal regions, which were equally divided into 800- μ m lengths. The x-axis represents the mediolateral orientation, determined from the ependymal layer of the third ventricle to the center of the cell. The mediolateral plane was parsed into two areas that represented the medial 200 μ m and the lateral area greater than 200 μ m from the midline. The y-axis was determined by measuring the distance from the center of each cell to the base of the brain for each section.

Statistical Analysis

We conducted one-way ANOVAs to investigate the photoperiod regulation of reproductive state, plasma testosterone concentration, and testicular volume. We conducted a 3 \times 6 two-way ANOVA to analyze the effects of photoperiod on the distribution of *GNRH1* cells in the POA of starlings. The two factors consisted of treatment group with three levels (prebreeding, breeding, and nonbreeding) by the six brain regions (rostromedial, rostralateral, intermediate medial, intermediate lateral, caudomedial, and caudolateral). Tukey pairwise tests were conducted for post hoc analyses. Significance was assessed at $P < 0.05$.

RESULTS

Male Starling Reproductive Condition

A one-way ANOVA revealed a significant difference in plasma testosterone concentration across treatment groups ($F_{2,17} = 4.08$, $P < 0.05$) (Fig. 1A). Post hoc analysis showed that breeding starlings had significantly greater concentration compared with prebreeding starlings ($P < 0.05$). However, there was no significant difference between prebreeding and nonbreeding starlings ($P = 0.79$) or between breeding and nonbreeding starlings ($P = 0.09$). There was a significant difference in testicular volume across treatment groups ($F_{2,17} = 31.62$, $P < 0.001$) (Fig. 1B). Tukey post hoc analysis demonstrated that breeding starlings had significantly greater volume compared with prebreeding and nonbreeding starlings ($P < 0.001$).

General Distribution and Photoperiodic Regulation of *GNRH1* mRNA

A survey of the positively labeled cells revealed a caudodorsal pattern in the POA commencing at the TSM split toward the anterior commissure in breeding starlings. Specifically, *GNRH1* mRNA was observed adjacent to the sub-commissural division of the OVLT. *GNRH1* mRNA was consistently observed across all treatment groups in the intermediate and caudal POA, comprising the precommissural division of the OVLT. *GNRH1* mRNA was also identified symmetrically to the midline in the septal region adjacent to the supracommissural division of the OVLT (data not shown). However, these cells were sparsely distributed and did not display a clear pattern across sections or within or across treatment groups.

The distribution of *GNRH1* mRNA varied significantly in male starlings across different reproductive states. Breeding starlings had a large number of cells (mean \pm SEM, 87.83 ± 10.18) extending laterally from the ventricle throughout the entire POA (Figs. 1C and 2). In nonbreeding starlings, there were significantly fewer *GNRH1* mRNA-expressing cells (mean \pm SEM, 9.42 ± 2.79) (Figs. 1C and 2). Prebreeding starlings exhibited intermediate levels of *GNRH1* mRNA-expressing cells (mean \pm SEM, 31.00 ± 7.53) (Figs. 1C and 2). The two-way ANOVA revealed a significant main effect of treatment group ($F_{2,102} = 39.80$, $P < 0.001$) (Figs. 1C and 2). Tukey pairwise tests demonstrated that breeding starlings had significantly more *GNRH1*-expressing cells compared with prebreeding and nonbreeding starlings ($P < 0.001$). Moreover, prebreeding starlings had significantly more *GNRH1* cells compared with nonbreeding starlings ($P < 0.05$). There was also a significant main effect of brain region ($F_{5,102} = 14.73$, $P < 0.001$). The intermediate medial POA region had significantly more *GNRH1* cells compared with the other five regions ($P < 0.001$), and the caudomedial POA region had significantly more *GNRH1* cells compared with the rostromedial, rostrolateral, intermediate lateral, and caudolateral regions ($P < 0.005$). There was a significant interaction between brain region and reproductive state ($F_{10,102} = 4.37$, $P < 0.001$) (Fig. 3, A and B). Post hoc analyses revealed that breeding starlings had more *GNRH1*-expressing cells in the intermediate medial POA compared with both prebreeding and nonbreeding starlings ($P < 0.001$) (Fig. 3A). Breeding starlings were also found to have significantly more *GNRH1* cells in the caudomedial region of the POA compared with prebreeding and nonbreeding starlings ($P < 0.001$) (Fig. 3A). Breeding starlings also had greater numbers of *GNRH1* cells in the intermediate lateral region compared with prebreeding and

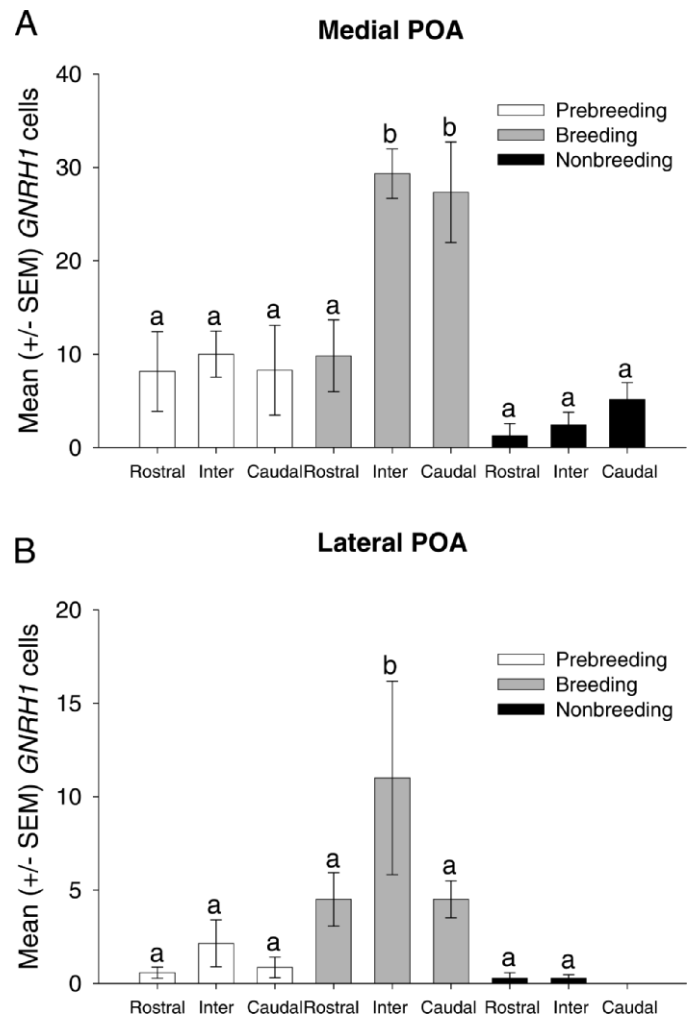


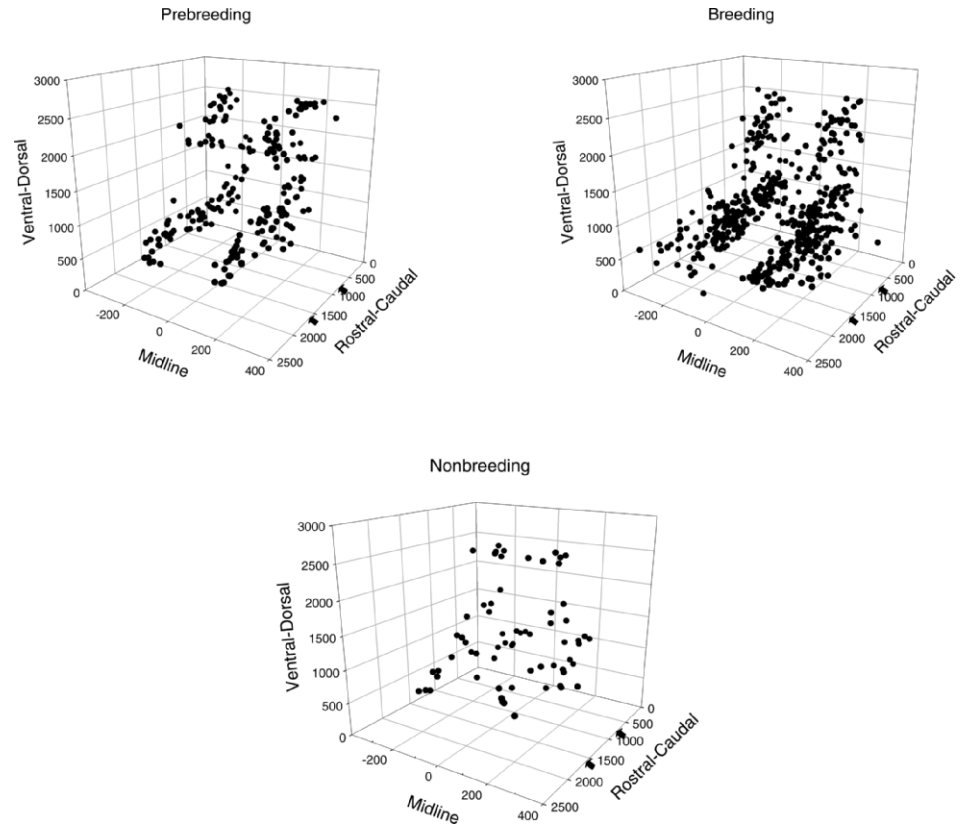
FIG. 3. Change in *GNRH1* mRNA-expressing cells in different subregions in the POA. Mean \pm SEM *GNRH1* cell number in the medial (A) and lateral (B) POA from the rostral, intermediate (Inter), and caudal regions across treatment groups. Lowercase letters represent significant differences; see Results for details.

nonbreeding starlings ($P < 0.01$ and $P < 0.05$, respectively) (Fig. 3B). A three-dimensional reconstruction of the anatomical location of every *GNRH1*-expressing cell from all starlings in each treatment condition confirmed the regional changes in distribution (Fig. 4).

DISCUSSION

Herein, we show that the distribution and number of *GNRH1* mRNA-expressing cells vary profoundly across reproductive states in male starlings. In breeding starlings, there is an overall increase in the number of *GNRH1* mRNA-expressing cells, with the predominant increase in the intermediate and caudomedial regions of the POA compared with prebreeding and nonbreeding birds. Additionally, there was a nonsignificant trend for *GNRH1* cell numbers to increase in the rostral and lateral directions in prebreeding and breeding starlings relative to nonbreeding starlings. These data suggest that the progression from prebreeding through breeding results in an increase in *GNRH1* expression by cells localized primarily along the midline of the POA, with a substantial regression in these populations in association with the onset of photorefractoriness (i.e., nonbreeding condition).

FIG. 4. Three-dimensional representations demonstrating anatomical distribution of *GNRH1* mRNA-expressing cells in the POA of starlings in prebreeding ($n = 7$), breeding ($n = 6$), and nonbreeding ($n = 7$) conditions. Graphs depict individual *GNRH1* cells (indicated as black dots) from all starlings. The x-axis represents the medio-lateral plane, the y-axis represents the ventrodorsal plane, and the z-axis represents the rostrocaudal plane. All measurements are in micrometers, and the z-axis corresponds to the area between the TSM split (2500 μm) and the anterior commissure (indicated as 0). Arrows along the rostrocaudal axis signify boundaries of anatomical subregions.



The pattern of *GNRH1* mRNA expression reported herein is consistent with the distribution recently reported in zebra finches [36], as well as immunocytochemical studies of *GNRH1* protein-expressing cells in starlings [9] and other songbird species [12, 14, 16, 30]. In starlings, the majority of *GNRH1* immunoreactive cells are localized in the subcommissural division of the OVLT, precommissural division of the OVLT, and supracommissural division of the OVLT [9]. The present analysis could not clearly differentiate between the three regions, and we therefore refer to this as the POA. Instead, we subdivided the distribution of *GNRH1* mRNA in the POA equally into three distinct regions and observed that breeding starlings have increased *GNRH1* cell numbers throughout the entire POA, with the vast majority observed along the midline, with a slight but nonsignificant increase in the rostral and lateral subdivisions. With the onset of a nonbreeding state, *GNRH1* cell numbers are significantly down-regulated, resulting in the retention of only a small population of cells in the intermediate and caudal regions of the POA. These findings are consistent with the observed changes in *GNRH1* protein measured via radioimmunoassay and immunocytochemistry in starlings [7, 9, 10, 15, 34]. Unlike many mammalian species, the control of seasonal breeding includes a gradual increase in *GNRH1* due to renewed synthesis in the POA in prebreeding birds [23, 33, 34]. Subsequent photostimulation by long day length then independently increases *GNRH1* synthesis and stimulates *GNRH1* secretion. The findings herein suggest that hypothalamopituitary control of reproduction by photoperiod involves two steps: 1) an increase in *GNRH1* mRNA that leads to greater *GNRH1* peptide synthesis and 2) photoperiod-induced secretion of *GNRH1* from the median eminence. However, further research investigating how the change in photoperiod independently regulates *GNRH1* mRNA expression in the POA and *GNRH1* secretion from the median eminence is required. For

example, studies [42, 43] in Japanese quail have demonstrated that photoperiodically driven changes in thyroid hormone metabolism set off a sequence of cellular events that regulate *GNRH1* release in the median eminence in a seasonal context. In quail, photorefractoriness related to the onset of a nonbreeding state is not associated with a marked reduction in *GNRH1* [19]. It would be useful to establish whether similar photoperiodically driven effects on *GNRH1* release are occurring in starlings along with the effects on *GNRH1* gene expression described in this article.

It is important to note that the change in *GNRH1* cell numbers detected via immunocytochemistry [9] and in situ hybridization (presented herein) is presumably not the result of a cycle of neurogenesis that is followed by apoptosis. Adult neurogenesis is widespread in the avian brain; however, it has not been observed in the POA [44, 45]. Instead, the change in *GNRH1* cell numbers seems to be driven primarily by substantial alterations in *GNRH1* transcription and translation within extant populations of cells, which facilitates or hinders their detection by immunocytochemistry or in situ hybridization. Whether the decline in cell numbers in nonbreeding birds represents a total inhibition of *GNRH1* gene expression or simply a reduction to undetectable levels is not yet clear.

Results of several investigations have suggested that subregions of the POA are functionally specialized based on the control of reproductive behaviors and that these functional subdivisions are associated with neurochemical and hodological specializations [35]. The findings reported herein provide further evidence for a functional topography underlying the organization of the POA. We recently observed that male starlings housed with females have significantly greater numbers of *GNRH1* immunoreactive cells compared with males housed alone [46]. Moreover, the increase in cell number occurred in the rostral and intermediate regions of the POA.

Taken together, the findings of these studies suggest that specific subregions of the POA integrate photoperiodic and social cues. However, an alternative hypothesis from these studies is that these cells only respond to some particular cues, or it could be that the intermediate population of *GNRHI* cells is significantly more plastic in general and readily changes expression patterns depending on environmental stimuli. This hypothesis is supported by findings in male ring doves (*Streptopelia risoria*), which demonstrate an increase in *GNRHI* mRNA cell numbers in the POA for 1 h after engaging in copulatory behaviors [47]. These authors did not report where the increase in *GNRHI* cells was localized.

In conclusion, the annual change in photoperiod has dramatic effects on *GNRHI* mRNA expression in starlings, which underlies observed variation in reproductive physiology in this species [28]. Herein, we show that overall changes in *GNRHI* mRNA reflect alterations in the number and distribution of cells expressing the *GNRHI* mRNA. It appears that *GNRHI* expression is up-regulated in specific subpopulations of cells within the POA to stimulate gonadal recrudescence among birds in breeding condition. Anatomical specificity of the *GNRHI* expression in these cells is either restrained or actively inhibited in prebreeding or nonbreeding birds to maintain gonads in a regressed state so as to prevent reproductive efforts at inappropriate times of the year. Last, previous findings in pigs suggest that subregions of GnRH1 immunoreactive cells in the rostral POA are activated during the GnRH surge [48], and results in rats indicate that there are separate GnRH1 subgroups regulating the LH surge vs. pulsatile release [49]. It appears that functional differences in the GnRH system may be more common than previously thought.

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