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Tuberal hypothalamic expression of the glial intermediate filaments, glial fibrillary acidic protein and vimentin across the turkey hen (*Meleagris gallopavo*) reproductive cycle: further evidence for a role of glial structural plasticity in seasonal reproduction

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Abstract

Glia regulate the hypothalamic-pituitary-gonadal (HPG) axis in birds and mammals. This is accomplished mechanically by ensheathing gonadotrophin-releasing hormone I (GnRH) nerve terminals thereby blocking access to the pituitary blood supply, or chemically in a paracrine manner. Such regulation requires appropriate spatial associations between glia and nerve terminals. Female turkeys (*Meleagris gallavapo*) use day length as a primary breeding cue. Long days activate the HPG-axis until the hen enters a photorefractory state when previously stimulatory day lengths no longer support HPG-axis activity. Hens must then be exposed to short days before reactivation of the reproductive axis occurs. As adult hens have discrete inactive reproductive states in addition to a fertile state, they are useful for examining the glial contribution to reproductive function. We immunostained tuberal hypothalami from short and long-day photosensitive hens, plus long-day photorefractory hens to examine expression of two intermediate filaments that affect glial morphology: glial fibrillary acidic protein (GFAP) and vimentin. GFAP expression was drastically reduced in the central median eminence of long day photosensitive hens, especially within the internal zone. Vimentin expression was similar among groups. However, vimentin-immunoreactive fibers abutting the portal vasculature were significantly negatively correlated with GFAP expression in the median eminence, which is consistent with our hypothesis for a reciprocal relationship between GFAP and vimentin expression. It appears that up-regulation of GFAP expression in the central median eminence of turkey hens is associated with periods of reproductive quiescence and that photofractoriness is associated with the lack of a glial cytoskeletal response to long days.

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Keywords

Meleagris gallavapo; GnRH; glia; GFAP; vimentin; photoperiod

1. Introduction

While glia were once believed to primarily serve an architectural purpose in the nervous system, research over the past few decades has shown that these cells regulate hypothalamic-pituitary-gonadal (HPG) axis activity. This view is evidenced by findings that the spatial relationship between glia and GnRH nerve terminals varies over vertebrate reproductive cycles. Electron microscopy revealed that in diestrus female rats, which have low levels of GnRH release, glial processes ensheath GnRH nerve terminals with much higher frequency than is observed in proestrus rats, when the GnRH surge occurs (Prevot et al., 1999). Tanycytes, glia whose processes extend from their point of origin in the ependymal layer of the third ventricle (VIII) to the external zone of the median eminence (ME-EZ), are a well-established source of this ensheathment (Prevot et al., 1999; Rodriguez et al., 2005). Wrapping of tancytic endfeet around GnRH nerve terminals in the ME-EZ is believed to create a barrier to neuropeptide release into the hypophyseal-portal-vasculature which prevents GnRH from reaching the pituitary and stimulating release of the gonadotrophins, thereby suppressing gonadal activity (Rodriguez et al., 2005). Astrocytes, glia characterized by the stellate shape that they may exhibit (Montgomery, 1994), have also been reported to ensheath GnRH nerve terminals in non-human primates (Witkin et al., 1995), humans (Baroncini et al., 2007) and possibly in sheep (Lehman et al., 1988), but the exact function of astrocytic–GnRH terminal ensheathment is poorly understood (Dhandapani et al., 2003). It is known, however, that tanycytes (Nakao et al., 2006; Prevot et al., 2003; Yoshimura et al., 2003) and astrocytes (Kuo and Micevych, 2012; Ojeda et al., 2003) produce factors, such as transport proteins, neurosteroids and enzymes, that influence GnRH release.

Changes in the spatial relationship between GnRH terminals are also observed in seasonally breeding birds. In male Japanese quail (*Cortunix japonica*), inducement of GnRH release and subsequent gonadal activation by exposure to reproductively stimulatory long days corresponds with a significant decline in the number of putative GnRH nerve terminals encased by glia, as compared with reproductively quiescent short-day quail (Yamamura et al., 2004). Turkeys (*Melagris gallovapo*) also use long photoperiods as a proximal cue for reproduction (Siopes, 1994). As with many other avian photoperiodic breeders, female turkeys (Siopes, 1994) develop a state of photorefractoriness in which previously stimulatory day lengths become insufficient to maintain reproductive activity and the HPG-axis shuts down in anticipation of unfavorable environmental conditions (Nicholls et al., 1988); exposure to short days for several weeks is necessary to reestablish photosensitivity (Siopes, 1989). In sharp contrast to reproductively inactive short-day but photosensitive hens, as well as photorefractory hens, the rostral and central ME of reproductively active long-day turkey hens express high levels of type 2-iodothyronine deiodinase, a glial derived enzyme that increases thyroid hormone activity (Steinman et al., 2008). Thyroid hormone in turn has been reported to induce tanycyte retraction from GnRH terminals in Japanese quail (Yamamura et al., 2006). Likewise long day turkeys show high expression of residual proteins marking recent expression of c-fos, a transcription factor and indirect marker of cellular activation (Morgan and Curran, 1991), in the anterior portion of the ME, while short day and photorefractory hens show little evidence of c-fos immunoreactivity (Millam et al., 2003; Steinman et al., 2008). Many of the c-fos-immunoreactive (ir) nuclei in the LD turkey appear inside stellate astrocytes (Steinman et al., 2008). These findings suggest that glia in the ME of photorefractory hens seem to themselves show a refractoriness to long days.

This apparent glial photorefractoriness coupled with the fact that reproductively mature turkeys have a seasonal cycle with one reproductively active state and at least two distinct inactive states makes turkeys a useful model of how glia regulate HPG-activity. Herein reproductive state refers to the combination of photoperiod and degree of photosensitivity. We sought to integrate this paradigm with an examination of the intermediate filaments glial fibrillary acidic protein (GFAP) and vimentin, as they are integral components of glial cytoskeletons that can influence glial spatial associations with neurons (Eng and Lee, 1995). Intermediate filaments (IF) support cytoarchitecture in glia and motility of free-moving astrocytes by influencing the dimension and density of glial processes. GFAP which is often used as a marker of astrocytes (Chiu et al., 1981; Eng and Ghirnikar, 1994), but also occurs in tanycytes and other glia (Chojnacki et al., 2009), is significantly less expressed in the ME-EZ of rats in the ovulatory phase of their estrous cycle (Parkash and Kaur, 2005). Vimentin occurs in tanycytes (Pixley et al., 1984) and immature astrocytes (Dahl et al., 1981; Voigt, 1989) and has been shown to exhibit reduced expression under short photoperiods in reproductively suppressed hamsters (Bolborea et al., 2011; Kameda et al., 2003). Therefore, reduced GFAP expression and increased vimentin expression may be associated with periods of high GnRH release. Consequently, we used immunohistochemistry to test the hypothesis that GFAP and vimentin expression occur in a reciprocal fashion, as turkeys transition between states of reproductive activity and quiescence. Specifically, we predicted that reduced GFAP expression would be accompanied by elevated vimentin expression in the ME of photostimulated long day hens as compared to photosensitive short day and photorefractory turkeys. We also examined expression of these two intermediate filaments in the infundibular area (IA), another photoresponsive portion of the tuberal hypothalamus dorsal to the ME (Meddle and Follett, 1995; Millam et al., 2003; Steinman et al., 2008). Results indicated that GFAP expression was in fact reduced in the central portion of the ME of long day hens, but surprisingly, the effect was most pronounced in the inner portion (ME-IZ). There was no effect of reproductive state on vimentin expression in any of the areas analyzed.

2. Materials and Methods

2.1 Experimental Animals

The study used somatically mature turkey hens (Nicholas 06 · 02 strain, Nicholas Turkeys, Lewisburg, WV, USA) that had completed fewer than two reproductive cycles. One group of photosensitive hens was maintained under a short day photoschedule (8L:16D; SD group) for 10–14 weeks before euthanasia. A subset of these hens were photostimulated by switching them from short to long days (16L:8D) for 48 h prior to euthanasia (LD group). A final group of hens (PR) was exposed to 35–46 weeks of long days until they became photorefractory as evidenced by a cessation of egg production and palpation of rigid, tight pubic bones. They were then placed on short days for 2 weeks, which is insufficient to reestablish photosensitivity [24], and again exposed to long days for 48 h before euthanasia. Upon post-mortem inspection, PR hens were observed to have completely regressed ovaries. This protocol is the same as has been previously used to test photoinducibility of turkey hen hypothalamic regions in our lab (Millam et al., 2003; Steinman et al., 2008). Hens were euthanized by an intravenous overdose of chloral hydrate (20%) in water, and then underwent perfusion fixation via the carotid artery by exsanguinating with sodium phosphate-buffered saline (PBS) and heparin and then fixing with 10 ml of cold 4% paraformaldehyde. Brains were removed and the hypothalamus was isolated by dissecting away the surrounding tissue, transferred to paraformaldehyde overnight, and cyroprotected in 20% sucrose in PBS for 24 h. Tissue was flash frozen on dry ice and stored at –80 °C until cryosectioning at 25 µm thickness, thaw-mounted (Superfrost; Fisher Scientific, Pittsburgh, PA, USA), and stored at 4° C in 95–100% ethanol. All animals were treated

according to protocols approved by the Institutional Animal Care and Use Committees of the University of California, Davis and North Carolina State University.

2.2 Immunohistochemistry

The rostral two-thirds of the ME (approximately 1.5 mm in distance, encompassing six sections chosen at every 250 μ m) was single-labeled for either GFAP or vimentin. This portion of the ME was examined because previous turkey work indicated that it is highly responsive to photoperiod in photosensitive, but not PR birds (Millam et al., 2003; Steinman et al., 2008). Slides were stained in batches that included at least two different reproductive states. Tissue was rehydrated in 0.1 M PBS then incubated in 200 μ g/ml trypsin in PBS for 20 min and washed in Tris-buffered saline (TBS, 1 M Tris, 1.5 M NaCl, pH 7.5). Slides were placed in 0.3% hydrogen peroxide for 30 min and washed in PBS. Tissue was blocked for 3 h at room temperature in Tris-for-Elmer, Bridgeport, CT, USA) with 5% filtered horse serum (TNB-HS). Slides were then incubated at 4 °C overnight in either a mouse anti-GFAP (AG230, from Chemicon, now Millipore, Bedford, MA, USA) or mouse anti-vimentin (ab8978, Abcam, Cambridge, MA, USA) diluted 1:1000 and 1:100 respectively in TNB-HS. The next morning slides were washed in TBS with 0.5% Tween 20 and then slides were incubated at room temperature for 3 h with a biotinylated horse anti-mouse secondary (BA-2000, Vector Laboratories, Burlingame, CA, USA). Slides were subjected to Tyramid Signal Amplification (Perkin-Elmer) according to the kit's instructions, which included use of streptavidin conjugated-horseradish peroxidase (HRP). Slides were each developed for 10 min in 2 ml of Vector VIP, a peroxidase substrate that forms a purple precipitate when it interacts with HRP, allowing for visualization of immunoreactive cells. Slides were then dried and coverslipped. The mouse anti-GFAP antibody has been previously validated for use in turkey (Steinman et al., 2008). The vimentin antibody used was the RV202 clone which has been shown to be specific for vimentin (Dent et al., 1989) and has previously been used in galliformes tissue (Christian et al., 2012). In our hands, omission of primary antibody abolished vimentin staining.

2.3 Image analysis

Sections were photographed using an Axiocam MRC camera (Carl Zeiss Meditec) attached to a Zeiss Axioimager (Carl Zeiss Meditec, Inc., Dublin, CA) and images were analyzed by an observer blind to treatment using Image J software (NIH, Bethesda, MD, USA). Rostral and central sections (2–3 sections each) were analyzed for each bird ($n = 6$ for GFAP staining, $n = 4$ for vimentin staining).

2.3.1. Quantification of GFAP and Vimentin-immunoreactivity—Percentage

staining was defined as the percent area occupied by black pixels on a white background after manually using the Threshold function in Image J to convert immunoreactive pixels to black in images taken on a 10X objective. The percentage staining for GFAP and vimentin-immunoreactivity in the ME was measured by placing a 0.055 mm² counting box with the top positioned along the bottom of the third ventricle to measure the ME-IZ and the bottom of the box along the ventral edge of the ME to measure the ME-EZ. Percentage staining in the IA was obtained by placing a 0.062 mm² box bilaterally in the center of the IA just dorsal to the ME-IZ and then averaging measurements for the left and right sides.

Stellate astrocytes were identified as GFAP-ir cells having polygonal-shaped somata with at least two processes extending from them (Toran-Allerand et al., 1991). Within the ME, stellate astrocytes tended to be more centrally situated rather than relegated to dorsal or ventral peripheries of the ME so the 0.213 mm² counting box was placed in the center of the ME in 10X images quantified in ImageJ. Stellate cells were hand-counted using the ImageJ cell counter application.

The number of finger-like vimentin-immunoreactive glial terminals that were abutting the edge of the ME-EZ where the basal lamina of the portal vasculature is found was performed using a previously described method (Steinman et al., 2012). Images were photographed on a 63X objective and the fibers contacting the edge of the ME-EZ were manually counted from within a box occupying 0.013 mm^2 along the left medioventral edge (right, if left was damaged) of the ME-EZ.

2.4 Statistical Analysis

Main effects of reproductive condition on GFAP and vimentin expression were assessed using one-way ANOVA. Planned contrasts were also used to test the hypothesis that LD turkeys have different levels of glial intermediate filament expression as compared with SD and PR turkeys, while such expression would not significantly differ in the latter two groups. Levene's test was used for all ANOVA to ensure homogeneity of variance. This condition was met for all dependent variables except for number of GFAP-ir stellate glia in the central ME, which was log-transformed for ANOVA but was graphed using untransformed data. Within groups, repeated measures ANOVA was used to examine whether GFAP percentage staining differed between the central ME-IZ and the central ME-EZ. For planned contrasts variance was always treated as homogeneous if it passed the Levene's test where $P > 0.05$. Spearman correlations were used to assess the relationship between number of vimentin-ir glial terminals in at the edge ME-EZ and GFAP percentage staining in the ME. We constructed 95% confidence intervals using Z-statistics for all results where $P > 0.05$ in order to provide data on statistical power ((Hoenig and Heisey, 2001; Levine and Ensom, 2001), Supplementary Data 1).

3. Results

3.1 GFAP Expression

GFAP was expressed variably throughout the ME and the IA and was rarely seen in the ependymal walls of the VIII (Figs. 1&4A). It was most frequently expressed in fibers with no clear somata, but was also observed in stellate astrocytes (Fig. 1D).

There was a main effect of reproductive condition on GFAP percentage staining in the central portion of the ME-IZ (Fig. 1; $F_{2,15} = 4.7$, $P = 0.026$) as well as the average of the combined ME-IZ+ ME-EZ (Fig. 1; $F_{2,15} = 4.6$, $P = 0.028$). Planned contrasts supported the hypothesis that when compared with non-breeding counterparts, LD hens exhibit reduced GFAP expression in the central ME-IZ (Fig 2A, $P = 0.008$) and the ME-IZ + ME-EZ averaged together (Fig. 2C; $P = 0.009$). There were no main effects of reproductive condition on GFAP percentage staining in any zone of the rostral ME, the central ME-EZ alone, or any coronal division of the IA (Table 2, P 's > 0.3), nor were there any significant planned contrasts (all P 's > 0.2) for GFAP percentage staining in the rostral ME (Fig 2. D–F) or infundibular area. The overall percentage staining for GFAP was significantly higher in the central ME-IZ than the ME-EZ (Fig. 2A&B, $F_{1,17} = 7.6$, $P = 0.013$). Consistent with the percentage staining results, there was a main effect of reproductive condition on number of stellate GFAP-ir astrocytes in the central ME ($F_{2,15} = 4.2$, $P = 0.037$), but not rostral ME ($P = 0.53$), with planned contrasts indicating that LD birds had far fewer detectable stellate glia in the central (Fig 3A, $P = 0.005$) but not rostral (Fig. 3B, $P = 0.308$) ME than birds of either gonadally-regressed group.

3.2 Vimentin Expression

Vimentin expression tended to be highly expressed throughout the ME and IA and was almost always present in the ependymal layer of the VIII (Fig. 4B). It consisted of dense, coarse fibers that terminated at the edge of the ME-EZ in the finger-like projections that are

characteristic of tanyctic endfeet (Mitchell, 1979; Prevot et al., 2003). The presence of vimentin expression in the ependyma and the finger-like projections suggests that both poles of tanyctes were visible, but we cannot rule out that some of the fibers that stained were of astrocytic origin.

There were no main effects of reproductive state on vimentin percentage staining in any zone (ME-IZ, ME-EZ or ME-IZ + ME-EZ) of either the rostral or central ME (Table 1, $P > 0.4$), nor did differing reproductive states correspond with significant differences in vimentin percentage staining in rostral or central IA (Table 2, $P > 0.81$). Similarly, there were no significant planned contrasts for percentage staining in the ME ($P > 0.2$) or IA ($P > 0.7$). Statistical analysis did not identify any main effects of reproductive state (Table 1, $P > 0.4$) or significant planned contrasts ($P > 0.2$) for number of vimentin-ir fibers contacting the edge of the ME-EZ and hence in a position to potentially interact with portal capillaries. There were, however, significant negative Spearman correlations between the number of vimentin immunoreactive fibers contacting the edge of the rostral ME-EZ and the GFAP percentage staining in the ME-IZ (Fig. 4D, $r = -0.65$, $P = 0.023$), ME-EZ (Fig. 4E, $r = -0.65$, $P = 0.023$), and ME-IZ averaged with the ME-EZ (Fig. 4C, $r = -0.74$, $P = 0.006$). Such correlations were not present in the central ME ($P > 0.05$, data not shown).

4. Discussion

The present study indicates that the perception of long day lengths by female turkeys is associated with drastic reductions in GFAP-immunoreactivity in the central ME. These results demonstrate that as with ovulating rats, turkey hens in the ovulatory portion of their circannual cycle have reduced GFAP expression in the ME. The appearance of such a phenomenon across such disparate approaches to reproductive timing provides further evidence that glial structural plasticity is a conserved feature of HPG-axis regulation in birds and mammals. Turkeys differed from rats, however, in that the decrease in GFAP expression in the ME of proestrus rats was specific to the ME-EZ (Parkash and Kaur, 2005), while in turkeys the effect was much more robust in the ME-IZ. One possible reason for the dampened response in the ME-EZ is that overall GFAP percentage staining is already much lower in the central ME-EZ than the ME-IZ in turkeys.

It is plausible that GFAP-immunoreactive glia and their processes may directly encase or release inhibitory paracrine factors onto GnRH terminals making neurohemal contacts in the ME-EZ of SD and PR hen. Indeed, GFAP knockout mice exhibit disruption in the tightness of their blood brain barrier, demonstrating that GFAP is a prerequisite for appropriate glial regulation of certain neurohemal interfaces (Liedtke et al., 1996; Pekny, 2001). The potential function of GFAP-ir glia in the ME-IZ, however, is less clear. There is evidence in several species that GnRH terminals can interface with capillaries in the ME-IZ (Page and Dovey-Hartman, 1984; Redecker, 1991), so without further work we cannot rule out the possibility that some GnRH is released within the ME-IZ of turkeys. Glia in the ME-IZ may be advantageously situated to coordinate signals between GnRH terminals in the ME-EZ and other regulators of the avian HPG-axis whose fiber tracts are located in the ME-IZ, such as thyrotrophin-releasing hormone (Jozsa et al., 1988), neuropeptide Y (Aste et al., 1991), and beta-endorphin (Contijoch et al., 1993). In rodents, kisspeptin, a regulator of mammalian GnRH release, is found in the ME-IZ but not the ME-EZ (Clarkson and Herbison, 2006; Kinoshita et al., 2005), and it has been suggested that this peptide may act on GnRH neurons via glial intermediaries (Uenoyama et al., 2011). Furthermore estradiol appears to mediate rat astrocytic and tanyctic regulation of GnRH release at the time of ovulation (Buchanan et al., 2000; de Seranno et al., 2010). However, in contrast to mammals, the steroidal positive feedback that drives ovulation in birds is progesterone (Wilson and Sharp, 1976). Nevertheless, progesterone receptors are located in the ME-IZ,

but not the ME-EZ of female chickens (*Gallus gallus*) suggesting that glia in the ME-IZ could transduce progesterone signals.

It is likely that expression of GFAP in central ME of turkeys is affected by gonadal steroids. There is abundant evidence in rodents (Garcia-Segura et al., 1994; Parkash and Kaur, 2005; Yin et al., 2009) and primates (Witkin et al., 1991; Witkin et al., 1995) that hormone level manipulations can cause glia to mimic phenotypes observed at corresponding levels of the reproductive cycle (Parkash and Kaur, 2005). It seems that glia are involved in a positive feedback loop on GnRH release as they respond to gonadal steroids by facilitating GnRH release which leads to further production of gonadal steroids (Garcia-Segura et al., 2008). It is intriguing to regard photostimulation in birds as a time of annual puberty during which the gonads reactivate after a period of quiescence (Nicholls et al., 1988). Mice that have a genetic defect that prevents them from undergoing puberty have been shown to develop and sustain a massive up-regulation of GFAP in the tuberal hypothalamus as compared to wild type mice (McQueen and Wilson, 1994). This difference becomes most pronounced as mice approach the age when they would normally attain reproductive competence. In a similar manner elevated GFAP in the central ME of SD and PR turkeys may reflect a return to a pre-pubescent like state of the HPG-axis in a somatically adult animal.

The present findings provide further evidence that reduced photosensitivity in PR turkey hens is associated with impaired glial photoresponsiveness. While it is clear that 48 hr long day exposure was insufficient to affect GFAP expression or number of stellate astrocytes in the central ME of PR hens, it remains possible that the 2 wk of short day exposure was necessary to cause PR hens to exhibit GFAP levels that were comparable to photosensitive SD hens. The effects of long days on GFAP expression in photosensitive hens was highly region-specific and was observed in the central ME but not the rostral ME or any part of the IA. Previous work in the turkey suggests that the rostral tuberal hypothalamus is the most photosensitive and becomes less so as it progresses caudally (Millam et al., 2003; Steinman et al., 2008) but these findings suggest that this may not be the case with all photoinducible genes and/or proteins. Nevertheless, these findings support a previous conjecture that the rostral-to-caudal extent of the turkey ME is heterogeneous in how it regulates the HPG-axis (Steinman et al., 2008). Our lab (Steinman et al., 2008) and others (Meddle and Follett, 1995) have demonstrated that photostimulation induces c-fos expression in GFAP-ir astrocytes in birds. GFAP expression is frequently associated with astrocytes that have been stimulated to interact with nearby cells (Ridet et al., 1997). Given that long days decrease overall GFAP expression while simultaneously inducing c-fos expression in a subset of GFAP-ir astrocytes, it appears that photoperiod drives a change in astrocyte physiology that is more complex than simply moving between binary states of “active” or “inactive.” In fact, it seems unlikely that c-fos expression is directly related to GFAP transcription (Brenner, 1994).

We examined vimentin expression hypothesizing that its expression would be inversely related to that of GFAP. Vimentin expression is generally tanycyte-biased (Chauvet et al., 1995; Kawakami, 2000). Although we did not observe any direct effects of reproductive condition on vimentin expression in any part of the tuberal hypothalamus examined, we did identify a striking negative correlation between the number of vimentin-ir fibers contacting the edge of the rostral external-zone of the median eminence and the percentage GFAP-staining in the rostral ME. This suggests that in the rostral ME there is in fact a reciprocal relationship between GFAP expression and the number of vimentin-ir fibers along the edge of the ME-EZ, although it does not appear to be photoperiodically driven. Identifying the precise significance of this correlation warrants further study, but perhaps it reflects individual differences in glial differentiation (Kalman et al., 1998). It is notable that in California mice (*Peromyscus californicus*) the number of putative tanycytic fibers contacting

the edge of the ME-EZ seems to be negatively associated with relative uterine weight (Steinman et al., 2012), so there is evidence for this glial parameter to serve as a measure of reproductive function.

4.1. Conclusions

The data presented here demonstrate that a reduction in the glial intermediate filament GFAP occurs in a subregion of the ME in LD turkey hens as compared with SD and PR hens. This supports the central hypothesis that changes in expression of proteins that affect glial morphology are associated with changes in reproductive potential in an avian species. It also suggests that photorefractoriness in turkeys results in part from reduced glial responsiveness to long days. More work needs to be done to establish the specific functions of glia of the ME in regulating the HPG-axis, particularly those found within the ME-EZ.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

(LD)	long day
(ir)	immunoreactive
(IA)	infundibular area
(ME)	internal (ME-EZ) and external (ME-EZ) zones of the median eminence
(PR)	photorefractory
(SD)	short day
(TNB-HS)	Tris-NaCl-blocking reagent with horse serum.

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- Glia regulate tuberal hypothalamic gonadotrophin releasing hormone secretion.
- Glial fibrillary acidic protein (GFAP) is reduced in hypothalami of breeding hens.
- Hypothalamic vimentin levels are consistent across the breeding cycle.
- Median eminence GFAP levels are inversely related to number of vimentin terminals.
- Photorefractory turkey hens exhibit reduced glial response to long days.

Fig 1.

Representative photomicrographs depicting the significant reduction in glial fibrillary acidic protein (GFAP) expression in the central median eminence of the long day photostimulated hens (B) as compared with short day photostimulated (A) and photorefractory hens (C). Panel (D) shows a stellate astrocyte (white arrow) under high power magnification in the boxed area from (A). EZ, external zone of the median eminence; IA, infundibular area; IZ, internal zone of the median eminence; VIII, third ventricle. A–C scale bars = 100 μ m, D scale bar 20 μ m.

Fig 2.

Bar graphs showing the GFAP expression as a percent of area stained in the internal zone, external zone and the internal + external zones (averaged together) in the central (A–C) and rostral median eminence (D–F). GFAP expression was significantly reduced in the internal (A) and the internal + external zones (C) of the central median eminence in long day-photosensitive hens (LD) compared with short day-photosensitive hens (SD) and long-day photorefractory hens (PR). * $p < 0.009$. HPG, hypothalamic-pituitary-gonadal (axis). Mean $\pm$ S.E is given for all data.

Fig. 3.

Bar graphs displaying the number of stellate astrocytes/mm² in the central (A) and rostral (B) median eminence. The number of GFAP -immunoreactive stellate astrocytes was significantly reduced in central median eminence of long day-photosensitive hens (LD) as compared with short day-photosensitive hens (SD) and long-day photorefractory hens (PR). *P = 0.005. HPG, hypothalamic-pituitary-gonadal (axis). Mean ± S.E is given for all data.

Fig 4.

Representative photomicrographs demonstrating glial acidic fibrillary protein [GFAP; (A)] and vimentin (B) expression in adjacent sections of the tuberal hypothalamus of a photosensitive long day hen. Panel (C) is a high power image of the boxed area from panel (B) depicting finger-like vimentin-immunoreactive fibers terminals most likely belonging to tanyctyes that abut the edge of the external-zone of the median eminence. (D–F) Dot plots showing the significant negative Spearman correlations between GFAP expression as a percentage of area stained and the number of vimentin-immunoreactive fibers terminals along a 1 mm edge of the external zone of the median eminence. A&B scale bars = 100 μ m, C scale bar 20 μ m. EZ, external zone of the median eminence; IA, infundibular area; IZ, internal zone of the median eminence; OT, optic tract; PT, pars tuberalis, VIII, third ventricle.

Table 1

One-way ANOVA indicated that birds of divergent reproductive conditions do not differ in vimentin expression in any part of the median eminence (all P's > 0.05). Mean $\pm$ S.E is given for all data.

	Vimentin Expression in the Median Eminence					
	% Stain (Internal-Zone)		% Stain (External-Zone)		# Terminals along 1 mm	
	Rostral	Central	Rostral	Central	Rostral	Central
Short Day	74.5 $\pm$ 3.7	65.2 $\pm$ 2.3	64.3 $\pm$ 4.2	66.4 $\pm$ 4.3	74.5 $\pm$ 44.8	146.9 $\pm$ 15.4
Long Day	68.0 $\pm$ 0.7	66.8 $\pm$ 3.1	67.5 $\pm$ 3.2	60.1 $\pm$ 2.7	154.0 $\pm$ 29.5	167.4 $\pm$ 13.4
Photorefractory	71.0 $\pm$ 4.0	67.7 $\pm$ 8.4	68.0 $\pm$ 5.7	64.5 $\pm$ 3.6	108.6 $\pm$ 47.5	165.9 $\pm$ 32.9

Table 2

One-way ANOVA did not identify any differences in intermediate filament expression within the infundibular area of birds of contrasting reproductive conditions (all P's > 0.05). Mean $\pm$ S.E. is given for all data.

Vimentin and GFAP Expression in the Infundibular Area						
	Vimentin		GFAP			
	% Stain		% Stain		# Stellate Glia/mm ²	
	Rostral	Central	Rostral	Central	Rostral	Central
Short Day	60.4 $\pm$ 3.3	65.8 $\pm$ 2.2	32.7 $\pm$ 6.5	21.5 $\pm$ 6.1	15.7 $\pm$ 7.4	4.4 $\pm$ 1.5
Long Day	62.5 $\pm$ 3.0	63.1 $\pm$ 3.2	29.5 $\pm$ 7.7	10.4 $\pm$ 5.1	11.7 $\pm$ 7.03	1.4 $\pm$ 0.9
Photorefractory	61.8 $\pm$ 3.1	63.3 $\pm$ 4.2	32.0 $\pm$ 3.9	32.0 $\pm$ 3.9	14.5 $\pm$ 4.8	3.7 $\pm$ 2.5