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Prior Experience with Photostimulation Enhances Photo-Induced Reproductive Development in Female European Starlings: A Possible Basis for the Age-Related Increase in Avian Reproductive Performance[1]

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ABSTRACT

Reproductive performance in female birds improves with age, and this is generally attributed to experiences obtained during breeding. In temperate-zone species, experience with photostimulation during the first breeding year may prime the hypothalamo-pituitary-gonadal axis to respond to photic cues more rapidly or robustly in subsequent years. To test this idea, we captured 32 photorefractory juvenile (hence naive to photostimulation) female European starlings (*Sturnus vulgaris*) and held half of them (naive group) on a photoperiod of 8L:16D for 32 wk and the other half (experienced group) on 8L:16D for 12 wk, 16L:8D for 12 wk, and then 8L:16D for 8 wk. When we subsequently transferred all birds to 16L:8D, the increase in body mass, which may presage egg laying in the wild, was more robust in experienced than in naive females. Experienced females also showed a more robust elevation in plasma concentrations of the yolk-precursor protein vitellogenin, although naive females showed an initial rapid but transient rise in vitellogenin that we attribute to their extended exposure to short-day photoperiods prior to photostimulation. Finally, the photo-induced increase in diameter of the largest ovarian follicle, in plasma concentrations of luteinizing hormone, and in the number of septo-preoptic fibers relative to the number of cell bodies immunoreactive to GnRH was greater in experienced than in naive females. Thus, prior experience with photostimulation enhances some initial phases of photo-induced reproductive development and may explain, in part, why reproductive performance improves with age in temperate-zone birds.

aging, behavior, environment, neuroendocrinology, seasonal reproduction

INTRODUCTION

Reproductive performance initially improves with age in most iteroparous animals [1]. Typically, the largest improvement occurs between the first and second breeding attempts and yields an increase in number and quality of offspring. For seasonally breeding birds, large improvements in reproductive performance occur between the first and second breeding seasons, whereby second-year and older females will usually initiate egg laying earlier and consequently lay more eggs [2] than will first-year females [3, 4]. In part, higher reproductive performance among second-year and older females may be due to differential survival rates that bias the representation of high-performance individuals among older members of the population [3, 5]. In addition, however, as a female gets older, she lays sooner in the season and more eggs than in her first year of breeding [3, 5–7]. This phenomenon has long been attributed to experiences obtained in the first year of breeding that are then applied in subsequent years to elevate reproductive efficiency [3, 4] or reproductive effort [8, 9]. But of the myriad potential experiences, which are important?

Virtually all temperate-zone bird species are photoperiodic, in that the seasonally changing proportion of daylight hours initiates both the gonadal development and gonadal regression that respectively mark the seasonal onset and termination of breeding [10, 11]. Young hatch in the spring or summer in the condition of photorefractoriness [12, 13]; they are incapable of responding reproductively to the stimulatory effects of the long photophases to which they are exposed at that time [14]. Through an unknown neural mechanism, exposure to the short photophases of fall and winter establishes the hypothalamic sensitivity necessary for the individual to mount a reproductive response to its first experience with photostimulation the following spring [10]. These long photophases of spring stimulate the hypothalamo-pituitary-gonadal (HPG) axis, which leads to gonadal secretion of estradiol-17β (estradiol). Estradiol then stimulates secretion of the yolk precursor vitellogenin from the liver [15–17]. Vitellogenin travels via the blood plasma to ovarian follicles, contributes to the follicular enlargement that must precede ovulation [18], and forms one of the major yolk resources for the developing embryo [19]. In most species, long photophases also initiate a process that eventually leads to the onset of photorefractoriness and regression of the gonads, beginning the annual cycle anew [10]. Thus, one of the most marked differences between a first- and second-year breeding bird is its experience with photostimulation. A first-year breeder is experiencing photostimulation for the first time in its life; that is, it is naive to this experience. A second-year breeder has had prior experience with photostimulation.

In European starlings (*Sturnus vulgaris*), gonadal maturation starts earlier in the second year than in the first [20], but whether this is the result of prior experience with photostimulation is not known. Using wild-caught, laboratory-housed, female European starlings of equal age, we tested

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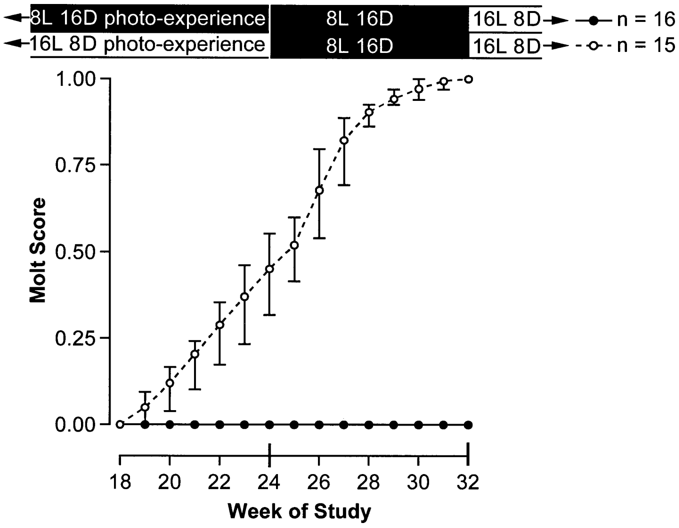

FIG. 1. Progress of prebasic molt of primary feathers in female European starlings (mean ± range). A timeline of different photoperiod exposures for each of two experiment groups is shown at the top and is aligned with the abscissa. Sample sizes refer to the number of females.

the hypothesis that an individual's having prior experience with photostimulation (hereafter photo-experience) elevates the rate or magnitude of reproductive development stimulated by photostimulation (hereafter photo-induced reproductive development). Like most bird species, wild-caught starlings will rarely lay eggs under photostimulation in small laboratory cages. However, laboratory photostimulation will drive many of the initial stages of reproductive development that must precede laying, were it to occur, and here we use these initial stages as a proxy for reproductive development in general. Specifically, we predicted that having photo-experience elevates the photo-induced increase in 1) body mass, associated in many species with preparation for laying; 2) secretion of GnRH; 3) secretion of LH; 4) development of the ovary, oviduct, and ovarian follicles; and 5) secretion of the yolk precursor vitellogenin. We assume in this study that changes in these measures in the laboratory reflect the beginnings of the suite of physiological processes that, in the wild, would eventually lead to egg laying.

MATERIALS AND METHODS

We conducted this study in accordance with the 1996 National Academy of Sciences *Guide for the Care and Use of Laboratory Animals* and with approval from the Johns Hopkins University Institutional Animal Care and Use Committee.

Premanipulation: Capture, Housing, and Initial Photosensitization

We captured European starlings in September 2001 on a farm near Baltimore, Maryland, and determined by their brown speckled plumage that they had all hatched that spring or summer [21]; we held them on a daily photoperiod of 8L:16D. Throughout the study we provided them with ad libitum access to food and water. In mid-November, we anesthetized (5 mg secobarbital injected i.m.) and sexed them by laparotomy.

Photo-Experience Manipulation

Twelve weeks after capture and onset of 8L:16D, we randomly paired 32 females in 16 sound-attenuation chambers, half on 8L:16D (naive group) spatially interspersed in one room with the other half on 16L:8D (experienced group; approximately 5 wk into this phase, one female in this group died and was excluded from analysis).

Beginning at Week 12, with the onset of this phase, and lasting through to Week 36 of the study, we periodically weighed individuals, scored prebasic primary feather molt using a previously described technique [22], and collected blood samples from wing veins into heparinized capillary tubes for assays of plasma vitellogenin and LH (see below). We stored blood on ice for a few hours prior to centrifugation for 9 min at 9000 rpm to separate plasma, which we stored at −20°C until assayed.

Eight weeks of 8L:16D is typically sufficient to ensure starlings are photosensitive [23], and 12 wk of 16L:8D is typically sufficient to drive starlings photorefractory [24]. However, one means of confirming photorefractoriness and thus to confirm that photocondition differed between the experienced and naive groups during the photo-experience phase is to quantify the progress of prebasic molt. The onset of prebasic molt presages and serves as an indicator of photorefractoriness because of the fact that both may be regulated by the same physiological mechanism [25, 26]. We determined that all experienced and no naive females initiated molt well in advance of the end of this photo-experience phase of the experiment (Fig. 1). This confirmed that, by the end of the photo-experience phase, all experienced and no naive individuals had been driven photorefractory.

Postmanipulation: Photosensitization Followed by Photostimulation of All Females

Twelve weeks after transfer to the chambers (and transfer to 16L:8D for the experienced group), we removed all females from the chambers and placed them in open wire cages together in one room on 8L:16D. After 8 wk, we changed the photoperiod to 16L:8D to simultaneously photostimulate naive individuals for the first time and experienced individuals for the second time in their lives. To summarize, after holding half the females (naive group) on 8L:16D for 32 wk and the other half (experienced group) on 8L:16D for 12 wk, 16L:8D for 12 wk, and then 8L:16D for 8 wk, we simultaneously photostimulated all of them. It is the period following Week 32 that is most relevant to our hypothesis (see *Introduction*), because during this time the naive group was experiencing photostimulation for the first time and the experienced group for the second time in their lives. In first-year female starlings, mean hypothalamic GnRH concentrations do not decline (i.e., females do not begin HPG regression) before 6 wk after transfer from 8L:16D to 18L:6D [27]. On 16L:8D, GnRH concentrations would decline even later. However, due to interindividual variation (from photo-experience or other factors) and to be sure that measurements occurred when photo-induced HPG capacity was still ascending, we anesthetized (6.5 mg secobarbital injected i.m.), killed, and collected the brains of all individuals for GnRH immunocytochemistry (see below) after 4 wk of photostimulation using procedures described previously [28]. Blind to the photo-experience of each individual, we also dissected and weighed ovaries and oviducts and estimated the diameter of the largest ovarian follicle.

Assays for Vitellogenin and LH

We assayed plasma vitellogenin indirectly using the zinc method developed for the domestic hen [29] and validated for passerines [16, 30]. Inter- and intraassay variation were 6% and 7%, respectively. Concentrations of vitellogenin-bound zinc depicted in the results are a reliable index for the concentrations of plasma vitellogenin (vitellogenin hereafter). We measured vitellogenin in the plasma of only one of the females from each photo-experience pairing (see above).

Using duplicate 20-μl aliquots of plasma, we assayed LH concentrations with a homologous chicken LH radioimmunoassay [31]. The antiserum was IRC M202 at a 1:5000 dilution. The sensitivity of the assay was 0.05 ng/ml, and 50% displacement was obtained with 2.4 ng/ml. All samples were assayed in one assay with 5.3% and 7.3% variation for a high- and a low-value pool, respectively.

GnRH Immunocytochemistry and Quantification

We performed immunocytochemistry for GnRH on 40-μm, sagittally cut brain sections, as previously described for the transcription factor ZENK [28], except we visualized the avidin-biotin horseradish-peroxidase complex in a 0.018% diaminobenzidine tetrachloride solution containing 0.01% H_2O_2. As part of another study, we initially labeled tissue for ZENK immunoreactivity using a different chromogen. The tissue was quenched with 0.5% H_2O_2 before incubation with a 1:5000 dilution of GnRH primary antibody (HU60 bleed H), which was provided by H.F. Urbanski (Division of Neuroscience, Oregon Regional Primate Center, Beaverton, Oregon). The details of this antibody have been described previously [32]. The rabbit-raised antibody recognizes intact but not fragment forms of both the GnRH-I and GnRH-II decapeptides found in birds. GnRH-I is the only form present in the area involved in regulating pituitary function in the bird, the septo-preoptic area, which contains cells projecting to the median eminence [33, 34]. We processed all of the tissue in one immunocytochemistry batch.

We conducted all quantification procedures blind to the photo-experience of each animal. Under 400× magnification and Köhler illumination, we summed the number of GnRH-immunoreactive (GnRH-ir) cells in the septo-preoptic area of every fourth-cut section (and then multiplied counts by 4 for graphical depiction). Using the section with the highest GnRH-ir cell count for each individual, we captured three nonoverlapping 640 × 480 pixel 8-bit grayscale digital images of the septo-preoptic area between the anterior commissure and the supraoptic decussatio. We captured a fourth image immediately adjacent to and caudodorsal to the supraoptic decussatio, a region with GnRH-ir fibers extending to the median eminence but with no cell bodies. Using ImageJ (National Institutes of Health, Bethesda, MD), we measured the diameter of each clearly labeled, nonoverlapping immunoreactive cell in each image and calculated a mean diameter for each individual. We then drew a horizontal line across the center of each image and summed across images the number of times a GnRH-ir fiber intersected the line. This served as our estimate for amount of fiber staining, a commonly used indicator of GnRH secretion [10, 35]. We did not include intersections with cell bodies.

Statistical Analyses

Due to our nested experiment design (pair of females within one chamber to which we independently assigned photo-experience treatments), the pair and not the individual female constitutes the experiment unit [36]. Therefore, for all dependent variables (except vitellogenin concentration, for which we determined values of only one female per pair; see above), we calculated the mean of the females within a single photo-experience chamber, and it was this mean that was used in all analyses and to which degrees of freedom refer.

To analyze body mass and LH vitellogenin concentrations, we used a general linear model repeated measures ANOVA (SPSS software version 11.0.1 for the Macintosh; SPSS Inc., Chicago, IL), with photo-experience (naive vs. experienced) as a factor. For oviduct and ovary mass; follicle diameter; and GnRH-ir cell counts, cell diameter, and fiber counts (i.e., terminal, Week-36-only measures), we used ANOVA with photo-experience as a factor and, where indicated in *Results*, Week-36 body mass or GnRH-ir cell count as a covariate. Although body mass and GnRH-ir cell count interact with the treatment factor (see *Results*), the general linear model procedure in SPSS adjusts for such interactions between the factor and covariate. We used linear contrasts and least significant difference (LSD) procedures for post hoc comparisons. LH concentrations were below the assay's lower limit of detection in numerous plasma samples from Weeks 12, 14, and 18, but in none from other weeks. For graphs, we conservatively use this limit as the value for samples below it. However, because this also reduces the variance, statistical analyses of LH include only Weeks 24 and 31–36. We square-root transformed vitellogenin concentrations on Weeks 32–36 to satisfy the assumptions of normal and homoscedastic distributions but were unable to satisfy these assumptions with transformation of Week-14 and -18 vitellogenin concentrations. Therefore, statistical analyses of vitellogenin do not include Weeks 14 and 18, although a graph does. We log-transformed oviduct mass and follicle diameter to satisfy the assumptions above. In graphs, we depict raw data or adjusted least-squares means when analyses include covariates.

RESULTS

Body Mass

Photo-experience affected how body mass changed over the course of the study (week × photo-experience effect, $F_{20,280} = 8.10$, $P < 0.00001$). Body mass did not reliably differ between photo-experience groups (post hoc contrast, $P > 0.2$) at the onset of the photo-experience phase of the experiment (Week 12). Body mass then dropped (post hoc LSD, $P = 0.0002$) in the experienced group to a level lower than that in the naive group (post hoc contrast, $P = 0.001$) by Week 14 (Fig. 2A). By Week 18, 6 wk after onset of this phase, body mass of the experienced group had returned to levels that did not reliably differ from those of the naive group (post hoc contrast, $P > 0.2$), and by the onset of the next phase, levels were nearly identical between groups (post hoc contrast, $P > 0.2$). By Week 26, body mass in the experienced group had increased to levels reliably greater (post hoc contrast, $P = 0.015$) than those of the naive group, although all individuals were under identical conditions. From that time, body mass in the experienced group remained elevated above levels in that group at the onset of this phase (Week 24) until at least Week 30 (post hoc LSD, $P < 0.002$ in each comparison).

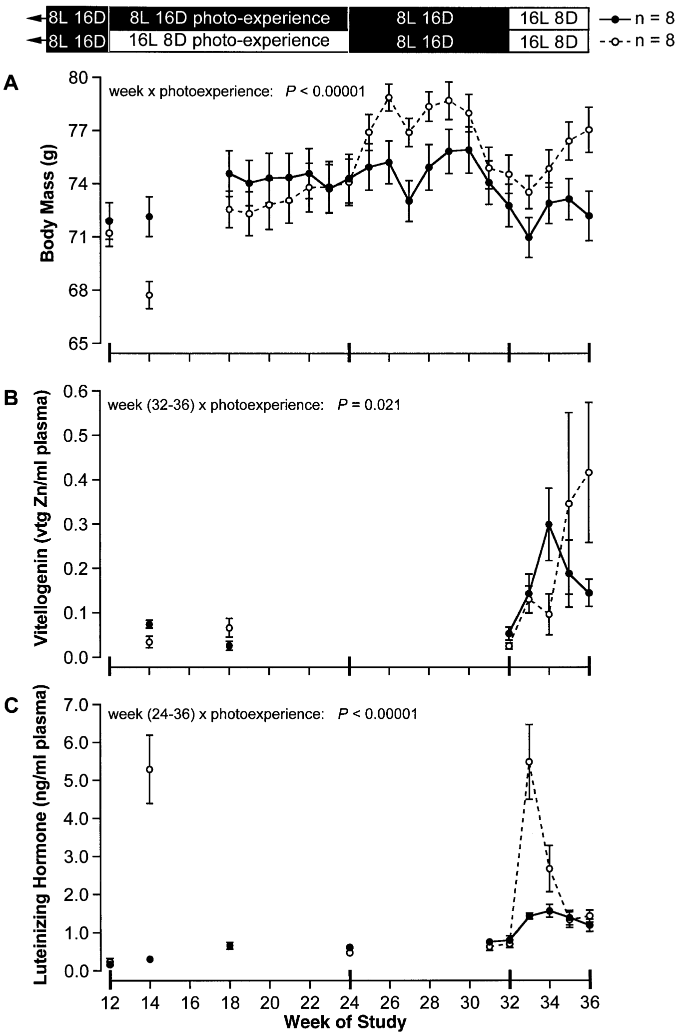

FIG. 2. Temporal change in body mass (**A**) and plasma concentrations of vitellogenic zinc (**B**) and LH (**C**) (mean ± SEM) as a function of photoperiod and photo-experience in female European starlings. A timeline of different photoperiod exposures for each of the two experiment groups is shown at the top and is aligned with the abscissa. Sample sizes refer to the number of experiment units.

At the onset of the time period of primary interest in this study (Week 32), body mass did not reliably differ between groups (post hoc contrast, $P > 0.2$). Although all individuals were under identical conditions, by Week 36 body mass had increased in the experienced group to levels reliably greater than both those of the naive group (post hoc contrast, $P = 0.022$) and those of the experienced group at Week 32 (post hoc LSD, $P = 0.012$). Body mass of naive females did not reliably differ between Weeks 36 and 32 (post hoc LSD, $P > 0.2$).

Plasma Concentrations of Vitellogenin and LH

During the photo-experience phase of the study, vitellogenin concentrations were very low, regardless of photo-

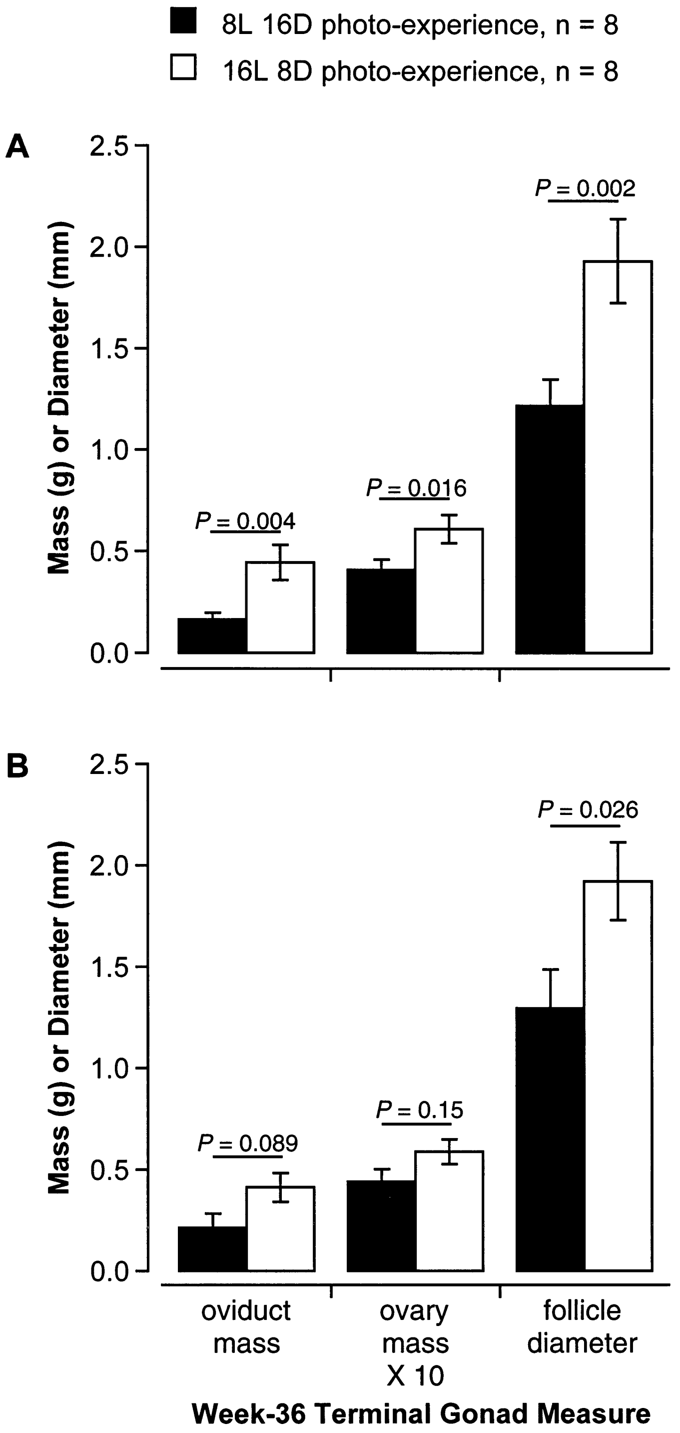

FIG. 3. Effect of prior photo-experience, (**A**) before and (**B**) after adjusting for body mass, on oviduct mass, ovary mass, and diameter of the largest ovarian follicle (mean ± SEM) 4 wk following onset of photostimulation (Week 36) in female European starlings. Ovary mass is depicted at 10 times the estimated total. Sample sizes refer to the number of experiment units.

experience, and they were similar between groups at the start of the time period of primary interest, Week 32 (post hoc contrast, $P = 0.14$; Fig. 2B). Photo-experience affected how plasma concentrations of vitellogenin changed over the remainder of the study (Week [32–36] × photo-experience effect, $F_{4,56} = 3.13$, $P = 0.021$). By Week 34, although all individuals were under identical conditions, vitellogenin in the naive group rose to concentrations reliably greater than both those in the experienced group at that time (post hoc contrast, $P = 0.026$) and those in the naive group at the onset of photostimulation (Week 32; post hoc LSD, $P = 0.0004$). Thereafter, however, vitellogenin in the naive group declined while it increased in the experienced group. By Week 36, vitellogenin concentrations in the experienced group were higher (post hoc contrast, $P = 0.10$), on average, than those in the naive group and reliably higher than they were at the onset of photostimulation (Week 32; post hoc LSD, $P = 0.0004$). In contrast, the difference between Week-32 and -36 vitellogenin concentrations in the naive group was not particularly reliable (post hoc LSD, $P = 0.13$).

Photo-experience affected how plasma concentrations of LH changed over the course of the study (Week [24–36] × photo-experience effect, $F_{6,84} = 12.66$, $P < 0.00001$). At the onset of the photo-experience phase of the study (Week 12), LH concentrations of the experienced group were nearly identical to those of the naive group (Fig. 2C). However, by 2 wk later, LH concentrations were substantially greater in the experienced group than in the naive group. By Week 18, LH concentrations in the experienced group had returned to the low levels of the naive group. Both groups remained similarly low through to the onset of the phase of primary interest at Week 32 (post hoc contrast, $P > 0.2$). Immediately following onset of 16L:8D at Week 32, LH concentrations in both groups increased, but the increase in the experienced group was substantially greater than that in the naive group, although all birds were under identical conditions. By 1 wk into the onset of this phase, LH concentrations in the experienced group were much higher than both the concentrations in the naive group at this time (post hoc contrast, $P = 0.001$) and the concentrations in the experienced group at the onset of this phase (Week 32; post hoc LSD, $P < 0.00001$). The high concentrations in the experienced group then decreased to the moderately elevated levels of the naive group at Weeks 35 and 36, when levels in both groups were reliably elevated above levels at the onset of 16L:8D (Week 32; post hoc LSD, $P < 0.037$ each comparison).

Oviduct Mass, Ovary Mass, and Follicle Diameter

At the end of the study, 4 wk after onset of photostimulation in all individuals, oviduct mass (photo-experience effect, $F_{1,14} = 11.52$, $P = 0.004$); ovary mass (photo-experience effect, $F_{1,14} = 7.48$, $P = 0.016$); and follicle diameter (photo-experience effect, $F_{1,14} = 14.68$, $P = 0.002$) were all reliably greater in the experienced group than in the naive group (Fig. 3a). However, some gonadal size differences between groups may have been the result of the greater body mass of experienced individuals at the time of sacrifice (see Fig. 2a). When Week-36 body mass was included in the model as a covariate, the effects of photo-experience on oviduct (photo-experience effect, $F_{1,13} = 3.37$, $P = 0.089$) and ovary mass (photo-experience effect, $F_{1,13} = 2.39$, $P = 0.15$) became somewhat less reliable (Fig. 3b). The effect of photo-experience on follicle diameter (photo-experience effect, $F_{1,13} = 6.34$, $P = 0.026$) remained quite reliable when we controlled for body mass, indicating a high likelihood that the larger follicles of experienced females were, at least in part, independent of their larger body mass.

GnRH-ir Cell Count, Cell Diameter, and Fiber Count

At the end of the study, 4 wk after onset of photostimulation in all individuals, the GnRH-ir cell count was greater in the naive than in the experienced group (photo-experience effect, $F_{1,14} = 8.12$, $P = 0.013$; Fig. 4). GnRH-ir cell diameter was nearly identical between groups (photo-experience effect, $F_{1,14} = 0.05$, $P > 0.2$), and between-group differences in fiber count (photo-experience effect, $F_{1,14} = 2.21$, $P = 0.16$) were not clearly reliable. Because cell and fiber counts may better reflect the quantity of GnRH sequestered than secreted [37], a more appropriate index of secretion in the absence of direct measures may be the number of fibers relative to the number of cell bodies (see *Discussion*). The number of GnRH fibers relative to the number of cell bodies would provide an index of the intracellular distribution (cell body versus fiber) of GnRH while controlling for degree of immunoreactivity. We analyzed the number of GnRH-ir fibers relative to the number of cell bodies by including the GnRH-ir cell count as a covariate in a general linear model in which fiber count was the dependent variable. We found that the number of fibers relative to cells in the experienced group was reliably greater than that in the naive group (photo-experience effect, $F_{1,13} = 5.15$, $P = 0.041$; Fig. 4).

DISCUSSION

We found that the photo-induced increase in body mass, septo-preoptic immunoreactivity for GnRH fibers relative to cells, initial LH concentrations, longer term vitellogenin concentrations, and body mass–adjusted ovarian follicle diameter is greater in females with prior photostimulation experience (experienced females) than in females experiencing photostimulation for the first time (naive females). Thus, prior experience with photostimulation enhances some initial phases of photo-induced reproductive development and may explain, in part, why reproductive performance improves with age in temperate zone birds.

Body Mass

As the onset of egg-laying approaches, female birds may become hyperphagic [38–40] and rapidly accumulate body mass [39–43]. Our findings suggest this elevation in body mass occurs more quickly or robustly in experienced than in naive females. We also observed differences in body-mass change irrespective of photostimulation or photo-experience. That is, experienced females were reliably heavier than naive females during Weeks 26–29, a period when all individuals were on the same 8L:16D photoperiod. This effect was most likely the result of the rapid change to this photoperiod that had just occurred for experienced but not naive females. Similarly, a rapid change in photoperiod from 8L:16D to 16L:8D at Week 12 triggered a drop in body mass in the experienced group. We do not know why these rapid changes in photoperiod drive rapid changes in body mass, but we speculate that such photoperiod changes drive metabolic changes [44] or changes in light-dependent activity for which females do not initially compensate.

HPG Axis and Vitellogenin Concentrations

Several converging lines of evidence in this study suggest that having photo-experience elevates or accelerates some aspects of photo-induced HPG activity. The surge in plasma LH concentrations that typically follows the onset of photostimulation was much more robust in experienced than in naive females. Shortly after this surge, LH declined to the moderately elevated concentrations in naive females. Thus, although both experienced and naive females mounted a photo-induced increase in plasma concentrations of LH, the initial increase in experienced females was much greater than that in naive females. But was this LH surge in experienced females due to their prior experience with photostimulation?

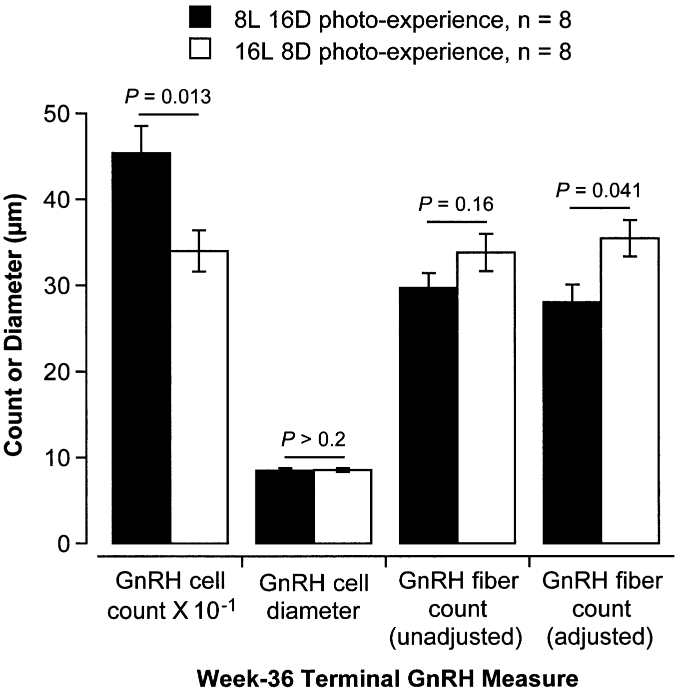

FIG. 4. Effect of prior photo-experience on number of septo-preoptic cell bodies immunoreactive for GnRH, mean GnRH-ir cell-body diameter, and number of septo preoptic GnRH-ir fibers before and after adjusting for the number of cell bodies 4 wk following onset of photostimulation (Week 36) in female European starlings (mean ± SEM). The number of GnRH-ir neurons are depicted at 10^{-1} of the estimated total. Sample sizes refer to the number of experiment units.

The highly elevated LH concentrations in experienced females 2 wk following their first experience with photostimulation (Week 14) suggests that such high concentrations do not require prior experience with photostimulation. Why then were concentrations so low 2 wk following onset of the first photostimulation for naive females (Week 34)? We do not know, but we note that prior to their first experience with photostimulation, naive females had spent 32 consecutive weeks on 8L:16D. Therefore, naive females were photosensitive for an extended time during which negative feedback from low, chronic, gonadal steroid activity may have desensitized the pituitary to GnRH. By comparison, experienced females had been on 8L:16D for 12 wk just prior to their first experience and 8 wk just prior to their second experience with photostimulation.

Due to the possibility that extended short-day exposure may have influenced the sensitivity of the pituitary in naive females, perhaps a more helpful comparison of LH concentrations would be that between the first and second exposures to photostimulation in the experienced group. The peak in LH during their first exposure is very similar in magnitude to that during their second exposure, but the timing or the duration of these peaks differs. We have no data from Week 13, 1 wk following onset of their first exposure to photostimulation, which would be the ideal time to compare to Week 33, 1 wk following onset of their second exposure. Nonetheless, the rapid drop from Week 33 to Week 34 in experienced females suggests that females do not sustain such peak levels even for as long as a week. Therefore, one might hypothesize that perhaps the major difference between first- and second-year photostimulated females is not the magnitude of the LH surge but rather its timing, with second-year females reaching that peak approximately 1 wk prior to first-year females. Alternatively, high LH concentrations in experienced females on Week 13 would indicate that the duration of the LH surge is attenuated with photo-experience, perhaps as a result of a more effective feedback system.

We would expect the elevated LH concentrations of experienced, photostimulated females to result in greater or earlier stimulation of the gonad compared with naive, photostimulated females. Indeed, experienced females had a larger ovary and oviduct, but this effect may have been the result of their larger body mass. However, follicle diameter was larger in experienced than in naive females, irrespective of body mass. This alone is perhaps our most convincing evidence that experience with photostimulation elevates the rate or magnitude of photo-induced reproductive development in the starling, because unlike the relatively transient changes in LH, vitellogenin, and GnRH concentrations, follicle diameter reflects the integration of numerous factors over a longer period of time. Consistent with these findings, recent photoperiodic experience can influence subsequent, photo-induced gonad and body growth in young mammals [45–47], suggesting that the ability to somehow ‘‘encode’’ photoperiodic history applies to multiple vertebrate taxa. It should be noted for our study, however, that oviduct mass, ovary mass, and follicle diameter were all much lower than values in females nearing lay [15], regardless of photo-experience. But the values we observed are typical for photostimulated, laboratory-housed, wild-caught female starlings [48]. We assume that differences between experienced and naive females in this experiment reflect those that would occur during the earliest stages of photo-induced reproductive development, differences which would eventually lead to an older, free-living female laying sooner and more eggs than a younger one.

Having photo-experience is not required for an initial, photo-induced elevation in vitellogenin, as vitellogenin in both groups initially increased with photostimulation at Week 32. The fact that no such increase occurred for experienced females exposed to photostimulation the first time (Week 14) suggests an age-dependent process underlying a female’s ability to respond to long days with vitellogenin secretion, although we may have missed an increase between Weeks 14 and 18. Where photo-experience appears to play a role is in the longer term elevation of vitellogenin, whereby experienced females have a more robust prolonged response than naive females. We offer this hypothetical explanation. Extended short-day exposure elevates liver stores but not synthesis of vitellogenin, possibly because short days inhibit secretion. Due to an accumulation of stores in naive females, photo-induced secretion is elevated initially but cannot be sustained with comparable synthesis. Females with normal short-day exposure respond more slowly because of low stores but, with photo-experience, can sustain the response because of elevated synthesis. Some evidence in birds for a so-called ‘‘memory effect’’ of the vitellogenin II gene provides support for this hypothesis [49]. Specifically, secondary estradiol exposure elevates mRNA concentrations over initial exposure. This would seemingly facilitate longer term high secretion rates. However, some caution is merited here as well. First, the memory effect shown previously occurred over a period of several hours, but was not tested over several weeks. Second, regardless of photo-experience or photoperiodic condition, vitellogenin concentrations in our study were much lower than those of a female starling undergoing rapid yolk deposition in preparation for ovulation [50, 51]. Still, as for gonad size (see above), we assume these low, rising levels reflect the initial changes that subsequently lead to egg laying in free-living females.

McNaughton et al. [52] found in starlings that repeated exposure to GnRH primes pituitary LH secretion, resulting in increasing concentrations of secreted LH with each additional exposure to GnRH. They also found that, in terms of LH secretion, the pituitary was substantially less responsive to GnRH injection in photorefractory juvenile starlings than in photorefractory adults, which would have had prior experience with high GnRH exposure. One interpretation of their findings is that GnRH upregulates its own receptors on the pituitary, a process that occurs in mammals [53]. These studies suggests a mechanism for the results presented here and indeed for the age-related increase in reproductive performance in birds. First-year breeders may be less responsive to high, photo-induced levels of GnRH secretion because of a deficiency in GnRH receptor. However, this initial GnRH exposure is suggested to upregulate receptor levels, so that with an individual’s second experience with GnRH secretion, the pituitary may be substantially more responsive. This might then result in the more rapid LH surge, which presumably leads to more rapid follicle growth and eventual egg laying.

Although there is no a priori reason that other mechanisms might not also be acting to enhance reproductive performance with age, this hypothesized mechanism itself suggests that GnRH secretion would not need to differ between first-year and older breeders. Rather, elevated sensitivity to GnRH at the level of the pituitary would be sufficient to drive elevated photo-induced reproductive performance. Therefore, we were surprised to find that having photo-experience influenced both the number of GnRH-ir cells as well as the relative intracellular distribution of GnRH-ir, as measured by the number of GnRH-ir fibers relative to the number of cells. Of course, interpreting results on immunocytochemically stained GnRH can be difficult. For example, one might not have expected more GnRH cells in naive than in experienced birds, as we found. However, the probability of counting a cell body or fiber labeled with immunocytochemistry is a function of the concentration of the antigen in that cell body or fiber—the more GnRH in the cell, the more likely labeling for that antibody will reach the threshold necessary for detection [37]. GnRH concentrations (measured by radioimmunoassay) in the preoptic area of photorefractory female starlings begin to increase with their transfer to short photophases and gradual acquisition of photosensitivity but without a corresponding increase in concentrations of plasma LH [54]. Photostimulation then elevates concentrations of plasma LH [10]. Together, this suggests that GnRH is synthesized during short-photophase exposure but not abundantly secreted until photostimulation. Thus, one would expect prolonged photosensitivity of naive females to elevate GnRH stores but not secretion, leading to greater sequestration of the peptide and the likelihood of detecting a cell body or fiber.

Our findings that GnRH cell counts were higher in naive than in experienced females are consistent with the reasoning above. Why then did we not detect more fibers in the naive females, where GnRH would also be sequestered, presumably? Although the likelihood of detecting a GnRH fiber should increase with GnRH sequestration, with increasing secretion, fiber relative to cell-body content of GnRH should also increase as the result of the one-way movement of GnRH molecules from cell bodies to cell fibers to the extracellular portal vascular system. In other words, with increasing secretion rates, we would expect relatively greater intracellular distribution of GnRH away from cell bodies and toward fibers and the portal vasculature. This is indeed what we found, and together with our results on LH concentrations, vitellogenin concentrations, and follicle size, this suggests that females with photo-experience have higher photo-induced GnRH secretion rates than females without such experience. However, confirmation of these findings awaits more direct measures of GnRH secretion.

We have found that photo-experience elevates the rate or magnitude of some of the initial stages of photo-induced reproductive development in a temperate zone, female songbird. We suggest that in free-living females, the elevation of these initial stages would give rise to laying earlier and more eggs and would explain, in part, why reproductive performance is often higher in older than in younger birds. Certainly other experiences, such as feeding young [55], may also enhance reproductive performance.

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