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Supplementary radio noise advances sexual maturity in domestic pullets exposed to 7-h photoperiods

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Abstract
Cage-reared, egg-type domestic pullets were given a 7L:17D regimen from 2 d of age. At eight weeks, 24 birds were transferred to individual cages in each of four controlled environment chambers and maintained on 7-h photoperiods. A radio was played at 75-80 dB from 10 weeks of age in two of the chambers during the 7 h immediately preceding the photoperiod, and the other two chambers remained as controls. Mean age at first egg for the radio birds (143 d) was 13 d significantly earlier than controls, but body weight at first egg was similar for the two groups. Although this suggests that the radio noise might have stimulated feeding activity and accelerated growth, there was no significant difference between the groups for a regression of body weight on age at first egg, indicating that accelerated growth did not contribute to the advance in maturity. A shift in the location of the photoinducible phase, so that it became partially illuminated by the normally non-stimulatory 7-h photoperiod, is another possible reason for the earlier sexual development. Plasma melatonin concentrations midway through the 7-h dark period in which the radio was played were similar to those of non-radio controls, indicating that this period was not regarded as being part of a 14-h subjective day.

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Introduction
The provision of radio noise prior to a non-stimulatory photoperiod has been reported to advance sexual maturity in both male and female quail (Millam *et al*., 1985; Li & Burke, 1987). Ishino *et al*. (1998) also noted that 3 h of 25-kHz ultrasound given from 93 d of age to White Leghorn pullets maintained on 14-h photoperiods advanced the median age at first egg by 8 d. In laying hens, the progressive replacement of the first 8 h of a 12-h photoperiod with radio noise, to leave only 4 h of light (4L:20D), supported rates of lay similar to controls maintained on 12-h photoperiods (Lewis & Perry, 1990). In this latter trial it was suggested that the radio noise had anchored the photoinducible phase (PIP, a 4-h period that induces a photosexual response when illuminated) to the position it had occupied in the original 12-h photoperiod, thus enabling it to be illuminated by light from the 4-h photoperiod. In contrast, if this had not happened, it is likely that PIP would have phase-shifted into the 20-h night of the new 4L:20D regimen, and the lighting been insufficient to sustain egg production at a rate similar to that of the 12-h controls (Lewis, 1996).

This paper reports the findings of a trial that investigated the effect on sexual maturation of playing 7 h of radio noise immediately prior to a 7-h photoperiod, from 10 weeks of age, to egg-type domestic pullets. The radio treatment was introduced at 10 weeks because, in *ad libitum*-fed egg-type pullets, the maximum advance in AFE in response to an increment in photoperiod is achieved at about this age (Lewis *et al*., 2002).

Materials and Methods
Ninety-six Hyline Silver brown-egg hybrids, that had been reared with a larger group of Hyline Brown pullets in 8-bird cages in light-proof rooms and maintained on 7-h photoperiods (08:00-15:00) following an initial 2 d of continuous illumination, were transferred randomly at 56 d of age to 24 individual-bird cages in each of four sound and light-proof environmental chambers. Illumination was provided by 60W incandescent lamps (33 ± 1.1 lux at feed trough) in the brooding rooms, and by 9W compact fluorescent lamps (21 ± 1.4 lux at feed trough) in the chambers, with lamps located at a height of 2 m from the floor in both units. From 71 d onwards, a radio, producing a mixture of vocal and musical noise at a sound pressure of 75-80 dB, was

played in two of the four chambers for 7 h immediately prior to, and for the first 30 min of, the 7-h photoperiod (radio on 01:00 - 08:30). The 30-min overlap provided insurance against any asynchrony in the clocks that operated the radios and the lights. The other two chambers acted as controls, with birds maintained on 7-h photoperiods but with no radio. The pullets that had been reared, for a separate investigation, in the same facilities as these birds to 56 d remained there, and were either maintained on 7-h photoperiods or transferred to 14-h photoperiods at 71 d. Data from these birds provided a fortuitous comparison of the effect of adding 7 h of light, with the provision of 7 h of radio noise, on age at first egg.

The feeding and drinking systems, and ambient temperatures (approximately 21 ºC) were similar for the two facilities, and conventional pullet starter (< 70 d) and grower (> 70 d) diets were fed *ad libitum*. A 2-mL blood sample was taken at 70 d (1 d before radio noise was introduced) from the brachial vein of eight birds from each treatment during the first 2 to 3 h of the photoperiod, and from the same birds at the similar times on days 73 and 77 (+2 and +6 d of radio noise), centrifuged at 500 g for 15 minutes, and the serum frozen. Subsequently, plasma luteinizing hormone (LH) concentrations were determined by radioimmunoassay using the method described by Sharp *et al.* (1987). At 77 d, further blood samples were taken between 4 and 5 h after the radio had started, but while the birds were still in darkness, and the sera chloroform-extracted and assayed for plasma melatonin concentration using an iodinated tracer and a modified method from Fraser *et al*. (1983).

Age (AFE) and body weight at, and weight of, first egg were recorded for individual birds, and, after a t-test had shown no significant difference between pairs of chambers subjected to the same treatment, the data were subjected to a one-way ANOVA using a model from Statistix version 8 (Analytical software, 2003). AFE data for the 7-h controls in this experiment and for the 7-h controls in the concurrently-run trial (reared together to 56 d) were also subjected to a one-way ANOVA with 'trial' as the variable to determine the validity of using the data for the photostimulated (7 to 14 h at 71 d) group in this other study as a measure of the extent to which the radio noise had advanced sexual maturity in this trial. Regressions of the standard errors of the mean (SEM) for plasma LH and melatonin concentrations on the corresponding means for each group indicated that, for each assay, the SEM increased in direct proportion to the mean; this heterogeneity of variance was removed by transforming concentrations to $\log_{10}$ values prior to statistical analysis.

Results

The mean AFE for the pullets given the radio noise was 13 d significantly earlier than the controls (Table 1), but the coefficients of variation were similar at 8.48 and 8.40% respectively; this is shown in Figure 1. Mean body weight at first egg for the birds given the radio noise was not significantly different from the controls (Table). Mean weight of first egg for the radio birds was 3.1 g significantly lighter than for the no-radio controls (Table), but a regression of both sets of egg-weight data on AFE showed no significant difference in slope or elevation, with egg weight increasing by 0.24 g for each 1-d delay in AFE ($P < 0.001$, Slope s.e. = 0.024, $r^2 = 0.504$). There were no significant differences in plasma LH concentration between the two groups at –1, +2 or +6 d of the introduction of the radio noise, nor for the change between -1 and +6 d (Table). Mean plasma melatonin concentration was similar for both groups midway through the 7-h period of darkness in which the treatment birds received the radio noise (Table 1).

Discussion

The significant 13-d advance in AFE for the pullets given the radio noise concurs with the earlier findings in quail (Millam *et al*., 1985; Li & Burke, 1987), and demonstrates that a radio played prior to a non-stimulatory photoperiod in some way provides a stimulus for initiating rapid gonadal development. However, the radio noise was not linked to the photoperiod to create a 14-h subjective-day, because the Hyline Brown pullets that had been reared with the current birds to 56 d, and transferred from 7- to 14-h photoperiods, matured 43 d earlier than constant 7-h controls (Figures 1a, 1c). Although different genotypes were used in these two trials, the comparison of the effect of 7 h of radio noise with that of a 7-h increment in photoperiod is still legitimate because an ANOVA using 'trial' as the variable showed that the mean AFE for the constant 7-h birds in the current trial (156.7 ± 1.90 d) and that for the constant 7-h controls in the other trial (155.4 ± 2.24 d) were not significantly different from each other.

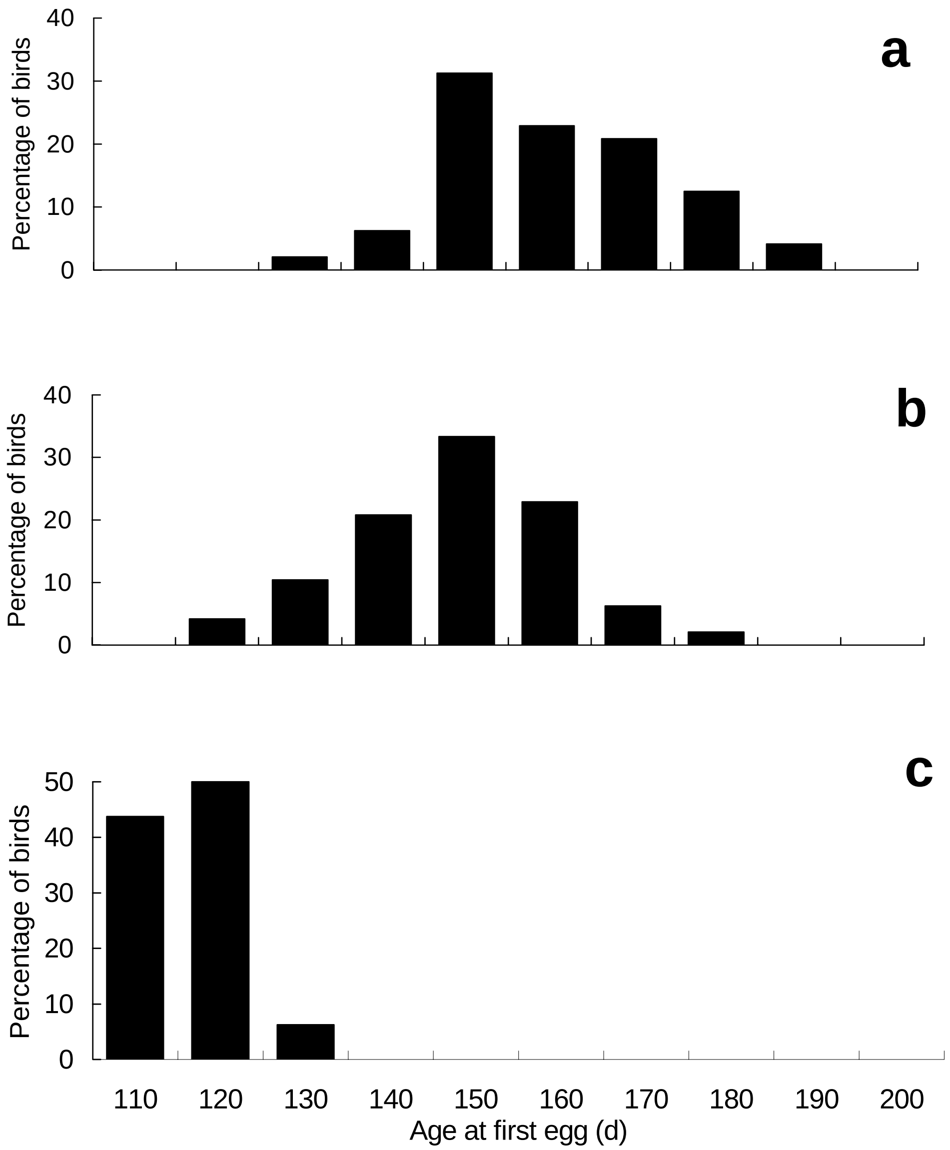

Figure 1 Distribution of ages at first egg (percentage in 10-d classes) for Hyline Silver pullets (a) maintained on 7-h photoperiods or (b) maintained on 7-h photoperiods but given 7 h of radio noise before the photoperiod from 71 d, and (c) Hyline Brown pullets transferred from 7- to 14-h photoperiods at 71 d in a concurrent trial

In birds exposed to a normal light-dark cycle, melatonin is synthesized at night in both the pineal gland and the retina, but suppressed by the activity of serotonin N-acetyltransferase during the day (Binkley *et al*., 1980). Serotonin production is initiated by retinally synthesized dopamine, a neurotransmitter whose own synthesis is dependent upon light falling on the retina (Morgan *et al*., 1995). However, within an intermittent lighting regimen, melatonin release is also suppressed in the scotoperiods located within the subjective day (Lewis *et al*., 1989). It is possible, therefore, that melatonin release would also be suppressed during the part of the scotoperiod in which the radio was being played if this phase were considered to be part of a subjective day. However, the similarity in plasma melatonin concentration for the radio- and control-birds in samples taken midway through this 7-h period dispels this hypothesis.

Another explanation for the 13-d earlier AFE of the radio-birds might be that the radio noise phase-shifted the PIP, normally located within the scotoperiod of a 7L:17D regimen, so that part of it became illuminated by light at the end of the photoperiod. This partial rather than complete illumination might explain the 13-d advance of the radio-birds compared with the 43-day advance in sexual maturity achieved when the PIP was fully illuminated by the transfer to a 14-h photoperiod in the other investigation. In support of this hypothesis an advance in the PIP was reported to have enabled a normally non-stimulatory photoperiod to become photoinductive when quail were exposed to different light/dark cycles (Juss *et al*., 1995; Follett *et al*., 1998), and a phase-shift in photosensitivity was also considered to be the likely reason for the 7-d advance in AFE observed when egg-type pullets were given, from 10 weeks of age, 8 h of very dim light (down to 0.1 lux) immediately prior to an 8-h photoperiod of 10-lux lighting (Lewis *et al*., 2001). The phenomenon is also the same as that suggested by Lewis & Perry (1990) to explain how 8 h of radio noise played immediately before the photoperiod of a 4L:20D regimen was able to sustain a rate of egg production similar to that of 12L:12D controls. However, in this case, the radio was thought to have anchored the PIP rather than phase-shifting it as suggested for this trial, because the hens had previously been exposed to 12-h photoperiods. It is possible that if the current birds had been given a 1 to 2 week conditioning period of 14-h daylengths simultaneous with the introduction of the radio treatment, and then returned to 7-h daylengths, the advance in AFE might have been closer to the 43-d recorded for the permanent transfer to 14-h photoperiods.

Table 1 Mean age and body weight at, and weight of, first egg, and plasma luteinizing hormone (LH) concentration for Hyline Silver pullets maintained on 7-h photoperiods with or without 7 h of radio noise played daily, from 71 d, for 7 h immediately before the photoperiod

	Radio	No radio
Age at first egg (d)	143.4 ± 1.76 [a]	156.7 ± 1.90 [b]
Body weight at first egg (g)	1683 ± 18.2	1697 ± 16.4
First egg weight (g)	43.5 ± 0.60 [a]	46.6 ± 0.70 [b]
LH concentration (ng/mL)		
70 d (-1 d of radio)	3.20 ± 0.43	3.37 ± 0.49
73 d (+2 d of radio)	2.29 ± 0.27	2.56 ± 0.38
77 d (+6 d of radio)	2.46 ± 0.44	2.25 ± 0.21
LH change (%)		
70 to 77 d (-1 to +6 d of radio)	-20.5 ± 9.6	-27.2 ± 9.6
Melatonin (pg/mL)	257 ± 109	129 ± 55

Within a row, means with different postscripts are significantly different ($P < 0.05$) for the 7-d advance

Another explanation could be that the radio noise stimulated feeding activity and accelerated growth to achieve an earlier attainment of the optimal body weight and body composition for spontaneous sexual development. Feed intake was not recorded in this trial, but the non-significant difference in body weight at first egg between the groups suggests that the birds given the radio noise did, indeed, have a faster body weight gain than the controls. Support for this supposition comes from unpublished data from a trial conducted by the senior author at the University of Bristol, where 8 h of radio noise played prior to a 4-h photoperiod stimulated laying hens to commence feeding as soon as the radio came on. However, increased feed intake and faster growth are unlikely to have been the sole reasons for the 13-d earlier sexual maturity of the radio-birds, because Lewis *et al*. (1996) reported that, whereas pullets transferred from 8- to 13-h photoperiods and allowed *ad libitum* access to feed matured 35 d earlier than un-photostimulated controls, sexual maturity was still advanced by 31 d when access to feed was denied during the extra 5 h of lighting. It is unlikely, therefore, with 5 h of illuminated feeding activity resulting in only a 4-day advance in AFE, that 7 h of radio noise during darkness would stimulate feed intake and growth to such an extent that AFE could be advanced by 13 d. Additionally, Lee *et al.* (1971) concluded that a 1% difference in feed intake results in

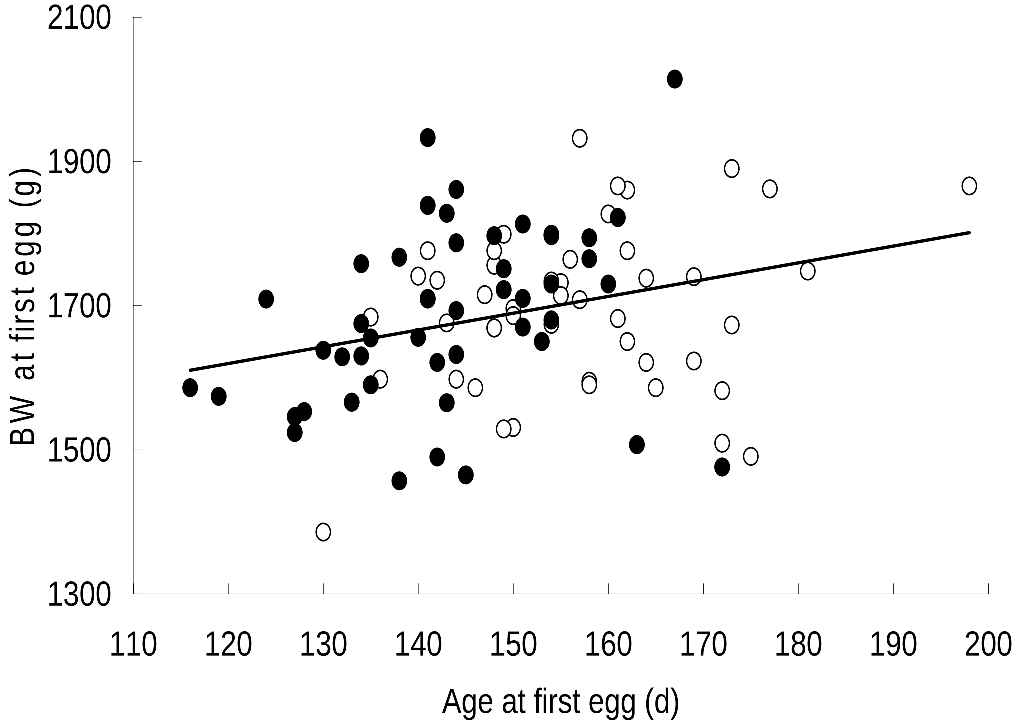

Figure 2 A regression of body weight (BW) at first egg on age at first egg for pullets exposed to a 7L:17D regimen with (●) or without (○) 7-h of radio noise played immediately prior to the photoperiod

a 0.5-d difference in sexual maturity, so the 13-d advance in AFE for the radio birds would have necessitated a 26% increase in feed intake. Such an increase in feed intake is not plausible because Lewis *et al.* (1996) recorded only an 8% increase in feed intake for the pullets that were given 5 h extra illuminated daily access to feed. Furthermore, a regression of body weight on age at first egg (BW at first egg (g) = 1341+ 2.33 AFE (d), P = 0.010) revealed no significant difference in either slope or elevation between the groups (Figure 2), which also indicates that the advance in maturity of the radio birds was not due to an acceleration of growth. The 2.33 g increase in daily body weight gain is a typical growth rate for brown-egg hybrids at the end of the rearing period.

The fall in plasma LH between the first and second samplings in both the experimental and the control groups was not unexpected, and is likely to have been a response to the stress of handling the birds during the initial blood sampling. However, the 0.17 ng/mL rise in LH that occurred between +2 and +6 d of the introduction of the radio noise, though not statistically different from the 0.31 ng/mL continued fall for the no-radio controls during the same period (Table), may be biologically significant. In the separate experiment that involved the concurrently brooded Hyline Brown pullets, plasma LH rose by 3.36 ng/mL between 2 and 6 d of the transfer from 7 to 14-h photoperiods, and AFE was advanced by 43 d. Although the rise in LH in the radio birds in the current trial was only a twentieth of this figure, it might still be indicative of a sexual response, albeit slower than a full photoperiodic one, because AFE was advanced by 13 d and this must have been associated with an earlier rise in gonadotrophin release than the controls.

The absence of a significant difference in slope or elevation between the two groups for the regression of egg weight on AFE (the common slope was an increase of 0.24 g in egg weight for each 1 d delay in AFE) indicates that radio *per se* had no direct effect on egg weight, but that the lower egg weight for the experimental birds was exclusively the consequence of their earlier AFE, with the 13-d earlier AFE fully accounting for the 3.1-g lower egg weight (13 x 0.24 = 3.1).

Notwithstanding the obvious benefits of reduced electricity usage, the replacement of light with radio noise during the part of the photoperiod that lies outside PIP, which has already been shown to sustain satisfactory egg production and improve feed conversion efficiency in laying hens (Lewis & Perry, 1990), might also impart welfare benefits to birds of all ages by encouraging them to be more docile and dissuading antisocial behaviour.

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