Effects of Electromagnetic Fields on Photophasic Circulating Melatonin Levels in American Kestrels

Kimberly Jan Fernie, David Michael Bird, and Denis Petitclerc

1Natural Resource Sciences, McGill University, Quebec, Quebec, Canada; 2Toxicology Centre and Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada; 3Agriculture and Agri-Food Canada, Lennoxtown, Quebec, Canada

Birds reproduce within electromagnetic fields (EMFs) from transmission lines. Melatonin influences physiological and behavioral processes that are critical to survival, and melan- tin has been equivalently suppressed by EMFs in mammalian species. We examined whether EMFs affect photophasic plasma melatonin in reproducing adult and fledgling American kestrels (Falco sparverius), and whether melatonin was correlated with body mass to explain previously reported results. Captive kestrel pairs were bred under control or EMF conditions for one (short-term) or two (long-term) breeding seasons. EMF exposure had an overall effect on plasma melatonin in male kestrels, with plasma levels suppressed at 42 days and elevated at 70 days of EMF exposure. The similarity in melatonin levels between EMF males at 42 days and controls at 70 days suggests a seasonal phase-shift of the melatonin profile caused by EMF exposure. Melatonin was also suppressed in long-term fledglings, but not in short-term fledglings or adult females. Melatonin levels in adult males were higher than in adult females, possibly explaining the sexually dimorphic response to EMFs. Melatonin and body mass were not associated in American kestrels. It is likely that the results are relevant to wild raptors nesting within EMFs. Key words: American kestrel, birds, electromagnetic fields, EMF, melatonin, photoperiod. Environ Health Perspect 107:901-904 (1999). [Online 13 October 1999] http://ehpnet1.niehs.nih.gov/docs/1999/107p901-904fernietable.html

Melatonin influences numerous functions critical to the survival of vertebrates. Melatonin acts as an antioxidant and free radical scavenger (1); only rats receiving high doses of melatonin were protected from the lethal effects of whole-body irradiation (2). In birds, melatonin is involved in the regulation of body temperature (3), seasonal metabolism (4), locomotor activity and feeding patterns (5), and migration (6). The hormone is associated with plumage color changes (7), which are important for mate selection in birds (8,9). Melatonin plays a key role in the growth and development of young birds (10).

Electromagnetic fields (EMFs) have suppressed the scotoperiod rise of plasma melatonin in some mammalian species (11-13), likely because EMFs were perceived as light (14,15). Birds, particularly birds of prey, are exposed to EMFs when they use transmission towers for roosting and nesting over extended (e.g., ≥3 months) and repeated (e.g., ≥3 years) periods (16). Male American kestrels (Falco sparverius) were heavier and began to molt sooner when exposed to EMFs (17). Female kestrels laid larger eggs with larger embryos, but hatching success was reduced under EMF conditions (18,19). Female nesting kestrels were heavier and larger in size when exposed to EMFs (20).

We examined whether EMFs affected plasma melatonin in captive adult and fledgling American kestrels. Plasma melatonin was also correlated with body mass of kestrels because EMF adult males and nesting females were heavier than controls, but food intake of adults was unaffected by EMF exposure (17,20). Melatonin and increases in body mass have been linked in hammers (Phodopus sungorus) and chickens (Gallus domesticus) (10,21).

Materials and Methods

Fifty-six reproducing pairs of captive American kestrels were used from the Avian Science and Conservation Centre of McGill University (Quebec, Quebec, Canada). Twenty-eight pairs were randomly assigned to the control (13 pairs) and EMF (15 pairs) rooms to identify short-term EMF effects (one breeding season). Another 13 control pairs and 15 EMF pairs, randomly selected, identified long-term EMF effects (two breeding seasons). Fledglings from short- or long-term adults will be referred to as short-term fledglings or long-term fledglings, respectively. Kestrel pairs were immediately exposed to EMFs from May 11 onward, for 91 days and for approximately 23.5 hr/day. This is comparable to the potential exposure of kestrels reproducing in the wild (18,22). Care and treatment of the birds was conducted in accordance with guidelines of the Canadian Council of Animal Care and McGill University.

Humidity, temperature, and photoperiodic clocks reflected natural conditions (45° N, 73° W) and were similar in both rooms (17,18). Noise levels and average light intensity at the birds’ head level were similar between rooms and when EMFs were on or off (*-tests, all p-values > 0.05; EMF 160 lux, control 150 lux). Noise levels indicate vibrations from EMF equipment (23).

In the EMF room, a 60-Hz electrical current created a magnetic field of 30 μT and an electric field of 10 kV/m (24). A computer provided consistent and uniform fields that were equivalent to what free-ranging kestrels are exposed to when nesting under 735-kV transmission lines running at peak capacity. The magnetic and electric fields in the control room were 2.0 μT and 0.03 kV/m, respectively.

Kestrel pairs were genetically unrelated. When paired, all adults within each sex were similar in age (2-5 years), size (wing chord) (18), body mass, and condition (body mass:wing chord index) (17). Housing and feeding conditions have been described by Fernie and Bird (17); each pair was housed in a pen of similar size (0.7 × 0.7 × 1.2 m).

Approximately 1.1 mL blood per bird was drawn from the jugular vein into a 1-mL heparinized syringe with a 27-gauge needle, then kept on ice and centrifuged (10 min, 16,000 × g) within 2 hr. Plasma was stored at -20°C until further analysis. Sampling of male kestrels occurred between 0800 and 1100 before morning feeding at 14, 42, and 70 days after pairing. Blood samples were collected between 1300 and 1500 from females at 70 days after pairing and from 35-day-old fledglings. Serum was analyzed for melatonin by the double antibody radioimmunoassay (25). The intra- and interassay coefficients of variation were 9.2 and 17.1%, respectively, which is typical of this assay (26,27).

Statistical analyses were conducted using SAS software (28) and performed separately by sex, age (adult, fledgling), and length of exposure period (short-term, long-term).

Address correspondence to K.J. Fernie, Natural Resource Sciences, McGill University, 2111 Lakeshore Rd., Ste. Anne de Bellevue, Quebec, Canada H9X 3V9. Telephone: (514) 398-7932. Fax: (514) 398-7990. E-mail: kfernie@yahoo.com

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One-way analyses of variance (ANOVA) (age) for adult males and two-way ANOVAs (laying status, age) for adult females determined potential differences before further analyses. Subsequently, melatonin data were analyzed using repeated measures ANOVA for males and one-way ANOVAs for females. Paired *t*-tests (29) were used to compare melatonin of adults between sexes. *t*-Tests were used to analyze melatonin of fledglings within and between sexes. Differences between sexes of long-term fledglings were not analyzed because of small sample sizes after treatment differences. Pearson product-moment correlation with Bonferroni correction was used to analyze associations between melatonin and body mass. Two outliers, individual observations from the first sampling period for adult males that were more than two standard deviations beyond the group mean (*p* < 0.05), were removed prior to analyses (29). Statistical significance was at the *p* < 0.05 level, with means ± standard errors reported.

**Results**

**Adults.** There were no age effects on plasma melatonin of short- or long-term adult males (one-way ANOVA, all *p*-values > 0.05); therefore, data were pooled within treatment. Short-term EMF exposure affected overall melatonin concentrations in males (*F* 2, 24 = 13.4, *p* < 0.001) (Figure 1). Specifically, plasma melatonin was similar at 14 days (*F* 1, 24 = 0.5, *p* > 0.05), then lower in EMF males than in controls at 42 days (*F* 2, 24 = 27.1, *p* < 0.001). After 70 days, melatonin in EMF males was higher than in controls (*F* 1, 24 = 9.3, *p* < 0.01) (Figure 1). Time (*F* 2, 23 = 41.4, *p* < 0.001) and treatment *×* time interactions (*F* 2, 23 = 20.3, *p* < 0.001) were significant.

Males with long-term EMF exposure showed a similar melatonin pattern (Figure 1). Overall treatment effects were significant (*F* 2, 24 = 25.6, *p* < 0.001), as were time (*F* 2, 24 = 6.1, *p* < 0.001) and treatment *×* time interactions (*F* 2, 24 = 25.6, *p* < 0.001) (Figure 1).

Again, plasma melatonin was similar between control and EMF males at 14 days (*F* 1, 25 = 1.4, *p* > 0.05), but significantly lower in EMF males than in controls at 42 days (*F* 1, 25 = 42.6, *p* < 0.001). At 70 days, plasma melatonin in EMF males was significantly higher than in controls (*F* 1, 25 = 4.9, *p* < 0.05).

Melatonin concentrations in short- and long-term EMF males at 42 days were similar to those of respective controls at 70 days (*t*-tests, all *p*-values > 0.05) (Figure 1).

Melatonin data for adult female American kestrels were pooled because there were no differences relating to age or laying status (two-way ANOVAs, all *p*-values > 0.05). Short- and long-term EMF exposure had no effect on plasma melatonin in adult females (one-way ANOVAs, all *p*-values > 0.05; overall mean 69.8 ± 2.1 pg/mL).

Within treatment groups, adult male kestrels had higher melatonin levels than did females at the end of the breeding season (paired *t*-tests, all *p*-values ≤ 0.05) (Table 1). There was no correlation between plasma melatonin levels and body mass at each sampling period for adult male or female kestrels (Pearson, all *p*-values > 0.05).

**Fledglings.** Short-term EMF exposure had no effect on plasma melatonin in 35-day-old fledglings, and there were no differences between sexes (*t*-tests, all *p*-values > 0.05). Overall mean melatonin levels were 62.3 ± 4.5 pg/mL for female fledglings and 72.5 ± 5.3 pg/mL for male fledglings.

Long-term EMF exposure suppressed plasma melatonin in 35-day-old fledglings. EMF female fledglings (44.8 ± 3.3 pg/mL) had lower melatonin levels than control females (65.4 ± 6.2 pg/mL; *t* = 3.1, *p* < 0.05). Plasma melatonin in EMF male fledglings (37.6 ± 1.5 pg/mL) was also lower than in controls (45.2 ± 2.4 pg/mL; *t* = 2.7, *p* < 0.05). Plasma melatonin and body mass were not correlated for fledglings (Pearson, all *p*-values > 0.05).

**Discussion**

To the best of our knowledge, this study is the first to examine EMF effects on melatonin in an avian species. Furthermore, our data likely represent the first melatonin profiles for a diurnal raptor species. Annual melatonin profiles have been reported for the nocturnal Indian spotted owllet (Athena brama) (27). The photophase melatonin values reported here for kestrels are consistent with other photophase pretreatment melatonin values reported for owlets (27), Japanese quail (Coturnix coturnix japonica) (30), and chickens (31,32). EMF exposure affected plasma melatonin in adult male kestrels, suppressing it midway through, and elevating it at the end of the breeding season. Long-term, but not short-term, EMF exposure of adults suppressed plasma melatonin in their fledglings. EMF exposure had no effect on plasma melatonin in adult females.

We obtained blood samples during the photophase to avoid exposing the birds to light during the scotophase. Nocturnal melatonin levels of domestic and wild birds are rapidly suppressed in < 15 min by light (33,34) and only gradually recover (31,34,35). Consequently, nocturnal blood sampling of kestrels using lights would have confounded potential EMF effects. Furthermore, by blood-sampling reproducing kestrels during the photophase, as opposed to the scotophase, we avoided disturbing hormonal patterns related to egg laying, possible reabsorption or breakage of eggs, cannibalism of eggs and young, and injury to the adults.

The timing of our blood sampling suggests that EMFs influenced melatonin levels. We cannot determine if a phase-shift occurred from our sampling design. Light for 12 or 80 min during the scotophase reduced melatonin levels to near daytime levels in pigeons (33). Control and EMF kestrels were blood-sampled 4–8 hr after lights-on, when plasma melatonin fluctuations in other birds are minimal (30,31). Furthermore, feeding, light, noise, and vibrations, which can act as

**Table 1. A comparison of photophase plasma melatonin concentrations between adult male and female American kestrels (Falco sparverius) at 70 days after pairing.**

| Experimental group | Sex   | No. | Melatonin 70 days after pairing (pg/mL) |
|--------------------|-------|-----|-----------------------------------------|
|                    |       |     | Mean ± SE t-Value (df) p-Value          |
| Short-term control | Males | 13  | 46.6 ± 3.5 -3.97 (12) 0.01             |
|                    | Females | 13 | 24.1 ± 3.0                            |
| Short-term EMF     | Males | 15  | 60.9 ± 3.1 -7.92 (14) 0.001            |
|                    | Females | 15 | 32.2 ± 4.4                            |
| Long-term control  | Males | 13  | 51.4 ± 4.8 -2.25 (12) 0.05             |
|                    | Females | 13 | 35.2 ± 4.0                            |
| Long-term EMF      | Males | 15  | 65.5 ± 4.1 -7.73 (14) 0.001            |
|                    | Females | 15 | 25.0 ± 3.5                            |

Abbreviations: *df* degrees of freedom; EMF, electromagnetic field; SE, standard error.
zeitgebers (36,37), were controlled during the experiment. Feeding, conducted at the same time daily, was alternately started in the control or EMF rooms. Light intensity, noise, and vibrations were similar between the two rooms and were similar when EMFs were on or off.

The seasonal melatonin pattern of male kestrels was altered by EMF exposure. Under EMF conditions, melatonin immediately and rapidly declined, reaching a seasonal low at midseason (42 days), then was elevated at the end of the season (70 days). In addition, the 42-day levels of EMF males were similar to those of control males at the end of the season. It is likely that the pattern is a seasonal phase-shift in the melatonin profile of the breeding season, and indicates that EMFs may be detected as light (14,15) by the birds. Birds see in ranges of the light spectrum that are invisible to humans (38); kestrels use ultraviolet light for hunting (39). Photoperiod and melatonin rhythms are closely correlated in birds (32,40), with longer photoperiods advancing photorefractoriness and molt (41,42). EMF exposure altered the birds’ response to the photoperiod, i.e., the birds responded as if it was a longer photoperiod. Consequently, the EMF males became photorefractory by 42 days and began to molt in advance of the controls (17).

Melatonin is associated with increased body mass in hamsters and chickens (10,21) but not in young Japanese quail (4). The results for the kestrel fledglings are consistent with the lack of association in young quail, but do not explain the increased body mass of EMF nesting females (20). The EMF male kestrels were heavier than controls at the end of the season, but this was not a function of increased food intake induced by EMF exposure (17). Plasma melatonin and body mass were not correlated at any time period in the reproductive season.

The higher melatonin levels of adult male kestrels as compared to female kestrels, also seen in the raptorial Indian spotted owlet (27), may partially explain the sexually dimorphic response of kestrels to EMF exposure. EMF effects on body mass in adults were observed only in male kestrels (17) and male gerbils (Meriones unguiculatus) (43). This sexually dimorphic response may indicate that reproducing adult male birds are more sensitive than females to environmental factors (44), including EMFs.

The need for prolonged continuous EMF exposure to occur before effects are observed would explain the delayed EMF effects on melatonin in adult males and long-term fledglings. Prolonged EMF exposure would occur for a minimum of 4 weeks, whereas continuous exposure is exposure without interruption. EMF effects in these kestrels only appeared after 4 weeks of continuous EMF exposure. This delay is consistent with previous EMF–melatonin research in mammals. EMF exposure suppressed serum or pineal melatonin in rats, but only after 4–6 weeks of EMF exposure (11–13,45).

It is likely that the results of this study are relevant to wild raptor populations that reproduce under EMF conditions because EMF levels and exposure times used in this study are similar to that experienced by wild kestrels (18,22). Molt and the feeding of young are energetically demanding events (46) with limited temporal overlap near the end of the nestling period in kestrels (47). The advancement of molting in adult EMF males resulted in molt overlapping with the feeding of young nestlings (17,18) when male kestrels are the sole food providers (48). However, this overlap did not affect the EMF males’ provisioning of young, as indicated by similar feeding rates (18,22), larger EMF male nestlings (20), and similar or higher EMF fledgling success (19), as compared to controls; the survival of adult EMF males was not compromised. Consequently, although an advancement of molt in wild males exposed to EMFs would likely occur, this should not have any adverse effects on adult or nestling survival.

The suppression of melatonin in long-term EMF fledglings is also expected in wild fledglings because of the similarity in EMF levels and exposure times between laboratory and natural situations. However, because melatonin may be involved in determining migratory direction (49,50), particularly during the initial migration (51), suppression of melatonin in wild fledglings raised near EMFs may affect their migratory success. This bears further investigation.

In summary, EMFs affected plasma melatonin in male American kestrels, suppressing it at 42 days and elevating it at 70 days of exposure. The similarity of plasma melatonin in EMF males at 42 days to control males at 70 days indicates a likely compression of the seasonal melatonin profile from EMF exposure. Melatonin was suppressed in long-term EMF fledglings only. Plasma melatonin in adult males was higher than in adult female kestrels, and may partially explain the sexually dimorphic response of these birds to EMFs. Melatonin and body mass were not associated in American kestrels. Changes in melatonin are likely to occur in wild raptors breeding under EMF conditions. Although melatonin changes in adult males are of seemingly minor importance to adult and nestling survival, suppression of melatonin in fledglings may be important for migration, although it bears further investigation.
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