Published in final edited form as:

Comp Biochem Physiol A Mol Integr Physiol. 2008 February ; 149(2): 181–187.

6-MBOA affects testis size, but not delayed-type hypersensitivity, in white-footed mice (*Peromyscus leucopus*)

Lynn B. Martin II^{*}, Eric M. Johnson, Chelsea R. Hutch, and Randy J. Nelson

Departments of Psychology, Neuroscience, and Evolution, Ecology, and Organismal Biology, The Ohio State University, Columbus, OH 43210, USA

Abstract

Many rodents use day length to time reproduction to occur when resources are abundant, but some species also use supplementary environmental cues. One supplementary cue is the plant-derived compound, 6-methoxy-2-benzoxazolinone (6-MBOA). Most rodents grow their gonads in response to 6-MBOA in their diets, but it is presently unknown whether they also use 6-MBOA to adjust other aspects of physiology, specifically their immune systems. 6-MBOA is structurally similar to melatonin, and seasonal changes in rodent immune activities are often mediated by melatonin. We therefore predicted that white-footed mice (*Peromyscus leucopus*), which breed seasonally and are reproductively sensitive to melatonin, would adjust their immune systems when fed 6-MBOA. 6-MBOA treated mice in long day lengths regressed their testes to a greater extent than mice fed a standard diet, or mice kept in short day lengths and fed 6-MBOA or a standard diet. One type of immune activity (delayed-type hypersensitivity) was not affected by 6-MBOA, however, although responses were greater in short versus long day mice. In sum, *P. leucopus* responded reproductively to 6-MBOA, although differently than other species; immune activity was unaffected. Other aspects of the immune system, especially in herbivorous rodents, may be affected by 6-MBOA and thus warrant further study.

Keywords

DTH; melatonin; photoperiod; reproduction; seasonal

Introduction

Seasonal changes in climate at intermediate and high latitudes lead to temporal fluctuations in resource availability for wild animals. These fluctuations are believed to have led to the evolution of seasonal breeding in many vertebrate species (Bronson 2001). Rodents often use day length, or photoperiod, to coordinate their reproductive efforts to coincide with peak food availability. Day length accurately portends changes in the environment and, unlike most other cues, is error-free (Goldman 2001). Many organisms also use other environmental cues to further adjust their phenotypes (Wingfield et al. 1980; Ball 1993; Hahn 1998). Ambient temperature (Demas et al. 1998b), precipitation (Hau et al. 2004), social interactions (Trainor

^{*}Current address and contact information for corresponding author: Lynn B Martin II, Division of Integrative Biology, Department of Biology, University of South Florida, Tampa FL 33620, USA, **Email:** lmartin@cas.usf.edu, **Phone:** (813) 974-0157, **Fax:** (813) 974-3263

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

et al. 2006), water (Nelson et al. 1995) and food quantity and quality (O'Brien et al. 2005) vary over the year and are also used to time breeding.

Plant-derived chemicals, particularly 6-methoxy-2-benzoxazolinone (6-MBOA), also fluctuate on a seasonal basis and are used to coordinate breeding in rodents (Berger et al. 1981; Sanders et al. 1981). 6-MBOA is a by-product of the spontaneous breakdown of 2, 4-dihydroxy-7-methoxy-2-11-4-benzoxin-3-(4H)-one (DIMBOA), a glucoside of growing monocotyledons (Smissman et al. 1957; Berger et al. 1981), which provides protection against herbivory and fungal and bacterial infection (Epstein et al. 1986). Because 6-MBOA is most abundant in growing plants, some rodents use the appearance of 6-MBOA in their diets as a signal that a resource flush is imminent. Free-living (Berger et al. 1981; Sanders et al. 1981) and captive (Gower et al. 1990) montane voles (*Microtus montanus*) are particularly responsive to 6-MBOA, perhaps because of their predominantly herbivorous diet; their reproductive organs grow rapidly upon exposure. Pine (*M. pinetorum*), prairie (*M. ochrogaster*), and meadow (*M. pennsylvanicus*) voles also grow their gonads in response to 6-MBOA (Schadler et al. 1988; Nelson 1991; Nelson et al. 1993; Meek et al. 1995), as do house mice (*Mus musculus*) (Nelson et al. 1990), rats (*Rattus norvegicus*) (Butterstein et al. 1985), and Syrian hamsters (*Mesocricetus auratus*) (Anderson et al. 1988; Vaughan et al. 1988). Some mammals, however, are minimally sensitive or unresponsive (Ginther et al. 1985; Willard et al. 2006).

Although the magnitude of effects of 6-MBOA varies markedly among species (Korn 1989), its pervasiveness in natural environments raises the possibility that animals might use it to orchestrate other seasonal phenotypic adjustments. Many behavioral, morphological and physiological traits besides those entailed in reproduction vary seasonally in rodents (Prendergast et al. 2001). Some aspects of immune activity are enhanced in short-day lengths and decreased in long day lengths (Nelson 2004; Martin et al. in press); oftentimes these fluctuations can be effected solely via differential photoperiod exposure. Given this seasonal fluctuation and the structural similarity between 6-MBOA and the pineal-derived hormone, melatonin, 6-MBOA may also influence one component of rodent immune activity, delayed-type hypersensitivity (DTH). Melatonin enhances several components of immune activity in vertebrates (Guerrero et al. 2002; Hotchkiss et al. 2002), and as the duration of the melatonin signal is extended by short day-lengths, melatonin is thought to be directly responsible for seasonal changes in immune activity.

If 6-MBOA acts through the same pathways as melatonin, given its structural similarity, it should enhance immune responses and inhibit reproductive activation. However, 6-MBOA is more common in the environment during long days (Smissman et al. 1957) when the melatonin signal is reduced and typically enhances, not reduces, reproductive function. Thus 6-MBOA may act through independent mechanisms to depress DTH in spite of its melatonin-like characteristics. In other words, 6-MBOA may instigate trade-offs between the immune and reproductive systems (Sheldon et al. 1996) in spite of its structural similarity to melatonin. We assessed effects of 6-MBOA on reproductive parameters and DTH in long-day breeding white-footed mice. This species was chosen because individuals i) exhibit seasonal changes in immune activity and reproductive capacity (Johnston et al. 1980b; Pyter et al. 2005b), ii) some of these changes are mediated by melatonin (Johnston et al. 1980a; Glass et al. 1988), and iii) individuals consume a variety of foods in the wild, including plants (King 1968). Other species known to be sensitive reproductively to 6-MBOA have not been as well characterized. Further, work on a congener (the deer mouse, *P. maniculatus*) indicated some reproductive sensitivity to 6-MBOA (Korn 1989), which given the similarities in ecology and evolutionary histories of each species, we expected that white-footed mice would be an appropriate model for testing our hypothesis that 6-MBOA influences aspects of immunity in rodents.

Material and Methods

Mice

Male white-footed mice were obtained from our breeding colony at The Ohio State University initiated from stock purchased from the *Peromyscus* Genetic Stock Center (University of South Carolina, Columbia, SC, USA). For the duration of the study, mice were housed in groups in polypropylene cages in a 16L:8D photoperiod (lights off 1500 h EST). At 18-21 days of age, mice were weaned and housed individually until the experiment. Ambient temperature and relative humidity were maintained at $22.5 \pm 1^\circ \text{C}$ and $50 \pm 5\%$, and all mice were given filtered tap water *ad libitum* before and throughout experiments and standard rodent chow (Harlan Teklad 8640) before the experiment. Each mouse was randomly assigned to an experimental group: MBOA, long photoperiod ($n = 8$), untreated food, long photoperiod ($n = 7$), MBOA short photoperiod ($n = 9$), untreated food short photoperiod ($n = 9$). Long photoperiod (LD) was 16L:8D and short photoperiod (SD) was 8L: 16D. All procedures were approved prior to the study by The Ohio State University and comply with the US-NIH *Guide for the Care and Use of Laboratory Animals*.

Experimental diet

A diet previously developed to assess effects of 6-MBOA on the reproductive system of prairie voles was used in the present experiment (Nelson 1991). This diet consisted (in g kg⁻¹) of ground wheat (585.8), corn (196.9), and rolled oats (196.9) supplemented with tricalcium phosphate (14.8), dyetose (5.0 (#401476) to aid pelleting), and a vitamin mix (0.6 (#300000); Dyets, Inc., Bethlehem, PA, USA) and was provided to all individuals one week after the initiation of the photoperiod exposure period and for the remainder of the experiment. The nutrient composition of this diet (% w/w) was 60.2% protein, 3.3% fat, and 11.2% carbohydrate. 6-MBOA (ChemBioChem, Salt Lake City, UT, USA) was administered (50 µg g⁻¹ food) to approximately half of the individuals in each photoperiod treatment (as above) by adding it directly to the custom-milled food during pelleting. This dosage of MBOA is comparable to the daily intake of 6-MBOA in free-living rodents of similar body size (Berger et al. 1981). Due to faulty pelleting, insufficient MBOA-treated diet remained for the last 5 days of the experiment. Thus, 250µg of MBOA (from the same stock as above) was dissolved in a small volume of 100% ethanol, and this solution mixed with distilled water. Then, 200µL of this solution was applied directly to 5g MBOA-free food (the vehicle diet above) for each mouse in the MBOA groups each day for remainder of the study; an identical volume of vehicle was added to 5g food for all remaining individuals. On most days, mice consumed all food given them. In a few instances, some food remained, and was discarded and replaced with fresh food.

Reproductive tissues

On the first day of photoperiod treatment, mice were lightly anesthetized and a small patch of fur was shaved on the abdomen to expose the right testis. Body mass (to the nearest 0.1g) and the width and length of the exposed testis were measured (to 0.01mm), and estimated testis volume (ETV) was calculated using the formula: (testis length) × (testis width)². This formula provides an accurate estimate of testis size in rodents and above a certain threshold is correlated with spermatogenic capacity of testis tissue (Pyter et al. 2005a). ETVs were then obtained for every other week for each individual over the next 6 weeks; means of intervening weeks were calculated so as to allow comparisons of ETVs of all individuals at all time points. Two weeks after the last ETV measurement, DTH was induced (see below). After DTH assessment, body mass was measured and mice were decapitated while under deep isoflurane anesthesia. Testes and epididymal fat pads were then collected, cleaned of connective tissue, and weighed to the nearest 0.1mg within 3 min.

Delayed-type hypersensitivity (DTH)

DTH was induced after 8 weeks of photoperiod/food treatments. All mice were sensitized to 2, 4-dinitro-1-fluorobenzene (DNFB; Sigma-Aldrich, St. Louis, MO, USA) by applying 50 μ L of DNFB [0.5% (w/v) in 4:1 acetone/olive oil vehicle] to a shaved area on the rump for two consecutive days while each mouse was under anesthesia (isoflurane in O₂ enriched-air) (Pyter et al. 2005b; Martin et al. 2006b). One week later, mice were again anesthetized, and thicknesses of pinnae were measured using a constant-loading micrometer (Mitituyo, Tokyo). Immediately afterwards, 20 μ L of DNFB (0.7% (w/v) in 4:1 acetone/olive oil) was applied to right pinnae, and left pinnae were treated with 20 μ L of vehicle (Martin et al. 2006b). Every 24h for the following 5d, thicknesses of both pinnae were measured; swelling was expressed as the ratio of right pinna each day relative to its pre-challenge thickness. All DNFB applications and pinnae measurements were performed between 10:00 and 14:00h, and all were made on the same region of the pinnae each by the same person. No swelling was induced in one individual, so it was not included in statistical analyses. Three mice (2 in LD and 1 in SD) exhibited swellings >2 SD larger than all other individuals (probably due to overexposure to DNFB) and were also not included in analyses.

Data analyses

All percentage data were square-root transformed prior to analysis. Transformation improved normality and variance equality (according to 1-sample Kolmogorov-Smirnov and Levene's tests, respectively); all other variables were amenable to parametric statistical analysis without transformation. ETV data and DTH were compared among groups using repeated-measures ANOVA with diet (6-MBOA versus 6-MBOA free diet) and photoperiod (LD versus SD) and their interaction as factors; subsequent *t*-tests were used to identify differences between treatment groups at different time points (after Bonferroni correction of alpha to account for repeated *t*-tests: adjusted $\alpha = 0.008$). Paired testes mass, paired epididymal fat pad mass, and terminal body mass were compared among groups using two-way univariate ANOVA with factors assigned as above. All statistics were conducted using SPSS v.12 with $\alpha \leq 0.05$.

Results

Reproductive tissues

ETV changed over the course of the experiment in all mice (time: $F_{5, 130} = 11.1$, $P < 0.001$; Fig. 1), but neither 6-MBOA treatment (time $\times$ 6-MBOA: $F_{5, 130} = 1.1$, $P = 0.37$) nor photoperiod (time $\times$ photoperiod: $F_{5, 130} = 0.33$, $P = 0.89$), nor the interaction of terms (time $\times$ 6-MBOA $\times$ photoperiod: $F_{5, 130} = 0.28$, $P = 0.92$) affected the rate of ETV change. No main effects were significant either. Comparisons of paired testes mass at the end of the experiment obviated a trend for more dramatic effects of 6-MBOA on ETV in LD (Fig. 2A). Paired testes mass after the DTH response (3 weeks after final ETVs) was larger in LD versus SD mice ($F_{1, 30} = 27.30$, $P < 0.001$), but food treatment had different effects on testes mass depending on photoperiod conditions (6-MBOA treatment $\times$ photoperiod interaction: $F_{1, 30} = 5.79$, $P = 0.02$; Fig. 2A). In LD, 6-MBOA treatment reduced paired testes mass ($t_{1, 13} = 8.76$, $P = 0.01$), but in SD, 6-MBOA had no effect ($t_{1, 17} = 1.08$, $P = 0.31$). Body mass at the end of the experiment was not affected by photoperiod ($F_{1, 30} = 0.59$, $P = 0.45$) or 6-MBOA ($F_{1, 30} = 0.17$, $P = 0.69$), nor was the interaction of 6-MBOA treatment and photoperiod significant ($F_{1, 30} = 0.22$, $P = 0.65$; Fig. 2B). Paired epididymal fat pad mass was also not affected by 6-MBOA ($F_{1, 29} = 0.65$, $P = 0.43$), photoperiod ($F_{1, 29} = 0.04$, $P = 0.85$), or the interaction of terms ($F_{1, 29} = 0.14$, $P = 0.72$; Fig. 2C).

Delayed-type hypersensitivity

DNFB challenges induced significant swelling in pinnae ($F_{5, 120} = 134.3$, $P < 0.001$), but only photoperiod affected DTH ($F_{5, 120} = 4.39$, $P = 0.001$); 6-MBOA treatment ($F_{5, 120} = 1.08$, $P = 0.38$) and the interaction of 6-MBOA treatment and photoperiod ($F_{5, 120} = 0.46$, $P = 0.80$) did not (Fig. 3). As in previous studies (Pyter et al. 2005b; Martin et al. in press), LD mice (Fig. 3A) exhibited weaker DTH to DNFB than SD mice (Fig. 3B).

Discussion

6-MBOA reduced testes mass in white-footed mice housed in long day lengths, but did not affect DTH in either photoperiod. DTH to DNFB was greater in short day versus long day-housed white-footed mice (Pyter et al. 2005b; Martin et al. 2007) in previous studies not involving 6-MBOA. Together, these results indicate that white-footed mice respond reproductively, but not immunologically (at least not in terms of DTH), to 6-MBOA. Most species, particularly herbivorous rodents, grow their gonads in response to 6-MBOA. Testes regression in LD white-footed mice suggests that this species may interpret 6-MBOA as an extension of melatonin duration unlike other species, but other hypotheses are viable. Further, it is possible that the diets used in this study may have contained phytoestrogens that obscured effects of 6-MBOA, which could explain the gonadal regression that occurred in LD-vehicle treated mice. Milling procedures prevented us from quantifying estrogens to test this hypothesis. Only a small amount of food was produced for the experiment, and no food remained at the end of our study to measure phytoestrogens directly. Regardless, 6-MBOA expedited gonadal regression, but did not influence DTH, in white-footed mice.

Melatonin mediates seasonal gonadal regression in white-footed mice. Indeed, some of the first evidence that melatonin drives seasonal gonad regression in small mammals comes from this species (Whitaker 1936). Simple SD exposure (Pyter et al. 2005a) or administration of melatonin in LD via timed injections (Hall et al. 1985) or subcutaneous (Johnston et al. 1980a) or central (i.e., anterior hypothalamus) implants (Glass et al. 1987) induce gonad regression in white-footed mice (Johnston et al. 1980a; Johnston et al. 1980b). As with other seasonal breeders, melatonin-induced gonad regression occurs when GnRH release from the hypothalamus is down-regulated (Glass et al. 1981; Glass et al. 1987), eliminating tonic LH stimulation of the gonads (Glass et al. 1988) and ultimately apoptosis of the testes (Young et al. 2000). It is surprising that others species do not respond to 6-MBOA as white-footed mice given the similarity in chemical structure between 6-MBOA and melatonin. However, to ensure that the gonadal regression in LD mice occurs via melatonin pathways, additional experiments including melatonin-treated and melatonin receptor antagonist experimental groups are critical. Indeed, it is quite possible that effects of 6-MBOA occur via β -adrenergic pathways and not as a melatonin agonist (Sweat et al. 1988).

Assuming that reproductive effects were mediated by melatonin, it is surprising that DTH was unaffected by 6-MBOA. In other rodents, prolongation of the melatonin signal by exposure to SD or via implant or timed injections enhances some aspects of immune activity (Guerrero et al. 2002; Hotchkiss et al. 2002). Circulating leukocyte numbers, *in vitro* leukocyte proliferation to mitogens, and sickness behavior (Demas et al. 1998a; Wen et al. 2007) all are enhanced by melatonin and thus would be expected to be increased in response to 6-MBOA. The effects of melatonin and/or 6-MBOA on DTH have not yet been investigated in mammals. In birds, artificial increases in melatonin increase DTH responses (Moore et al. 2003). However, a mitogen was used to induce DTH in that study, which activates the immune system via different pathways than DNFB and thus may preclude meaningful comparisons with the present study (Martin et al. 2006a).

A viable explanation of the lack of 6-MBOA effect on DTH is that the immune and reproductive systems of this species respond differently to melatonin analogs. In Siberian hamsters (*Phodopus sungorus*), reproductive and immune responses to melatonin can be dissociated. Photoperiod conditions during gestation (and consequently the duration of fetal melatonin exposure) magnified the effects of adult photoperiod on reproductive, but not immune, parameters (Prendergast et al. 2004). Another study detected similar outcomes when perinatal (i.e., from gestation until weaning) and adult photoperiods were manipulated. Hamsters exposed to SD perinatally and in adulthood exhibited the largest DTH responses, but perinatal photoperiod exposure had no additive influence on reproductive parameters (Weil et al. 2006b). Similar capacity for dissociation of photoperiod effects on reproductive and immune traits has been found in collared lemmings (*Dicrostonyx groenlandicus*) (Weil et al. 2006a) and in individual Siberian hamsters that do not respond reproductively to SD (Drazen et al. 2000).

A final interpretation of our data involves differential sensitivity to 6-MBOA among species. Omnivorous rodents, including *P. leucopus*, may be less likely than herbivorous ones to coordinate seasonal changes in phenotype using plant-derived substances (King 1968). A close congener of white-footed mice (the deer mouse) is sensitive to 6-MBOA (Macmillan 1983; McClenaghan 1987), but not very strongly. Some larger mammals are completely unresponsive (Ginther et al. 1985; Willard et al. 2006). Perhaps only rodents that are predominantly herbivorous, such as voles, lemmings, and rabbits, would use 6-MBOA to adjust their immune systems.

In conclusion, 6-MBOA induced gonadal regression in LD white-footed mice, but did not affect one measure of immune activity. Future experiments should quantify multiple aspects of immune activity in both omnivorous and herbivorous species to assess exhaustively potential effects of 6-MBOA on immunity in rodents. In sum, it is premature to conclude that rodents do not use supplementary diet cues, such as 6-MBOA, to adjust their immune systems. Given the extensive enhancive effects of 6-MBOA on reproduction in herbivorous rodents, the structural similarity of 6-MBOA and melatonin, and the pervasive effects of melatonin and photoperiod on rodent immune systems, further studies are warranted.

Acknowledgements

The authors thank Stephanie Kidder for assistance with animal care and experimental procedures. Funding for this work comes from NSF IOB grant 04-16897 to RJN.

References

- Anderson KD, Nachman RJ, Turek FW. Effects of melatonin and 6-methoxybenzoxazolinone on photoperiodic control of testis size in adult male golden-hamsters. *J Pineal Res* 1988;5:351–365. [PubMed: 3210136]
- Ball GF. The neural integration of environmental information by seasonally breeding birds. *Am Zool* 1993;33:185–199.
- Berger PJ, Negus NC, Sanders EH, Gardner PD. Chemical triggering of reproduction in *Microtus montanus*. *Science* 1981;214:69–70. [PubMed: 7025210]
- Bronson FH. Mammalian reproduction: an ecological perspective. *Biol Reprod* 2001;32:1–26. [PubMed: 3882162]
- Butterstein GM, Schadler MH, Lysogorski E, Robin L, Sipperly S. A naturally-occurring plant-compound, 6-methoxybenzoxazolinone, stimulates reproductive responses in rats. *Biol Reprod* 1985;32:1018–1023. [PubMed: 3926010]
- Demas GE, Nelson RJ. Exogenous melatonin enhances cell-mediated, but not humoral, immune function in adult male deer mice (*Peromyscus maniculatus*). *J Biol Rhythms* 1998a;13:245–252. [PubMed: 9615288]

- Demas GE, Nelson RJ. Photoperiod, ambient temperature, and food availability interact to affect reproductive and immune function in adult male deer mice (*Peromyscus maniculatus*). *J Biol Rhythms* 1998b;13:253–262. [PubMed: 9615289]
- Drazen DL, Kriegsfeld LJ, Schneider JE, Nelson RJ. Leptin, but not immune function, is linked to reproductive responsiveness to photoperiod. *Am J Physiol* 2000;278:R1401–R1407.
- Epstein WW, Rowsemitt CN, Berger PJ, Negus NC. Dynamics of 6-methoxybenzoxazolinone in winter-wheat - effects of photoperiod and temperature. *J Chem Ecol* 1986;12:2011–2020.
- Ginther OJ, Bergfelt DR, Scraba ST, Pivonka PR, Nuti LC. Effects of 6-MBOA on reproductive function in ponies, mice, rats and mink. *Theriogenology* 1985;24:587–595. [PubMed: 16726112]
- Glass JD, Dolan PL. Melatonin acts in the brain to mediate seasonal steroid inhibition of luteinizing-hormone secretion in the white-footed mouse (*Peromyscus leucopus*). *Proc Soc Exp Biol Med* 1988;188:375–380. [PubMed: 2969112]
- Glass JD, Knotts LK. A brain site for the antigonadal action of melatonin in the white-footed mouse (*Peromyscus leucopus*) - involvement of the immunoreactive GnRH neuronal system. *Neuroendocrinology* 1987;46:48–55. [PubMed: 3302747]
- Glass JD, Lynch GR. Melatonin - identification of sites of anti-gonadal action in mouse brain. *Science* 1981;214:821–823. [PubMed: 7292016]
- Goldman BD. Mammalian photoperiodic system: Formal properties and neuroendocrine mechanisms of photoperiodic time measurement. *J Biol Rhythms* 2001;16:283–301. [PubMed: 11506375]
- Gower BA, Berger PJ. Reproductive responses of male *Microtus montanus* to photoperiod, melatonin, and 6-MBOA. *J Pineal Res* 1990;8:297–312. [PubMed: 2203893]
- Guerrero JM, Reiter RJ. Melatonin-immune system relationships. *Curr Top Med Chem* 2002;2:167–179. [PubMed: 11899099]
- Hahn TP. Reproductive seasonality in an opportunistic breeder, the red crossbill, *Loxia curvirostra*. *Ecology* 1998;79:2365–2375.
- Hall ES, Makoul GT, Lynch GR, Anderson G. Effects of timed melatonin injections on reproduction in pinealectomized *Peromyscus leucopus*. *Gen Comp Endocrinol* 1985;58:407–414. [PubMed: 4007479]
- Hau M, Wikelski M, Gwinner H, Gwinner E. Timing of reproduction in a Darwin's finch: temporal opportunism under spatial constraints. *Oikos* 2004;106:489–500.
- Hotchkiss AK, Nelson RJ. Melatonin and immune function: Hype or hypothesis? *Crit Rev Immunol* 2002;22:351–371. [PubMed: 12803316]
- Johnston PG, Zucker I. Antigonadal effects of melatonin in white-footed mice (*Peromyscus leucopus*). *Biol Reprod* 1980a;23:1069–1074. [PubMed: 7193487]
- Johnston PG, Zucker I. Photoperiodic regulation of reproductive development in white-footed mice (*Peromyscus leucopus*). *Biol Reprod* 1980b;22:983–989. [PubMed: 7397311]
- King, J. Biology of *Peromyscus* (Rodentia). In: Special Publication No. 2. The American Society of Mammalogists; Stillwater, OK: 1968.
- Korn H. A feeding experiment with 6-methoxybenzoxazolinone and a wild population of the deer mouse (*Peromyscus maniculatus*). *Can J Zool* 1989;67:2220–2224.
- Macmillan BT. The effects of 6-methoxybenzoxazolinone (6-MBOA) on testis growth in prepubertal deer mice raised in an inhibitory photoperiod. *Fed Proc* 1983;42:614–614.
- Martin LB, Han P, Lewittes J, Kuhlman JR, Klasing KC, Wikelski M. Phytohemagglutinin (PHA) induced skin swelling in birds: histological support for a classic immunoeological technique. *Funct Ecol* 2006a;20:290–300.
- Martin LB, Weil ZM, Kuhlman JR, Nelson RJ. Trade-offs between cutaneous immune responses in female white-footed mice (*Peromyscus leucopus*). *Funct Ecol* 2006b;20:630–636.
- Martin LB, Weil ZM, Nelson RJ. Immune defense and reproductive pace of life in *Peromyscus* mice. *Ecology* 2007;88:2516–2528. [PubMed: 18027755]
- Martin LB, Weil ZM, Nelson RJ. Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. *Phil Trans Roy Soc Lond B*. in press
- McClenaghan LR. Lack of effect of 6-MBOA on reproduction in *Dipodomys merriami*. *J Mammal* 1987;68:150–152.

- Meek LR, Lee TM, Gallon JF. Interaction of maternal photoperiod history and food type on growth and reproductive development of laboratory meadow voles (*Microtus pennsylvanicus*). *Physiol Behav* 1995;57:905–911. [PubMed: 7610143]
- Moore CB, Siopes TD. Melatonin enhances cellular and humoral immune responses in the Japanese quail (*Coturnix coturnix japonica*) via an opiate mechanism. *Gen Comp Endocrinol* 2003;131:258–263. [PubMed: 12714007]
- Nelson RJ. Maternal diet influences reproductive development in male prairie vole offspring. *Physiol Behav* 1991;50:1063–1066. [PubMed: 1805270]
- Nelson RJ. Seasonal immune function and sickness responses. *Trends Immunol* 2004;25:187–192. [PubMed: 15039045]
- Nelson RJ, Blom JMC. 6-methoxy-2-benzoxazolinone and photoperiod - prenatal and postnatal influences on reproductive development in prairie voles (*Microtus ochrogaster ochrogaster*). *Can J Zool* 1993;71:776–789.
- Nelson RJ, Gubernick DJ, Blom JMC. Influence of photoperiod, green food, and water availability on reproduction in male California mice (*Peromyscus californicus*). *Physiol Behav* 1995;57:1175–1180. [PubMed: 7652040]
- Nelson RJ, Shiber JR. Photoperiod affects reproductive responsiveness to 6-methoxy-2-benzoxazolinone in house mice. *Biol Reprod* 1990;43:586–591. [PubMed: 2289012]
- O'Brien S, Hau M. Food cues and gonadal development in neotropical spotted antbirds (*Hylophylax naevioides*). *J Ornithol* 2005;146:332–337.
- Prendergast BJ, Bilbo SD, Dhabhar FS, Nelson RJ. Effects of photoperiod history on immune responses to intermediate day lengths in Siberian hamsters (*Phodopus sungorus*). *J Neuroimmunol* 2004;149:31–39. [PubMed: 15020062]
- Prendergast BJ, Kriegsfeld LJ, Nelson RJ. Photoperiodic polyphenisms in rodents: Neuroendocrine mechanisms, costs, and functions. *Q Rev Biol* 2001;76:293–325. [PubMed: 11561508]
- Pyter LM, Hotchkiss AK, Nelson RJ. Photoperiod-induced differential expression of angiogenesis genes in testes of adult *Peromyscus leucopus*. *Reproduction* 2005a;129:201–209. [PubMed: 15695614]
- Pyter LM, Neigh GN, Nelson RJ. Social environment modulates photoperiodic immune and reproductive responses in adult male white-footed mice (*Peromyscus leucopus*). *Am J Physiol* 2005b;288:R891–R896.
- Sanders EH, Gardner PD, Berger PJ, Negus NC. 6-methoxybenzoxazolinone - a plant derivative that stimulates reproduction in *Microtus montanus*. *Science* 1981;214:67–69. [PubMed: 7025209]
- Schadler MH, Butterstein GM, Faulkner BJ, Rice SC, Weisinger LA. The plant metabolite, 6-methoxybenzoxazolinone, stimulates an increase in secretion of follicle-stimulating-hormone and size of reproductive-organs in *Microtus pinetorum*. *Biol Reprod* 1988;38:817–820. [PubMed: 3135852]
- Sheldon BC, Verhulst S. Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol Evol* 1996;11:317–321.
- Smissman EE, Lapidus JB, Beck SD. Isolation and synthesis of an insect resistance factors from corn plants. *J Am Chem Soc* 1957;79:4697–4698.
- Sweat FW, Berger PJ. Uterotropic 6-Methoxybenzoxazolinone Is an Adrenergic Agonist and a Melatonin Analog. *Mol Cell Endocrinol* 1988;57:131–138. [PubMed: 2899525]
- Trainor BC, Martin LB, Greiwe K, Kuhlman JR, Nelson RJ. Social and photoperiod effects on reproduction in 5 *Peromyscus* species. *Gen Comp Endocrinol* 2006;148:252–259. [PubMed: 16626709]
- Vaughan MK, Little JC, Powell DC, Puigdomingo M, Reiter RJ. Melatonin and 6-methoxy-2-benzoxazolinone (6-MBOA) alter the response of the male Syrian hamster to natural photoperiod. *Int J Biometeorology* 1988;32:103–107.
- Weil ZM, Martin LB, Nelson RJ. Photoperiod differentially affects immune function and reproduction in collared lemmings (*Dicrostonyx groenlandicus*). *J Biol Rhythms* 2006a;21:384–393. [PubMed: 16998158]
- Weil ZM, Pyter LM, Martin LB, Nelson RJ. Perinatal photoperiod organizes adult immune responses in Siberian hamsters (*Phodopus sungorus*). *Am J Physiol* 2006b;290:R1714–1719.

- Wen J, Dhabhar FS, Prendergast BJ. Pineal-dependent and independent effects of photoperiod on immune function in Siberian hamsters (*Phodopus sungorus*). *Horm Behav* 2007;51:31–39. [PubMed: 17022983]
- Whitaker WL. Effect of light on reproductive cycle of *Peromyscus leucopus noveboracensis*. *Proc Soc Exp Biol Med* 1936;34:329–330.
- Willard ST, Dickerson T, Dodson R, Weis A, Godfrey RW. Administration of 6-methoxybenzoxazolinone (MBOA) does not augment ovulatory responses in St. Croix White ewes superovulated with PMSG. *Anim Reprod Sci* 2006;93:280–291. [PubMed: 16154300]
- Wingfield JC, Farner D. Environmental and endocrine control of seasonal reproduction in temperate zone birds. *Prog Reprod Biol* 1980;56:62–101.
- Young KA, Zirkin BR, Nelson RJ. Testicular regression in response to food restriction and short photoperiod in white-footed mice (*Peromyscus leucopus*) is mediated by apoptosis. *Biol Reprod* 2000;62:347–354. [PubMed: 10642572]

Fig. 1.

- 6-MBOA expedites regression of estimated testes volume (ETV) in long-day (A), but not short-day (B) *Peromyscus leucopus*. Bars are means $\pm$ SEM.

Fig. 2.
- 6-MBOA affects final paired testes mass (A), but not body mass (B) or epididymal fat pad mass (C) in *Peromyscus leucopus*. Bars are means $\pm$ SEM. Asterisks denote significance by independent samples *t*-test ($P \leq 0.05$); horizontal bar indicates significant photoperiod effect by ANOVA ($P \leq 0.05$).

Fig. 3.
 - 6-MBOA does not affect delayed-type hypersensitivity (DTH) in *Peromyscus leucopus*, although DTH is reduced in long day (A) versus short day (B) housed individuals. Bars are means $\pm$ SEM.