Short Days Increase Hypothalamic-Pituitary-Adrenal Axis Responsiveness

Leah M. Pyter, Jaimie D. Adelson, and Randy J. Nelson

Departments of Neuroscience and Psychology, and Institute of Behavioral Medicine Research, Ohio State University, Columbus, Ohio 43210

Individuals dramatically alter physiology and behavior to adapt to seasonal changes in their environment. To cope with winter stressors such as reduced food availability and low temperatures, central stress responses are presumably modulated at the level of the hypothalamic-pituitary-adrenal (HPA) axis, but the details remain unspecified. We examined the effects of long or short photoperiods (day lengths) on corticosterone responses to restraint, HPA negative feedback sensitivity, glucocorticoid receptor gene expression in the hippocampus, the role of corticosterone in spatial learning, and corticosterone responses to stressors associated with the spatial water maze task in adult male white-footed mice (Peromyscus leucopus). Short days increased corticosterone responses to restraint, increased hippocampal glucocorticoid receptor expression, enhanced corticosterone negative feedback on the HPA axis, and increased sensitivity to dexamethasone suppression of corticosterone. Although spatial learning and memory performance (via water maze) of all mice was impaired after pharmacological corticosterone inhibition, both water maze exposure and treatment injections alone were sufficient to increase short-day, but not long-day, corticosterone concentrations. Thus, the effects of corticosterone on spatial learning in these mice may be complicated by photoperiodic differences in stressor response to the learning task itself. Overall, these results suggest that photoperiod-evoked modification of the HPA axis and its potential behavioral consequences may be adaptive for winter survival. (Endocrinology 148: 3402–3409, 2007)

MANY ANIMALS USE day length (photoperiod) to predict and adjust to seasonal changes in the environment. Among seasonal phenomena, regulation of seasonal breeding is best understood (1). Most nontropical rodents stop breeding in the autumn when day lengths fall below a critical minimum. Light information is converted into a neural signal in the retina, travels to the suprachiasmatic nuclei of the hypothalamus, and then to the pineal gland in which it is transduced into a hormonal signal encoding day length (1, 2). Melatonin is secreted into circulation from the pineal exclusively in the dark; thus, the nightly duration of melatonin secretion provides photoperiodic information to all cells in the body (3, 4). In nontropical rodents, short days (i.e. long nightly durations of melatonin secretion) inhibit the hypothalamus-pituitary-gonadal axis, causing autumnal regression of the reproductive system, adjustments in other physiological systems, and dramatic changes in behavior (5).

For example, short days reduce brain size, impair hippocampal-based spatial learning and memory, reduce hippocampal volume, and decrease CA1 hippocampal spine density in adult male white-footed mice (Peromyscus leucopus) (6). Impaired spatial performance and reduced brain tissue outside of the breeding season, when home range size decreases and mate-seeking behaviors diminish in rodents, may conserve energy in the winter and promote survival similar to winter suppression of breeding (7). The mechanisms underlying seasonal plasticity of brain and behavior remain unspecified. Because spatial learning and memory performance and adult hippocampal plasticity are influenced by steroid hormones including glucocorticoids (8–10), we hypothesized that photoperiod-evoked changes in glucocorticoid regulation and stress responses underlie seasonal plasticity in brain and learning and memory performance.

The hypothalamic-pituitary-adrenal (HPA) feedback loop involves glucocorticoids produced in the adrenal cortex, which feed back on the hypothalamus and pituitary to inhibit CRH and ACTH, respectively. Glucocorticoids also bind to receptors in the hippocampus, which suppresses the HPA axis (11). The HPA axis regulates energy availability within the body by continuously readjusting mobilized energy levels after disruptions to homeostasis (i.e. allostatics) (12). This fragile balance is challenged vigorously by seasonally changing environmental conditions (i.e. seasonal fluctuations in temperature, food availability, agnostic interactions, etc.) (13). However, seasonal regulation of the HPA axis and response to stressors are poorly understood. We report here that short days increase corticosterone responses to restraint, alter negative feedback regulation of the HPA axis as measured by differential glucocorticoid receptor (GR) expression in the brain and dexamethasone suppression of corticosterone, and may alter spatial learning and memory via task-associated stress responses.

Materials and Methods

Animals

One hundred forty-three (>55 d of age) male white-footed mice (P. leucopus) from our breeding colony were used in these experiments. Animals were housed individually in polypropylene cages (27.8 × 7.5 × 13 cm) with a constant temperature and humidity of 21 ± 5 C and 50 ±
10%, respectively, and ad libitum access to food (8640 rodent diet; Harlan Teklad, Indianapolis, IN) and filtered tap water. Mice were housed in either long days [16 h light/d; lights illuminated at 2300 h Eastern Standard Time (EST)] or in reversed short days (8 h light/d; lights illuminated at 0700 h EST). Siblings were pseudorandomly distributed among all groups. The reproductive system of one mouse from experiment 3 did not respond to short days and was removed from the study. All animals were housed with approval of the Ohio State Institutional Animal Care and Use Committee and were conducted in compliance with all United States federal animal welfare requirements.

**Experiment 1: effects of photoperiod on corticosterone responses to restraint**

Thirty-eight mice were used in this experiment. All mice were exposed to their respective photoperiod conditions for 12 wk before testing. We operationally define the acute restraint procedures used in this experiment as a physical and psychological stressor based on the subsequent elevation of corticosterone concentrations (14). Mice from each photoperiod were further divided into three subgroups: 1) 1 h of restraint followed by an immediate blood collection (long day, n = 6; short day, n = 6); 2) 1 h of restraint with delayed blood collection (long day, n = 6; short day, n = 7); or 3), no restraint (long day, n = 6; short day, n = 6). Restrained mice were placed in well-ventilated, Plexiglas tubes without compression for 1 h (0900–1000 h EST). Immediately before these treatments, a 50 μl retro-orbital baseline blood sample was collected from all mice (0800–0900 h EST). Posttreatment trunk blood was collected after rapid decapitation. Mice with delayed blood collections were returned to their home cage for 1 h after restraint before trunk blood was collected. Unrestrained mice remained undisturbed in their home cage while the other mice were restrained and trunk blood was collected at the same time as the immediate group. Body mass was recorded, and paired testes were removed and weighed for all mice to determine reproductive responsiveness to photoperiod treatment. All blood samples were stored on ice until centrifuged at 3000 rpm for 30 min at 4°C. Plasma was removed from the samples and stored at −70°C until hormone analyses.

**Experiment 2: effects of photoperiod on corticosterone negative feedback**

Corticosterone receptor [GR and mineralocorticoid receptor (MR)] mRNA expression in the brain. Tissues from 24 mice were used for this experiment. The mice were a random subset of mice from a previous study used to look at the effects of photoperiod on gene expression in the testis (15). All mice were exposed to their respective photoperiod conditions for either 7 wk (n = 6 per photoperiod) or 14 wk (n = 6 per photoperiod). Body mass was recorded biweekly. At 7 or 14 wk, mice were rapidly decapitated (1000–1200 h EST), brains were removed, and the hippocampus and hypothalamus were dissected and stored in RNA Later solution (Qiagen, Valencia, CA) at −70°C until RNA processing. These regions of the brain are involved in the feedback loop that regulates corticosterone release (12).

**RNA extraction.** Total RNA was extracted from at least 30 mg of individual hippocampi and hypothalami using a homogenizer (Ultra-Turrax T8; IKA Works, Wilmington, NC) with an RNeasy Mini kit according to the protocol of the manufacturer (Qiagen). Extracted RNA was suspended in 30 μl ribonuclease-free water, and RNA concentration was determined by spectrophotometer (SmartSpec 3000; Bio-Rad, Hercules, CA). All RNA samples were stored at −70°C until additional analysis. cDNA was created via reverse transcription of 2 μg of RNA from each sample with Moloney murine leukemia virus reverse transcriptase enzyme (Invitrogen) according to the protocol of the manufacturer.

**Gene sequencing.** To design primers and a probe for quantitative PCR (qPCR) with high specificity for this species, a portion of each gene of interest was sequenced. To sequence portions of these genes, semiquantitative PCR was conducted on 1 μl of pooled white-footed mice hippocampal cDNA with Tag DNA Polymerase enzyme (Invitrogen) according to the protocol of the manufacturer in a thermocycler for 40 cycles (Bio-Rad). Degenerate primers were designed based on conserved regions among multiple species with known gene sequences (GenBank) using PrimerExpress software (Applied Biosystems, Foster City, CA). PCR gene product amplification was visualized on 2% Tris-acetate-EDTA agarose gels containing ethidium bromide using a CCD camera. To verify amplification of correct gene, PCR products were purified (Centricor-100; Millipore, Billerica, MA) and sequenced at the Plant Genomics Center at Ohio State University. The resulting amplicon sequences that were more than 90% homologous to the Mus gene of interest were assumed to be the correct P. leucopus gene of interest. Sequencing information was entered in GenBank database: GR, accession no. DQ358741; MR, accession no. DQ358742.

qPCR. After confirmation of gene products, primers and probes for qPCR were designed using PrimerExpress. Primers and probes were synthesized as follows, with probes labeled with 6-carboxyfluorescein and minor groove binder (nonfluorescent quencher) at the 5′ and 3′ ends, respectively: GR forward, 5′-CCCAAGTGAACAGACAGAAA-GATGA-3′; GR reverse, 5′-GCCCATTTCTTCTGTTAAATACC-3′; GR probe, 5′-TTCATGAATTTGCCACCC-3′; MR forward, 5′-GCCGTTTCTCGTGTCCTAC-3′; MR reverse, 5′-TCTGCTTTTCTCCTGTCCT-3′; and MR probe, 5′-TCCAAAACACAGTACTCAACG-3′. A TaqMan 18S ribosomal RNA primer and probe set (labeled with VIC; Applied Biosystems) was used as the control gene for relative quantification. Amplification was performed on an Applied Biosystems 7000 Sequencing Detection System by using Taqman Universal PCR Master Mix. The universal two-step RT-PCR cycling conditions used were as follows: 10 min at 95°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. Relative gene expression of individual samples run pseudorandomly in duplicate was calculated by comparison with relative standard curve consisting of serial dilutions of pooled *P. leucopus* hippocampal cDNA (1:1, 1:10, 1:100, and 1:1000), followed by normalization to 18S rRNA gene expression.
and 2020 PLUS tracking software (HVS Image, Buckingham, UK) was used. Mice were handled using a small fishing net to avoid the stress of direct handling. On d 1, mice were allowed to swim freely for 60 sec without a platform to acclimate to the pool. On d 2–5, a platform (9-cm diameter) was hidden 0.5 cm below the water surface in one quadrant. Mice were given two blocks of trials per day for a total of eight blocks of trials. Each block consisted of three 60-sec trials during which the mice were trained to locate the hidden platform from random release points around the pool to “escape” from the water. On reaching the platform or after 60 sec, mice were placed on the platform for 10 sec and then returned to the home cage. The intertrial intervals were approximately 15 sec during which the pool was skimmed of debris (e.g., feces or bedding). Latency to reach the platform, the distance of the mouse’s path, and the path resolved were recorded by the system for each trial to assess acquisition of the spatial task. After each block of trials, mice received a piece of tissue paper in their cage to expedite drying. On d 6, the platform was removed, and a 60-sec probe trial was run to examine retention of spatial memory. The percentage of time spent in each quadrant (including the quadrant in which the platform had been) was recorded. To evaluate reversal learning, the platform was repositioned in a different quadrant, and retraining to the new location on d 7–8 (a total of four blocks of trials) was completed as described previously. A second probe trial followed reversal training on d 9. On d 10, a single 60-sec visible platform trial was run to determine general visual acuity of the mice in this paradigm. The visible platform (9-cm diameter) was raised 0.5 cm above water level and was encircled with a black rim. Latency to reach the platform was recorded. Testing and treatments were divided into two groups of mice representing all experimental treatments and were staggered by 1 wk.

**Experiment 4: effects of water maze experience and injection exposure on corticosterone concentrations**

Based on the results from the previous experiments, this experiment was designed to determine whether or not parameters of the treatments themselves, i.e. swim speed and water maze experience, produce different stress responses between photoperiod groups and therefore potentially confound the behavioral results. Mice used in this experiment were described previously for the water maze study (experiment 3; see below for description of how mice were balanced for use in experiments 3 and 4).

**Water maze as a stressor.** Three days before water maze, a 50 µL retro-orbital blood sample was collected (between 1400 and 1430 h EST) from each mouse to compare basal corticosterone concentrations. Additional 50 µL retro-orbital blood samples were collected 1 h after (between 1400 and 1430 h EST) the 60-sec free swim and both 60-sec probe trials (see description of water maze paradigm). Samples were collected after these trials because all mice swim in the water maze for the same duration regardless of treatment (60 sec). Blood samples were collected and stored as described previously.

**Injection exposure as a stressor.** To determine corticosterone responses to an injection alone, mice received a single ip injection of 0.1 ml saline, and blood samples were taken 1 and 2 h after injection. Half of the mice representing all experimental treatments received the saline injection 2 d before all water maze testing and drug injections to measure the “naïve” corticosterone response to an injection. The remaining mice received the saline injection 1 d after the completion of water maze testing and injection treatment (“experienced”) to measure the potential habituation of corticosterone responses to injection. Blood samples were collected and stored as described previously.

**RIAs**

Plasma corticosterone concentrations were determined using a single 125I kit purchased from ICN Biomedicals (Costa Mesa, CA) for each experiment. Each sample was assessed in duplicate in a single assay according to the protocol of the manufacturer with two exceptions. Because corticosterone concentrations in Peromyscus are high relative to Mus musculus and Rattus rattus, serum was diluted 5.2-fold more than recommended for domestic rodents, and two additional standard dilutions were added to the low end of the standard curve (5 and 10 ng/ml). Cross-reactivity with other steroid hormones is less than 0.5%.

**Statistical analyses**

Within-subject, repeated-measures ANOVAs were used to compare water maze performance over time. Within-days, pairwise post hoc comparisons were planned a priori in the analysis models and were conducted using two-tailed Student’s t tests (18). Two-way ANOVAs or Student’s t tests were used for physiological comparisons depending on the number of groups. Data with unequal variances were compared using nonparametric Mann-Whitney tests to compare photoperiod differences. All comparisons were considered statistically significant when P < 0.05. StatView software was used for all analyses (version 5.0.1; SAS, Cary, NC).

**Results**

**Experiment 1**

Twelve weeks of short days reduced mean ± SEM relative and absolute testes masses (absolute: long day, 336.1 ± 23.8 mg; short day, 180.4 ± 24.6 mg; relative: long day, 16.7 ± 1.7 mg/g body mass; short day, 9.1 ± 1.2 mg/g body mass) (P < 0.001 in both cases, main effect of photoperiod). Body mass did not differ between photoperiods at wk 12 (P > 0.05; data not shown).

Based on two-way ANOVA tests, immediately after 1 h restraint, corticosterone concentrations increased in both short- and long-day mice (Fig. 1) (P < 0.001, F(1,31) = 9.49, main effect of restraint). However, the increase in short-day mice relative to unrestrained controls was greater than in long-day mice (Fig. 1) (P < 0.05, post hoc, t(9) = 3.02). Corticosterone concentrations in unrestrained mice did not differ between photoperiods (Fig. 1) (P > 0.05, post hoc t test). Corticosterone concentrations remained elevated in long-day mice 1 h after restraint but decreased significantly in short-day mice (P < 0.005, post hoc, t(10) = 3.7) to concentrations comparable with those of unrestrained mice. The same statistical differences were achieved after normalization of corticosterone responses to those of unrestrained controls within the same photoperiod. Baseline corticosterone values are high in P. leucopus relative to other rodent species, although our values fall within the normal baseline range for the species (51, 52).

![Graph showing corticosterone concentrations](image-url)
**Experiment 2**

**GR gene expression.** The mice used for this experiment were a random subset of mice used in a previous study that reported significant decreases in reproductive tissue masses after 7 and 14 wk of short days and changes in gene expression in reproductive tissue (18).

Based on two-way ANOVA tests, GR or MR gene expression in the hippocampus did not differ between photoperiod after 7 or 14 wk (Fig. 2, A and B) (P > 0.05 in all cases). However, both MR and GR expression increased between 7 and 14 wk in short-day hippocampi (Fig. 2, A and B) (P < 0.05 in both cases, post hoc, t(9) = 5.0 and t(9) = 4.3). In the hypothalamus, time spent in either photoperiod treatment reduced GR gene expression (Fig. 2A) (P < 0.05 in both cases, main effect of time, F(1,24) = 3.5), although photoperiodic differences were not significant within individual weeks (P > 0.05, post hoc t tests). Short days significantly reduced hypothalamic MR gene expression relative to long days after 14 wk (P < 0.05, post hoc, t(12) = 2.0), and time spent in photoperiod treatment increased hypothalamic MR expression (Fig. 2B) (P < 0.001, main effect of time, F(1,24) = 28.2).

**Dexamethasone suppression test.** Dexamethasone significantly reduced corticosterone concentrations in short-day mice (Fig. 2C) (P < 0.05, post hoc, t(9) = 2.9) but not long-day mice (P > 0.05). Saline-treated long- and short-day mice did not differ in corticosterone concentrations based on a post hoc t test (Fig. 2C) (P > 0.05).

**Experiment 3**

Fourteen weeks of short days reduced relative and absolute testes (absolute: long day, 383.6 ± 29.0 mg; short day, 112.7 ± 8.7 mg, t(34) = 9.4; relative: long day, 17.8 ± 1.1 mg/g body mass; short day, 6.2 ± 0.4 mg/g body mass, t(34) = 10.4) and epididymal fat pad (absolute: long day, 357.3 ± 39.3 mg; short day, 139.2 ± 16.5 mg, t(34) = 5.3; relative: long day, 16.3 ± 1.5 mg/g body mass; short day, 7.6 ± 0.8 mg/g body mass, t(34) = 5.4) masses relative to long days (P < 0.001 in all cases). Twelve and 14 wk of short days also significantly reduced body mass compared with long days (12 wk: long day, 20.3 ± 0.6 g; short day, 18.2 ± 0.5 g, t(34) = 2.6; 14 wk: long day, 21.3 ± 0.6; short day, 18.1 ± 0.4, t(34) = 4.4; P < 0.05 in both cases).

The latency to reach the hidden platform decreased over blocks of trials in all groups (Fig. 3, A and B) (P < 0.001, main effect, F(7,252) = 19.0). Short days decreased the latency to reach the hidden platform compared with long days on blocks 5 and 6 in control (noninjected) mice (Fig. 3A) (P < 0.05, post hoc, t(12) = 2.9 and t(12) = 2.4). Corticosterone synthesis inhibition increased the latency to reach the hidden platform compared with saline-injected controls (Fig. 3B) (P < 0.05, main effect, F(7,196) = 2.2). Post hoc analysis revealed that short days reduced the latency to reach the hidden platform compared with short metyrapone-treated mice on block 7 (Fig. 3B) (P < 0.05, F(7,196) = 2.2). The path length to reach the hidden platform decreased over blocks of trials in all groups (data not shown) (P < 0.001, main effect, F(7,252) = 10.0). Short days increase the path length compared with long days in control mice on blocks 5 and 6 (data not shown) (P < 0.05, post hoc, t(12) = 2.8 and t(12) = 2.3). Short days also reduced the path length over blocks of trials (P < 0.05, main effect, F(7,196) = 2.4), whereas metyrapone increased the path length compared with saline treatment (data not shown) (P < 0.05, main effect, F(7,196) = 2.4). Swim speed increased over blocks of trials (P < 0.001, F(7,252) = 7.0), but metyrapone decreased swim speed relative to saline treatment (data not shown) (P < 0.05, F(7,196) = 2.4). Mice from all groups spent more time in the quadrant of the pool from which the platform had been removed (quadrant 1) during the first memory probe trial compared with all other quadrants (data not shown) (P < 0.01, F(3,126) = 30.2). Although metyrapone appeared to decrease the time spent in quadrant 1, this difference was not statistically significant (P = 0.2).

Throughout reversal training to a hidden platform, latency to reach the hidden platform decreased over blocks of trials in all groups (Fig. 3C) (P < 0.002, F(3,210) = 5.7). Metyrapone significantly increased the latency to reach the platform compared with saline treatment (Fig. 3C) (P < 0.05, F(1,28) = 6.8). No significant differences in reversal parameters were observed between long- and short-day control mice (data not shown) (P > 0.05). Similarly, the path length to reach the platform decreased over blocks of trials in all groups, and metyrapone increased the path length (data not shown) (P < 0.05, F(3,210) = 24.4 and F(3,84) = 3.0). Swim speed was not significantly affected by metyrapone (P = 0.07), but post hoc analyses revealed that metyrapone decreased swim speed on blocks 1 and 3 of reversal training (data not shown) (P < 0.05, t(26) = 10.4 and t(26) = 10.9). Mice persisted in spending more time in the quadrant from which the platform had been removed from the original hidden platform trials (quadrant 1).
1; data not shown) \( P < 0.05, \text{F}(3,120) = 4.4 \) and did not spend more time in the quadrant from which the platform had been removed during the more recent reversal training (quadrant 3; data not shown) \( P > 0.05 \). However, saline-treated mice in long days spent less time in quadrant 1 and more time in quadrant 3 than metyrapone-treated mice in long days, although the interaction between time spent in these quadrants and drug treatment was not statistically significant (data not shown) \( P = 0.1 \). No differences in latency or path length to reach the visible platform were observed among groups (data not shown) \( P > 0.05 \) in all cases), although metyrapone reduced swim speed \( P < 0.05, \text{F}(1,28) = 4.6 \).

**Experiment 4**

**Water maze stressor.** Short days failed to significantly increase baseline corticosterone concentrations relative to long days 30 min before lights off (long day, \( 487.1 \pm 65.0 \) ng/ml; short day, \( 555.6 \pm 69.8 \) ng/ml; \( P > 0.05, \text{F}(1,28) = 0.9 \)). After the initial exposure to water maze (60-sec free swim), corticosterone concentrations were increased in short-day mice \( P < 0.002, \text{post hoc, } t(12) = 2.3 \) but not long-day mice (Fig. 4A) \( P > 0.05 \). The elevation in short-day corticosterone concentrations in response to a 60-sec probe trial mid-water maze was attenuated to concentrations similar to baseline and long-day mice (Fig. 4A). Similarly, no differences in corticosterone responses to a 60-sec probe trial at the end of water maze training were observed between photoperiods or compared with baseline concentrations (Fig. 4A) \( P > 0.05 \). Metyrapone significantly decreased corticosterone concentrations in all mice in the beginning (free swim), middle (probe 1), and end (probe 2) of water maze training compared with baseline and saline-treated control concentrations (Fig. 4A) \( P < 0.001, \text{F}(1,28) = 236.1; \text{F}(1,28) = 192.7, \text{and F}(1,28) = 40.6 \). The differences in corticosterone concentrations observed during the water maze testing remained significant after normalization of all values to baseline concentrations.

**Injection stressor.** One hour after a saline injection, short-day mice displayed an elevated corticosterone response compared with long-day mice before exposure to water maze/drug paradigm (Fig. 4B) \( P > 0.05 \). Corticosterone concentrations increased 2 h after a saline injection compared with 1 h \( P < 0.05, \text{F}(1,63) = 3.9 \); post hoc analyses revealed that this increase was primarily attributable to the prewater maze groups (Fig. 4B) \( P < 0.05, \text{post hoc, } t(132) = 5.7 \). However, the short-day increase in corticosterone concentrations 2 h after saline injection was not significantly greater than the long-day increase \( P > 0.05 \). Short-day mice that had completed the water maze training displayed a greater corticosterone elevation compared with long-day mice 2 h after saline injections (Fig. 4B) \( P < 0.05, \text{post hoc, } t(15) = 2.6 \).

**Fig. 4.** Water maze experience and injection exposure differentially affects corticosterone concentrations between photoperiod. A, Water maze-induced corticosterone concentrations at the beginning (free swim), middle (probe 1), and end (probe 2) of training in mice injected with metyrapone (met) or saline-injected mice. *, \( P < 0.05 \) between injection treatments within photoperiod; #, \( P < 0.05 \) between photo periods of saline-treated mice. B, Saline injection-induced corticosterone concentrations 1 and 2 h after injection in mice naive (Pre-MWM (Morris water maze)) and experienced (Post-MWM) to water maze training. *, \( P < 0.05 \).
Discussion

These experiments demonstrate a link among photoperiod, stress responsiveness, brain receptiveness, and behavior. The results have physiological, behavioral, ecological, and clinical relevance. Short days increased HPA responsiveness to restraint, as well as increased sensitivity to HPA negative feedback illustrated by increased GR and MR expression in the hippocampus, faster return to basal corticosterone concentrations after restraint, and increased sensitivity to dexamethasone suppression of corticosterone. These short-day alterations in HPA regulation likely have functional consequences with respect to spatial learning and memory. Short-day, noninjected controls display impaired spatial learning and memory compared with long-day controls, which may be attributable to the observed increased glucocorticoid response to the water maze task relative to long days. However, additional stressors, such as repeated daily injections before water maze training, complicated the effects of glucocorticoids on water maze performance; daily saline injection stressors, when combined with the stressor of the water maze itself, improved learning in short-day mice. Overall, these results suggest that photoperiod-evoked modification of the HPA axis and brain receptivity and their behavioral consequences may be adaptive for winter survival.

Physiologically, short days promoted a rapid, more efficient HPA feedback system demonstrated by increased GR expression in the brain, dexamethasone suppression, corticosterone responses to stressors, and a faster return to baseline corticosterone concentrations after restraint. Baseline glucocorticoid concentrations display a seasonal pattern in many vertebrates (19). Photoperiodic differences in baseline rodent corticosteroid concentrations have been reported, but differences in circadian sampling time make these results difficult to interpret (20–24). No statistically significant differences were observed in baseline corticosterone concentrations just before lights off in the present study. However, seasonal regulation of the HPA response to stressors may exist. After restraint, short days elevated corticosterone more than long days. These data support previous findings in which short days increased cortisol responses to restraint compared with long days in Siberian hamsters (*Phodopus sungorus*) (25). Additionally, the speed of return to basal corticosterone concentrations in short-day mice after restraint stress in this study agrees with similar findings in short-day Syrian hamsters (*Mesocricetus auratus*), another photoperiod-responsive species, after an anesthetic stressor (26). The potential ecological significance of a more efficient HPA feedback loop in response to stressors during the winter is discussed below.

Because exogenous glucocorticoids trigger the HPA negative feedback loop and dampen endogenous glucocorticoid production, the dexamethasone suppression test indicates relative sensitivity of the HPA negative feedback loop (15). In this study, corticosterone suppression in short-day, but not in long-day, mice was observed at a low dose of dexamethasone. These results suggest that the sensitivity of the negative feedback loop upstream of adrenal corticosterone production (*i.e.* hippocampus, hypothalamus, and pituitary) is enhanced in short-day mice. Rodents living in arctic conditions (a possible parallel to short-day rodents) also display greater sensitivity and efficiency of HPA negative feedback compared with non-arctic confamilars (27). Based on the increase in short-day hippocampal GR gene expression, it is likely that the more sensitive negative feedback loop observed in short-day mice is attributable to increased GR in the brain (11).

Photoperiod-induced changes in GR gene expression in the brain provide additional evidence of seasonal changes in adult mammalian brains. Fourteen weeks of short days (time frame necessary to induce gonadal regression in this species) increased hippocampal GR and hippocampal hypothalamic MR expression compared with initial exposure to short days (7 wk). These findings support a previous study in which short days increased hippocampal MR mRNA expression in Syrian hamsters (28) that was associated with increased cortisol binding to MR in the hippocampus and hypothalamus and rapid cortisol recovery after an anesthetic stressor (26). The functional or adaptive significance of these data was not tested, but, in the present study, alteration of the HPA axis (pharmacologically or via stressors) was associated with changes in hippocampal-based learning. The observed changes in both hippocampal and hypothalamic GR may greatly impact seasonal regulation of HPA axis negative feedback and therefore contribute to the numerous differences in corticosterone responses observed in other experiments. Comparable intracellular corticosteroid receptors in the brains of white-crowned sparrows decrease during the winter (29), although the specific regions within the brain in which these changes occur is unknown. MR has a higher affinity for corticosterone, but GR is thought to mediate stress effects on the brain (30). Therefore, the short-day increase in hippocampal GR expression may mediate the proposed stress-induced changes in spatial learning. The changes in glucocorticoid gene expression in long-day hypothalami from 7 to 14 wk were unexpected because of the absence of other phenotypic changes. It is possible that these slight changes in the hypothalamus (or hippocampus) were attributable to circadian differences in gene expression based on the time of day of tissue collection (31) or an effect of aging. Additionally, it is possible that the duration of short-day exposure required to affect receptor expression was between 7 and 14 wk. Because of the physiological cascade of events that may be upstream of photoperiodic changes, some changes are not evident until weeks/months after initial exposure (*e.g.* changes in body mass, reproductive status, responsiveness to melatonin, etc.) (18, 32, 33). The increased HPA feedback sensitivity observed in short-day mice in the present study is comparable with the increased hypothalamus-pituitary-gonadal feedback sensitivity described previously in short-day rodents (34–36).

On a behavioral level of analysis, the present study suggests that regulation of the HPA axis may be one pathway by which photoperiod affects behavior. The water maze task itself is considered to be a stressful behavioral test (37). Similar to previous studies (6, 38, 39), short-day control (noninjected) mice in the present study displayed impaired spatial learning compared with long-day controls. This short-day impairment of spatial learning corresponds with the decrease in home range size and
spatial behaviors (e.g., foraging, searching for mates, and patrolling territories) observed in field studies (6). In contrast to our predictions, the saline-injected mice did not display the characteristic photoperiodic differences in water maze performance that were observed in control mice. However, short days increased the corticosterone response to a saline injection compared with long days. The photoperiodic difference in corticosterone response of injection-experienced mice (Fig. 4B, Post-MWM) suggests that long-day, but not short-day, mice may habituate to daily injections, although later sampling times are necessary to determine when corticosterone concentrations return to baseline values. Compared with restraint, an injection stressor appears to have longer-lasting effects on corticosterone concentrations in short-day mice. The significance of corticosterone (or stressors) on water maze performance is confirmed in this study by the impairment of all metyrapone-treated mice and other manipulations from previous studies (40, 41). Therefore, it is likely that the effects of the injection stressor before each block of behavioral training masked the photoperiodic differences in water maze performance in the saline-treated mice. Decreased swim speed in metyrapone-treated mice during some of the blocks of training may have increased the latency to reach the platform on some of the blocks of trials but would not have affected the comparable differences observed in path length. Based on the corticosterone measurements throughout water maze training and the performance differences among treatment groups, it appears that basal (unmanipulated) short-day corticosterone responses to water maze training are responsible for impaired short-day learning, whereas the basal long-day corticosterone responses to water maze promote spatial learning. Additional evidence to support this idea is that blocking corticosterone responses in metyrapone-treated mice slightly enhanced water maze performance in short-day, but not long-day, mice (Fig. 4B, block 7). These corticosterone-dependent water maze results, however, are not unexpected given the photoperiodic differences in HPA regulation and receptivity observed in the other experiments.

Ecologically, the HPA axis is an important physiological system that mediates both adaptive and maladaptive responses in nature (12). For example, acutely elevated glucocorticoids mobilize emergency energy necessary for survival from stressors (e.g., predator avoidance), whereas continuously elevated glucocorticoids impair brain and immune function and consume extensive energy reserves that are crucial for winter survival (12, 42). Therefore, photoperiodic regulation of the HPA axis may help to modulate the balance between beneficial and detrimental effects of glucocorticoids. The numerous differences between long- and short-day HPA responses to stressors, brain receptiveness, and the effects of corticosterone on spatial learning reported in this study suggest that an altered HPA feedback axis is important for seasonal adaptation (19). Photoperiodic regulation of the HPA axis may underlie many other physiological and behavioral adjustments reported in seasonality research (43–46). Studies in wild populations of mice are necessary to test the functional consequences (including, but not limited to, spatial learning and memory) of a seasonally changing HPA axis. It is possible that a more efficient HPA response in short days represents a coping strategy evolved specifically for winter stressors (42). In contrast, studies in white-crowned sparrows suggest that short days decrease baseline and stress-induced corticosterone concentrations and reduce behavioral responsiveness (activity) to corticosterone treatment compared with long days (47, 48). Thus, photoperiodic changes in HPA responsiveness may vary by taxa because of differences in life history strategies (i.e., length of breeding season, migration, etc.). Additionally, seasonal changes in cortisol concentrations, tissue sensitivity to glucocorticoids (49), and dexamethasone suppression have been reported in normal and seasonally depressed humans (50), suggesting that mechanisms underlying seasonal regulation of the HPA may be applicable to treatment of seasonal cognitive and affective disorders.

Acknowledgments
We thank Stephanie Bowers, Brenda Reader, Erica Glasper, Lynn Martin, Jessica Trent, Erin Schiegel, and Zachary Weil for technical assistance and advice and A. Courtney DeVries for use of her behavioral equipment.

Received October 26, 2006. Accepted March 20, 2007.

Address all correspondence and requests for reprints to: Randy J. Nelson, Department of Psychology, Ohio State University, Columbus, Ohio 43210. E-mail: rnelson@osu.edu

This work was supported by National Institutes of Health (NIH) National Research Service Award MH 73379, NIH Grants MH 57535 and MH 66144, and National Science Foundation Grant IBN 04-16897. Additional support was received from NIH Grant P30 NS045758.

Disclosure Statement: All authors have nothing to disclose.

References
2. Goldman BD 2001 Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. J Biol Rhythms 16:283–301
31. Turek FW 1977 The interaction of the photoperiod and testosterone in regulating serum gonadotropin levels in castrated male hamsters. Endocrinology 101:1210–1215
32. Ellis GB, Turek FW 1979 Time course of the photoperiod-induced change in sensitivity of the hypothalamic-pituitary axis to testosterone feedback in castrated male hamsters. Endocrinology 104:625–630
44. Romero LM, Ramenofsky M, Wingfield JC 1997 Season and migration alter the corticosterone response to capture and handling in an Arctic migrant, the white-crowned sparrow (Zonotrichia leucomyza gambiae). Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 116:171–177

Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.