Photoperiod alters autonomic regulation of the heart

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Outside of the tropics, environmental conditions fluctuate in a generally predictable manner across the year. Many small mammals have evolved mechanisms, such as seasonal breeding and annual adjustments in physiology, morphology, and behavior, that promote winter survival when food is scarce and thermoregulation is challenging. Photoperiod (day length) is a cue used by many seasonal breeders to predict seasonal changes in environmental conditions. One system that is uniquely situated to mediate photoperiod-induced alterations in physiology is the autonomic nervous system (ANS). The 2 branches of the ANS are key regulators of immune responses, thermoregulation, and energy balance, functions that undergo marked shifts in baseline and reactivity following acclimation to short day lengths. Although previous studies have investigated the effects of photoperiod on ANS endpoints, this study examined the direct effects of photoperiod on integrated ANS function. To test the hypothesis that short day lengths increase parasympathetic and sympathetic tones, we maintained adult male Siberian hamsters (*Phodopus sungorus*) to either long or short photoperiods and then analyzed electrocardiogram recordings. Short day lengths increased both parasympathetic tone, as measured by respiratory sinus arrhythmia, and sympathetic control of the heart, measured with autonomic blockade. Additionally, short day lengths enhanced the withdrawal of parasympathetic control and the increase of sympathetic tone in response to acute restraint stress. Finally, these effects were discovered to be independent of circulating androgens. These data indicate that the ANS of Siberian hamsters undergoes profound changes following prolonged exposure to short winter-like day lengths.

autonomic nervous system | cardiovascular | parasympathetic | sympathethic | day length

For animals living outside of the tropics, life is characterized by dramatic daily and seasonal fluctuations in illumination, ambient temperatures, food availability, and predation pressures; adapting to this variation is critical for reproductive success and survival (1). Although variation in environmental conditions is inexorable, it is also generally predictable, and many vertebrate species have evolved mechanisms that allow for adjustment of physiology, behavior, and morphology to anticipate favorable and unfavorable energetic conditions at different times of the year (2). Because many such processes require significant time to develop, natural selection has favored individuals that have the capacity to measure day length (photoperiod) to predict annual variation in the environment (3).

Seasonal reproduction, which is characterized by long-day breeding during spring and summer, when environmental conditions are relatively mild, and regression of reproductive function during short, winter-like day lengths, when energy demands are high, is canonical among the photoperiodic and circannual adjustments made by small mammals (4). Although reproductive function is the most studied response to changes in day length, numerous nonreproductive seasonal adaptations exist among small mammals. For instance, prolonged short-day treatment alters various aspects of the immune and nonreproductive neuroendocrine systems and is also associated with changes in nervous system structure and behavior (5–8).

Day length conveys the necessary seasonal information even in the absence of other environmental cues, such as temperature and food availability. Thus, day length manipulations can be used as a simple, noise-free, and ecologically relevant cue to probe phenotypic plasticity in the absence of the proximal signals that provoked the evolution of photoperiodism. This approach allows for the elucidation of specific physiological adaptations associated with coping with winter conditions, and it provides additional insights into vertebrate evolutionary, ecological, and behavioral plasticity. This is particularly important given the critical role of seasonality and season-of-birth phenomena in human disease.

From a proximate perspective, research on the mechanisms of seasonal adjustments has focused on the pineal melatonin system, which is the primary coordinator of photoperiodic adaptations. However, other downstream effector mechanisms are almost certainly engaged by melatonin signaling. The role of one such effector system, the autonomic nervous system (ANS), and in particular the parasympathetic branch, has received little attention. It is known that increased sympathetic outflow in short days is responsible for reductions in body fat, alterations in the immune system, and elevated thermoregulatory capacity in hamsters (9, 10). However, the classical "rest and digest" functions of the parasympathetic branch are consistent with a shift in physiological priorities from reproduction in the summer to cellular maintenance and survival during winter (11, 12). Additionally, parasympathetic activity partially mediates the slowing of metabolic and cardiovascular activity in hibernating mammals (13, 14). Previous attempts to quantify sympathetic tone by using biochemical indicators of norepinephrine metabolism have produced equivocal results, depending on the tissue, species, and time of day studied; virtually no studies have addressed seasonal adjustments of the parasympathetic branch (15–17).

To address the effects of photoperiod on autonomic tone, we used electrophysiological recordings of the heart as an accessible and physiologically relevant proxy for overall autonomic activity (18, 19). Specifically, we measured respiratory sinus arrhythmia (RSA), an index of heart rate variability in the high-frequency domain, as a relatively pure measure of parasympathetic vagal control over the heart (18, 19). Additionally, we used pharmacological blockades to assess the tonic- and stress-induced changes in the regulation of the heart by the parasympathetic and sympathetic branches of the ANS (19). Regulation of the cardiovascular system by both branches of the ANS individually and in concert is a potent predictor of several important health outcomes (20). In contrast to the classic model of the reciprocal central regulation of the sympathetic and parasympathetic

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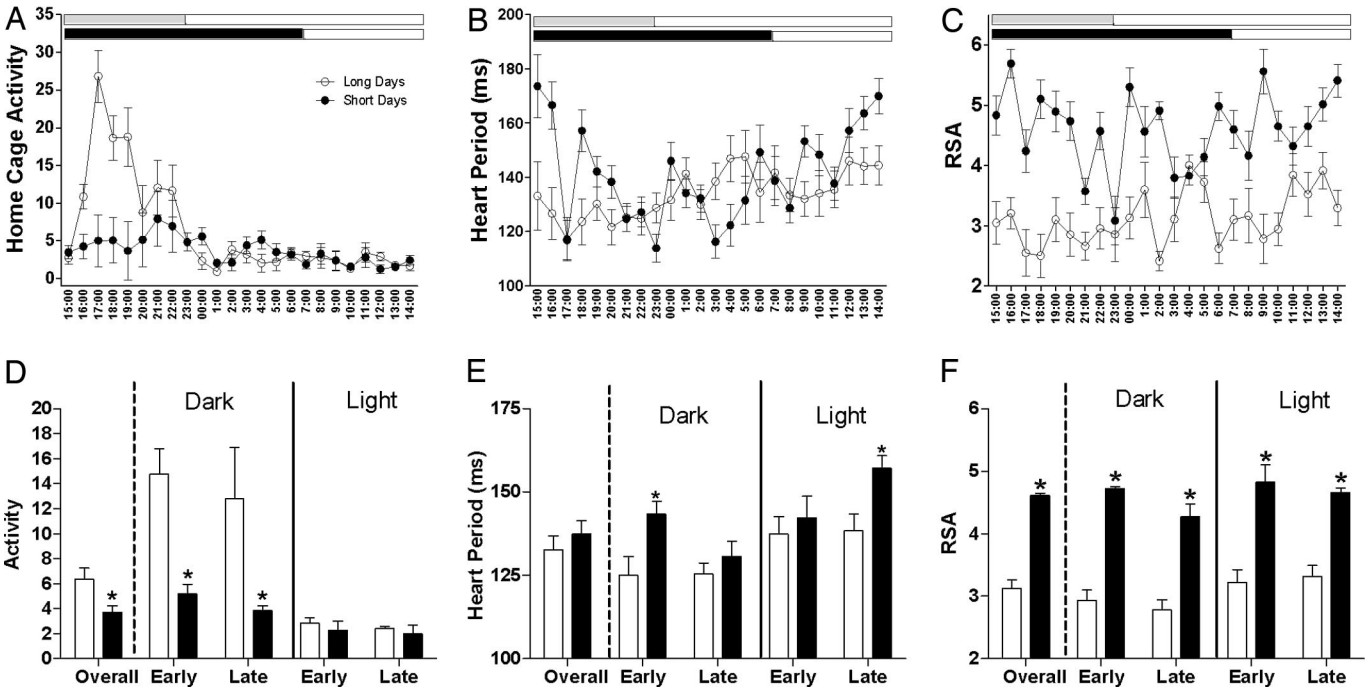

Fig. 1. Photoperiod effects on locomotor and cardiovascular parameters in Siberian hamsters. Gray and black bars represent lights out for long- and short-photoperiod hamsters, respectively. Home cage locomotor activity across the day (*A*) and broken up across early and late active and inactive periods (*D*). HP in milliseconds (60,000/heart rate) across the day (*B*) and divided across the early and late active and inactive periods (*E*). Respiratory sinus arrhythmia (RSA) plotted around the clock (*C*) and broken up across early and late active and inactive periods (*F*). Data are presented as mean ± SEM and mean differences were considered statistically significant when $P < 0.05$; *, significantly different from long days.

branches, adaptive patterns of autonomic control may entail coactivation of both autonomic branches (20). Based on the assumption that individuals trade off investing in survival mechanisms against investing in reproductive function during the winter, we hypothesized that short day lengths would enhance sympathetic tone and also potentially increase parasympathetic activity in adult male Siberian hamsters.

Results

Reproductive Responses. Photoperiod-induced regression of the reproductive tract in short-day hamsters is summarized in supporting information (SI) *Text* and Table S1.

Locomotor Activity. Photoperiod altered the daily pattern of locomotor activity. Specifically, short day lengths blunted the daily rhythms of home cage activity (Fig. 1*A*), and long-day hamsters were more active then short-day hamsters ($F_{1,13} = 6.26$, $P < 0.05$). This was mediated almost completely by a reduction in locomotor activity during the early ($F_{1,13} = 19.43$, $P < 0.001$) and late active periods ($F_{1,13} = 4.80$, $P < 0.05$) in short-day hamsters Fig. 1 *A* and *D*), and there were no detectable locomotor differences during the early ($F_{1,13} = 0.45$, $P > 0.05$) or late inactive periods ($F_{1,13} = 0.35$, $P > 0.05$).

Cardiovascular Parameters. Changes in photoperiod necessarily produce an alteration in the timing of daily patterns of activity. Therefore, we plotted heart period, activity, and RSA across the same time scale to determine whether increased locomotor activity could mediate alterations in autonomic control of the heart. Heart period varied across the day in both long- and short-day hamsters (Fig. 1*B*). Mean heart period did not differ systematically between long- and short-day hamsters when averaged across the day ($F_{1,10} = 0.76$, $P > 0.05$; Fig. 1*E*), but when the day was divided into early and late portions of the light and dark phases, respectively, a photoperiod difference emerged. Heart period was similar in the early portion of the light period ($F_{1,10} = 0.32$, $P > 0.05$) or at the end of the dark period ($F_{1,10} = 0.81$, $P > 0.05$). However, during the late portion of the light phase ($F_{1,10} = 9.16$, $P < 0.05$) and the early portion of the dark ($F_{1,10} = 7.50$, $P < 0.05$), short-day hamsters had longer heart periods.

RSA, an index of parasympathetic control of the heart, was markedly elevated in hamsters housed in short compared with long day lengths. Short-day hamsters had significantly higher RSA scores at all times of the day ($F_{1,10} = 118.28$, $P < 0.0001$), which were elevated during the early ($F_{1,10} = 19.95$, $P < 0.01$) and late ($F_{1,\ 10} = 56.13$, $P < 0.0001$) light periods, as well as the early ($F_{1,10} = 143.01$, $P < 0.0001$) and late ($F_{1,10} = 31.79$, $P < 0.0001$) dark periods.

To determine the relative sympathetic and parasympathetic control over the heart, we conducted blockade experiments isolating each branch of the ANS. As expected, blockade of the parasympathetic cardiac sinoatrial innervation with atropine decreased heart period and reduced RSA relative to saline injections. There were no photoperiod differences in RSA in atropine-treated hamsters ($F_{1,13} = 0.62$, $P > 0.05$; Fig. 2*A*), but heart period was significantly shorter in short-day hamsters following atropine treatment ($F_{1,13} = 5.48$, $P < 0.05$). Conversely, blockade of the sympathetic innervation of the heart with atenolol increased heart period relative to saline injections but had no noticeable effect on RSA. As in saline control injection sessions, short-day hamsters had higher RSA scores after atenolol than did long-day hamsters ($F_{1,9} = 83.12$, $P < 0.0001$); heart period did not differ significantly but tended to be numerically longer in short-day hamsters ($F_{1,9} = 4.54$, $P > 0.05$). The double blockade also slightly increased heart period and RSA relative to saline injections. However, there were no photoperiod differences in heart period (the intrinsic heart period) following the double blockade ($F_{1,13} = 0.97$, $P > 0.05$) or RSA ($F_{1,13} = 0.38$, $P > 0.05$). When the heart period responses

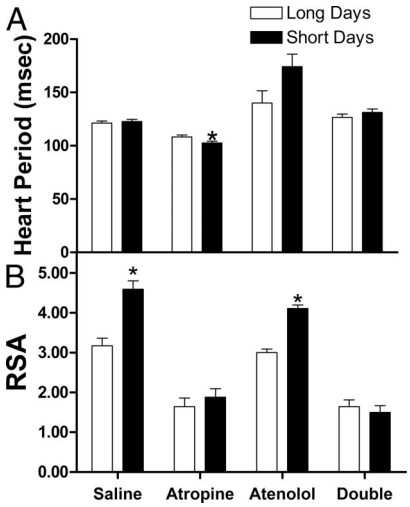

Fig. 2. Heart period (HP) and RSA under conditions of autonomic blockade. Hamsters were treated on separate days with either sterile saline or the muscarinic antagonist atropine sulfate (1 mg/kg intraperitoneally) and the β1 adrenergic antagonist (atenolol, 10 mg/kg) and atropine followed by atenolol. Immediately after drug injections, HP (*A*) and RSA (*B*) were assessed. Data are presented as mean ± SEM, and mean differences were considered statistically significant when $P < 0.05$; *, significantly different from long days.

under autonomic blockade conditions were analyzed with the equations below, it was evident that short photoperiods increased both parasympathetic ($F_{1,9} = 7.89$, $P < 0.05$; Fig. 3) and sympathetic ($F_{1,9} = 6.70$, $P < 0.05$) outflow to the heart.

Restraint. Restraint produced marked increases in heart period, followed by a gradual recovery toward baseline ($F_{4,28} = 59.20$, $P < 0.0001$; Fig. 4*A*). At the peak stress response, heart periods of the short-day and long-day animals were comparable. The heart period of short-day hamsters was longer at baseline, so the decrease in period length was of a greater magnitude in short days ($F_{4,28} = 6.45$, $P < 0.05$). Similarly, restraint dramatically reduced RSA, followed by a gradual increase back toward baseline ($F_{4,28} = 27.32$, $P < 0.0001$; Fig. 4*B*). Short-day hamsters had elevated RSA scores throughout the stressor and recovery periods, as indicated by repeated measures testing ($F_{4,28} = 3.974$, $P < 0.01$) and multiple comparisons ($P < 0.05$ in all cases). The change in RSA from prestress baselines did not differ between photoperiods in terms of percent changes ($F_{4,28} = 0.85$, $P > 0.05$).

Restraint combined with pharmacological blockade was used to determine the autonomic responses to restraint. Short day

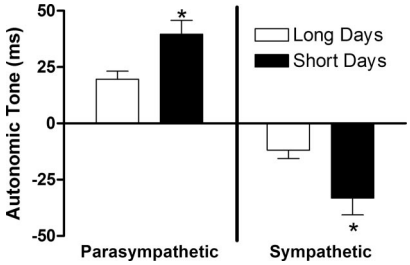

Fig. 3. Basal autonomic tone in long- and short-day hamsters. Short photoperiods augment both sympathetic and parasympathetic tone; that is, when considered independently from the other branch, the parasympathetic branch would increase heart period and the sympathetic branch decrease HP more in short than in long days. Data are presented as mean (milliseconds of heart period) ± SEM, and mean differences were considered statistically significant when $P < 0.05$; *, significantly different from long days.

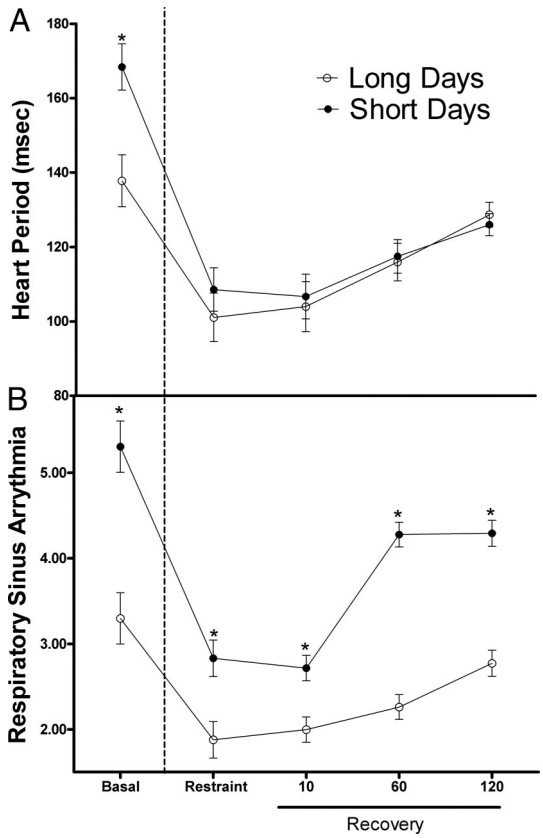

Fig. 4. Cardiovascular responses to acute restraint stress in short- and long-day hamsters. Acute restraint stress decreased heart period (*A*) and RSA (*B*). Short-day hamsters had higher RSA scores before and during restraint and 10, 60, and 120 min after the conclusion of restraint. Data are presented as mean ± SEM, and mean differences were considered statistically significant when $P < 0.05$; *, significantly different from long days.

lengths augmented both the increase in sympathetic outflow ($F_{1,10} = 5.91$, $P < 0.05$; Fig. 5) and reduction in parasympathetic activity in response to restraint ($F_{1,10} = 20.95$, $P < 0.01$). Fig. 6 is a diagram indicating basal and stress-induced changes in autonomic tone. Gonadal hormones did not mediate photoperiod differences in RSA (see *SI Text* and Fig. S1 for details).

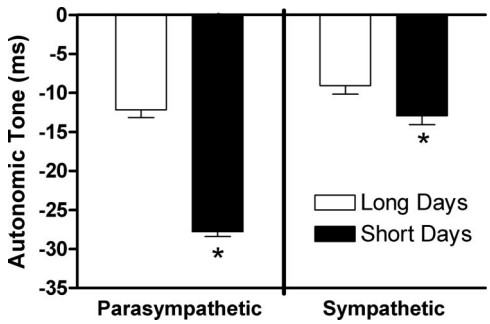

Fig. 5. Autonomic control during the restraint session was altered by day length. Autonomic blockade studies were conducted as above, but hamsters were restrained immediately after injections. Short photoperiods enhanced the withdrawal of parasympathetic tone and the increase in sympathetic control over the heart. Data are presented as mean (milliseconds of heart period) ± SEM, and mean differences were considered statistically significant when $P < 0.05$; *, significantly different from long days.

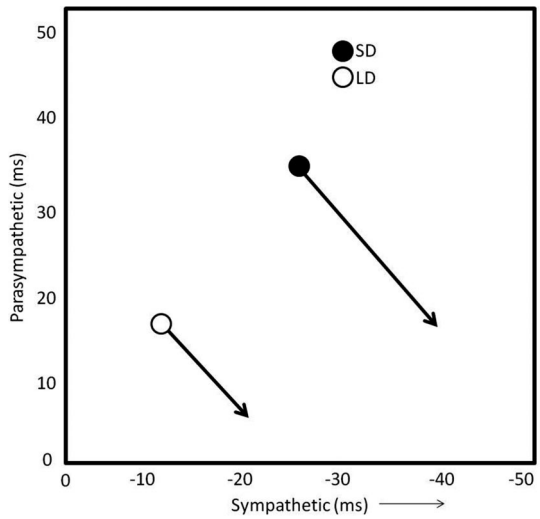

Fig. 6. Basal and stress-induced autonomic control. Data are plotted across a 2D autonomic space, with circles indicating basal autonomic tone and arrowheads indicating responses to acute restraint. Short photoperiods enhance both sympathetic and parasympathetic control over the heart under basal conditions. Following restraint stress, the withdrawal of parasympathetic control and the increase in sympathetic regulation are both enhanced by exposure to short day lengths. The *x* axis is sympathetic control over heart period (milliseconds), and the *y* axis indicates parasympathetic control over heart period.

Discussion

The short-day phenotype in Siberian hamsters is characterized by regression of the reproductive tract and other adaptations that putatively evolved to aid in winter survival. It is now clear that in concert with immunological and metabolic adaptations, a marked reorganization of autonomic control of the heart is part of the transition into the short-day phenotype. The autonomic determinants of basal heart period were markedly different between photoperiods. First, high-frequency heart rate variability (RSA), a relatively pure index of vagal-parasympathetic control of the heart, was markedly enhanced by chronic exposure to short days. Pharmacological blockade of sympathetic and parasympathetic branches indicated that short day lengths enhanced both vagal and sympathetic tone and confirmed that RSA was sensitive to inhibition by atropine. This is in contrast to the classical model of central reciprocal regulatory control of the ANS, in which increases in activity of one autonomic branch are associated with decreases in the other. The present findings, however, are in accord with a growing literature that indicates coactivation of the 2 autonomic branches may have adaptive advantages (20). Because this pattern of response may be associated with minimal differences in heart period (as observed here), the adaptive significance of this pattern may lie elsewhere. Heart period is only one limited dimension of cardiovascular function. Increased sympathetically mediated myocardial contractility, for example, could increase stroke volume and cardiac output in the absence of a change in heart period. At the same time, parasympathetic cholinergic vasodilator effects may further assist in improving circulation. These are possibilities that await further investigation. What is clear, however, is that the formal properties of autonomic coactivation, which include a greater dynamic range and reactive lability in autonomic regulation, may have adaptive significance (20).

In addition to basal autonomic function, day length also altered the responses of both branches of the ANS to restraint. Specifically, short day lengths enhanced both the increase in sympathetic activity and withdrawal of vagal tone during restraint, which were associated with an increased heart period response to stress. This is in accord with the increased reactive lability in the autonomic regulatory mode of coactivation alluded to above (31).

Melatonin is a relatively potent vasodilator and inhibitor of sympathetic activation and acutely elevates vagal tone, as indicated by elevated RSA, and as such has been proposed as a clinical treatment for hypertension (21–23). Thus, one potential explanation for the pattern of autonomic reorganization in this experiment would be that short days lengthen the duration of the pineal melatonin signal; however, this possibility can likely be ruled out, because short-day hamsters displayed elevated RSA at all times of the day, even when pineal melatonin production was likely inhibited by light. We cannot rule out the possibility that melatonin receptors might mediate reorganization of autonomic control over the course of photoperiod treatment, a hypothesis that requires future research. The ANS is controlled by a distributed neural network involving nuclei in the forebrain, midbrain, medulla, and spinal cord. Melatonin receptor expression has been reported in several hypothalamic nuclei that send projections to autonomic control nuclei (24). Melatonin receptor mRNA has been detected in cells that give rise to a multisynaptic pathway sending sympathetic projections to peripheral tissues (25), indicating that melatonin signaling could be responsible for remodeling autonomic function. A potential role for melatonin is also suggested by the apparent independence of photoperiod modulation of parasympathetic activity on gonadal steroids.

The heart, as in many organs, is dually innervated by both branches of the ANS, and together they are the principal determinants of heart period deviations from the intrinsic rate. The parasympathetic nervous system exerts a tonic inhibitory influence over the sinoatrial node. This is exemplified by the evidence that the intrinsic heart period (under both parasympathetic and sympathetic blockades) is shorter than resting heart period under basal conditions (26). Other cardiovascular parameters are also under the regulation of the ANS, including myocardial contractility and vascular resistance. These parameters cannot be directly assessed from the current measures, but they will be important for future studies because they contribute to the adaptive regulation of circulation.

As noted, the 2 branches of the ANS have long been considered to act in opposition and be subject to reciprocal central control. Many basic homeostatic cardiovascular reflexes of brainstem origin, including the baroreceptor-heart rate reflex, are neurologically organized to yield a reciprocal pattern of activity across the 2 branches. It is now clear, especially with higher-level central regulatory systems, that the autonomic branches may be more flexibly regulated and that autonomic activation may represent more of a 2D bivariate space (bounded by sympathetic and parasympathetic axes) rather than a simple continuum extending from sympathetic to parasympathetic dominance (27). In accordance with this model, short days in the present study resulted in a tonic coactivation of both the sympathetic and parasympathetic branches of the ANS. Diminished heart rate variability and other indicators of low parasympathetic tone are predictive of disease states in a variety of contexts. However, evidence is mounting that the pattern of activation of both branches of the ANS taken together is also an important predictor of health outcomes (20, 28). High activity in both branches of the ANS may allow for a greater dynamic range over which the cardiovascular system can be adjusted in response to stressors or other perturbations. Heart period was longer in short-day hamsters during the late portion of the light phase and the early portion of the dark phase, an effect that could be mediated in part by the greater locomotor activity in long-day hamsters during that period. The way in which the heart period set point was attained differed markedly across photoperiods. Exposure to short day lengths enhanced both sympathetic and parasympathetic tone, presumably reflecting increased energetic

investment in the regulatory capacity of peripheral processes. Short-day hamsters exhibited longer basal heart periods during some but not all parts of the day. This is further illustrated by the response of the autonomic branches to stress; in both photoperiodic conditions, heart period decreased in concert with enhancement of sympathetic and withdrawal of parasympathetic activity. Moreover, both the increase in sympathetic activity and the withdrawal of parasympathetic tone were enhanced by short day lengths. Similarly, stress responses in the hypothalamic–pituitary–adrenal axis are also enhanced by short day lengths; circulating cortisol is elevated more in short- compared with long-day animals following restraint stress (7). Despite these larger stress responses in short-day animals, restraint did not reduce the RSA to either the level of restrained long-day hamsters or the nearly complete withdrawal produced by atropine. Therefore, the enhanced parasympathetic and sympathetic activities allowed hamsters to maintain greater parasympathetic tone even under stressful conditions, a strategy that seems likely to help maintain flexibility in the regulation of local energy expenditure during environmental challenges.

The extent to which humans vary seasonally in autonomic tone has been studied little, but some indication that heart rate variability may be lower during summer with a concomitant increase in sympathetic tone has been reported (29, 30). This phenomenon of annual variation in autonomic function, if present in human populations, may help explain why human cardiovascular diseases fluctuate in severity, prevalence, or incidence across the year. Low temperature-induced increases in sympathetic tone have been postulated as a potential mediator of increased cardiovascular disease during the winter (31, 32). This study would suggest that predictive cues, such as day length, may be as important a mediator of autonomic changes as environmental conditions themselves. This perspective is supported by the winter increase in cardiovascular mortality in geographic locations that experience seasonal changes in day length but do not experience seasonal extreme temperature fluctuations (33, 34).

The only moderate photoperiod-induced changes in heart period suggest that alterations in the regulation of other dimensions of cardiovascular function (e.g., cardiac output, blood flow distribution) or even noncardiovascular parameters (e.g., immunity, glucose metabolism) may be the primary target for why the evolution of plasticity in this system occurred. For Siberian hamsters, short days are associated with dramatic reductions in ambient temperature and a concomitant reduction in food availability. (*i*) Reduced food availability and hypometabolism are associated strongly with high activity in the parasympathetic branch, so this pattern of autonomic function may serve directly to help maintain a positive energy balance (35). (*ii*) Increased sympathetic activity serves to elevate thermogenic capacity at least in part by increasing metabolic activity in brown adipose tissue, and it also serves to reduce fat pad masses, both of which can aid in thermoregulation (16, 36). (*iii*) Finally, remodeling of autonomic control of the periphery might have evolved to increase neural control of immune function, allowing for more efficient regulation of immune responses. The sympathetic nervous system is thought to fine tune many immune responses (37), and a role for the sympathetic branch in mediating photoperiod-induced changes in other aspects of the immune system has already been firmly established (10, 38). Similarly, the finding of increased vagal cholinergic transmission may serve to increase neural control over immunity through prevention of runaway inflammation after sepsis (37). Indeed, short-day animals display reduced cytokine production and sickness responses following peripheral administration of LPS (a component of Gram-negative bacterial cell walls), and they are also protected from lethal endotoxemia (39, 40). Taken together, it seems likely that a number of environmental pressures shaped the evolution of annual adaptations in the nervous system. It is important to note that the changes associated with short day lengths in the laboratory occurred in the absence of any of the proximate conditions that putatively lead to the initial evolution of these adaptations. The function of the ANS in nature is likely different from that for individuals fed ad libitum and housed under mild laboratory temperatures. Future studies should examine the effects of day length in combination with other environmental and hormonal variables related to energy balance on autonomic function. In conclusion, this is one of the first studies to uncover a profound and pervasive change in the autonomic regulation of the heart by a single ecologically relevant cue, and this may have important implications for understanding annual plasticity in health and in disease states across vertebrate taxa.

Methods

Animals. All procedures were approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee and conducted in accordance with National Institutes of Health guidelines. Siberian hamsters (*Phodopus sungorus*) used in this study were bred in our colony at Ohio State University. All hamsters were bred in long day lengths (16:8 light–dark) and maintained in long days until weaning (21 days). At that point, hamsters were randomly assigned to either remain in long day lengths or were transferred to short (8:16 light–dark) days. All hamsters were housed individually after weaning.

At ≈60–70 days of age, hamsters were anesthetized with isoflurane vapors, a radiotelemetric transmitter was implanted (ETA-F20; Data Sciences International) subcutaneously, and 1 of the leads was sutured to the muscle on each side of the rib cage. Mean activity and ECG were collected in 10-min bins every hour. To avoid comparing groups with differing time points, we used an equal sampling strategy in which we randomly chose 5 time points from both short-day and long-day animals across both light and dark periods. The light and dark periods were divided into early and late phases for each photoperiod such that the early portion of the light phase was 4 h for short days but 8 h for long days.

RSA. High-frequency heart rate variability was derived by spectral analysis of the interbeat interval (RR) series derived from the ECG by using commercial software (Mindware), and it was ensemble averaged for each minute. Analysis followed procedures recommended by the SPR committee on Heart Rate Variability (18) and was adapted for use in rodents. Briefly, the RR intervals were sampled at 20 Hz to yield an equal-interval time series. The time series was detrended (second-order polynomial), tapered (Hamming), and submitted to a fast Fourier transform. High-frequency spectral power was integrated over the respiratory frequency band (1–5 Hz) and digitized.

Blockade Studies. After baseline recordings, the contributions of the 2 branches of the ANS to both RSA and heart period were determined by using a specific muscarinic receptor antagonist (atropine sulfate; 1 mg/kg intraperitoneally to block parasympathetic inputs; Sigma–Aldrich), a β1 antagonist (atenolol; 10 mg/kg to block sympathetic inputs; Sigma–Aldrich), and both drugs in combination to determine the intrinsic heart period. Doses were selected to yield asymptotic levels of cardiac blockade in the rodent (41). Each hamster received each of the 3 drug treatments on separate days and in a counterbalanced order: (*i*) atropine injection alone, (*ii*) atenolol injection followed 30 min later by atropine, and (*iii*) a saline (vehicle) injection.

Basal Autonomic Control. Whereas HR measures of autonomic control are inherently biased by nonlinear interactions among the sympathetic and parasympathetic branches (42), heart period (HP; 60,000/HR) maintains a relatively linear relationship with autonomic control (43, 44). Based on previous findings (19), 2 equations were used to estimate autonomic control based on HP in each of the blockade conditions. The averages of the 2 estimates were derived for each autonomic branch. The first was taken as the change in HP from saline to the HP under blockade of the relevant branch (e.g., atropine for the parasympathetic branch), and the other (residual estimate) was derived as the difference between the HP level after double autonomic blockade and after blockade of the other (nontarget branch—e.g., atenolol for the parasympathetic estimate). The former provides an estimate based on the change in HP with blockade of the target branch, and the latter provides a second estimate represented by the residual autonomic effects of the target branch after blockade of the other.

1) $\text{cardiac vagal control}_{\text{estimate}} = [(\text{HP}_{\text{saline}} - \text{HP}_{\text{atropine}}) + [(\text{HP}_{\text{atenolol}} - \text{HP}_{\text{double}})]/2$

2) cardiac sympathetic control$_{estimate}$ = [(HP$_{saline}$ − HP$_{atenolol}$) + [(HP$_{atropine}$ − HP$_{double}$)]/2

Restraint Responses. To determine the autonomic responses to acute stressors, hamsters were confined in adjustable acrylic rodent restrainers (25-mm internal diameter) that prevented lateral movement but allowed the hamsters to breathe freely. Cardiovascular responses (RSA and HP) were measured during the restraint session and for the 2 h after the hamsters were returned to their home cages.

A separate group of hamsters from each photoperiod were stressed in concert with autonomic blockade to determine the responses of the sympathetic and parasympathetic branches of the ANS to restraint. Hamsters were treated on separate days with atropine (1 mg/kg), atenolol (10 mg/kg), or vehicle immediately before a 1-hour restraint session. The sympathetic and parasympathetic responses to restraint were derived from the 2 previously described equations (19), where HP$_{x/y}$ represents the mean HP value during a particular drug condition *x* and experimental condition *y*. Similarly to the determination of basal autonomic control above, the change in each branch was determined through the averaging of both residual and subtractive measures of autonomic control.

3) Δ Parasympathetic activity = [(HP$_{atenolol\text{-}restraint}$ − HP$_{atenolol\text{-}baseline}$) + (HP$_{saline\text{-}restraint}$ − HP$_{atropine\text{-}restraint}$)]/2

4) Δ Sympathetic activity = [(HP$_{atropine\text{-}restraint}$ − HP$_{atropine\text{-}baseline}$) + (HP$_{saline\text{-}restraint}$ − HP$_{atenolol\text{-}restraint}$)]/2

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