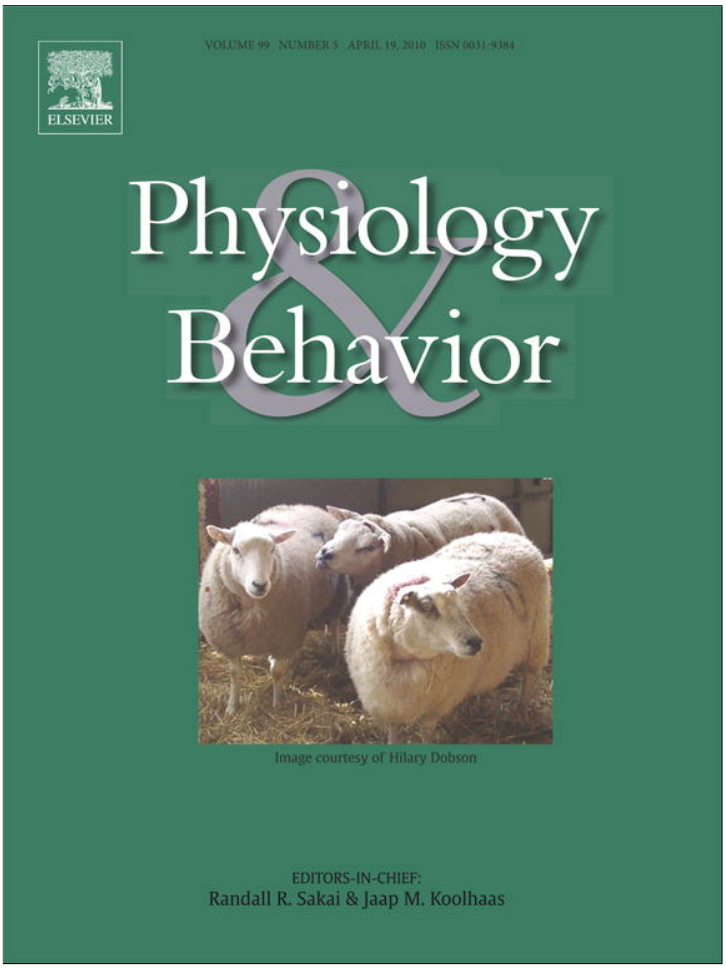

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Environmental enrichment enhances delayed-type hypersensitivity in both short- and long-day Siberian hamsters

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ABSTRACT

Darwinian fitness reflects trade-offs between reproduction and survival. Mechanisms have evolved in small nontropical mammals and birds to maximize reproductive output during the summer when thermoregulatory demands are relatively low and food is abundant and to shunt energy to processes that presumably increase the odds of survival during the winter when thermoregulatory demands are high and food is scarce. In order to predict the onset of winter, many seasonally-breeding mammals use day length (photoperiod) information. Seasonal adjustments of immune responses may be one mechanism to enhance survival; short days enhance cell-mediated immune function in seasonally-breeding rodents. The goal of the present study was to determine whether delayed-type hypersensitivity in hamsters is constrained or if photoperiod merely establishes a baseline level of immune response that can then be fined tuned by other environmental conditions. To test this, we used environmental enrichment, a manipulation that enhances many aspects of immune function. Hamsters were assigned to either long or short photoperiods and further assigned into either singly-housed or environmentally-enriched cages. After 10 weeks of concurrent photoperiod and housing treatment, delayed-type hypersensitivity (DTH) was induced. Although short days enhanced DTH responses compared with long days, environmental enrichment enhanced swelling responses in both short days and long days, suggesting that even during potential energetic bottlenecks or during maximal reproductive investment, hamsters can modulate their investment in immune function.

1. Introduction

In order to cope with the energetic demands of winter, seasonally-breeding mammals inhabiting nontropical climates undergo many physiological and behavioral changes in the trade-off between reproduction and survival, two key components of fitness. During winter, reproductive function and behavior of small animals wane, whereas some components of immune function are enhanced [28]. To initiate these changes, rodents primarily attend to two cues: day length (or photoperiod) and whether day lengths are lengthening or shortening. In the laboratory, short photoperiods can be used to induce a winter-like phenotype in seasonal breeders [37]. In response to short-day lengths, the duration of nightly melatonin secretion lengthens and is responsible for transducing the environmental signal of day length into a physiological signal. In short photoperiods in the lab (and potentially during winter in nature), Siberian hamsters (*Phodopus sungorus*) divert energy away from the reproductive system and toward processes that putatively enhance survival, such as thermoregulation and some components of immune function.

Specifically, short-day lengths in the laboratory enhance cell-mediated immune function [36,43]. One hypothesis explaining this phenomenon is that short days bolster cell-mediated immune function to compensate for elevated corticosteroid concentrations in response to intense winter stressors which may otherwise dampen immune function [23,26]. However, mounting an immune response is energetically expensive, a metabolic challenge that, if prolonged, can compromise survival by draining energetic resources [21]. Thus, an "energy bottleneck" may exist during winter when food availability is scarce and because of increased thermoregulatory costs and extended foraging bouts for food, energy requirements for survival are high [5]. Previous research has demonstrated that metabolic challenges can compromise immune function in seasonally-breeding rodents. For example, food restriction compromises DTH responses in Siberian hamsters housed in short, but not long days [4]. In deer mice (*Peromyscus maniculatus*), low temperatures can compromise splenocyte proliferation in short days, but only when food was restricted [6]. Together, these results suggest that environmental cues interact to alter immune function. A competing hypothesis that attempts to explain why short days enhance cell-mediated immune function is

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that the energetic constraints of breeding compromise immune processes [23]. Long days signal to seasonally-breeding rodents to maximize investment in reproduction, which may diminish investment in cell-mediated immune function [23]. The extent to which environmental cues interact to bolster immune function is unknown. The purpose of the present study was to determine whether long-day investment in reproductive function constrains immune function or whether photoperiod sets a basal level of immune function that can be fine tuned by additional environmental factors.

Additionally, short days may limit the capacity for enhancement in cell-mediated immune responses because further investment may place organisms at risk for autoimmune dysfunction so have been selected against in an evolutionary context. For instance, melatonin is associated with autoimmune disorders in humans [22]. Melatonin may alter immune function directly through melatonin receptors in lymphatic tissues and immune cells and may also act indirectly through downstream hormones, such as glucocorticoids, androgens, prolactin, and leptin [10,29]. Alternatively, short days may limit the capacity for enhancement of DTH responses because energy requirements prevent further investment in immune function. The extent to which DTH responses are bolstered may reflect either the maximal amount of available energy to fuel costly immune processes or the optimal regulation of ideal trade-offs among reproductive, thermogenic, or immune functions. This study investigates whether other factors known to increase immune function are influenced by photoperiod to alter DTH responses.

Environmental enrichment has long been used to study the effects of environmental on physiology and behavior. Environmental enrichment was first defined as a 'combination of complex inanimate and social stimulation' [39]. Although more attention has been directed toward enrichment, brain plasticity, and learning and memory processes [16,20,30,40,41] and stress and affective responses [2], enrichment can also alter immune parameters [1,2,19].

Specifically, environmental enrichment increases natural killer (NK) cell activity [2], reduces tumor growth, and increases survival in tumor-bearing mice [1]. Additionally, enriching variables (most notably, exercise and social interactions) can enhance immune function by themselves. Vigorous, voluntary exercise can protect immune function from suppressive effects of stress [12] and social housing can alter central and peripheral inflammatory processes [17]. In the present study, we used environmental enrichment as a tool to test the capacity of hamsters to respond to factors known to elevate aspects of immune function, in addition to photoperiod.

The immune measure employed here, DTH, has been extensively used as an assay of cell-mediated immune function and, as previously mentioned, has been used to measure cell-mediated immune function as a consequence of environmental and metabolic signals. Within 24 h of the challenge phase of DTH, mononuclear cells infiltrate the dermis where the antigen is applied; usually on the pinna [31]. The swelling response is correlated with resistance to infection [13,44]. However, enhancement of this process may not always be beneficial to the organism, as it may represent a pathological or allergic response. DTH is an integrative measure of cell-mediated immune responses that is dependent on antigen processing and presentation, memory formation and T cell-mediated inflammatory responses. Although this assay does not provide information about the specific component of the immune response altered by day length and enrichment it does serve as an overall index of cell-mediated immune function.

2. Methods

2.1. Animals

Thirty-three male Siberian hamsters (*Phodopus sungorus*) were used in this study. Hamsters were obtained from our breeding colony at the Ohio State University and were weaned at approximately 21 days in two cohorts directly into either short (8:16 LD) or long photoperiod (16:8 LD; with lights-off at 1500 Eastern Standard Time [EST] in both rooms) and either environmental enrichment or standard housing conditions, such that there were four groups: long-day, standard: $n=6$, long-day, enriched: $n=8$, short-day, standard: $n=8$, and short-day enriched: $n=11$. Hamsters were housed in their respective environmental conditions for 10 weeks before induction of delayed-type hypersensitivity (DTH). All hamsters had *ad libitum* access to food (Harlan Teklad Rodent Diet 8640) and filtered tap water. Environmental enrichment consisted of large polypropylene cages (45 cm × 24 cm × 20 cm), each equipped with a running wheel, a nest box, and a Habitrail® tube. Standard cages also consisted of polypropylene (28 × 17 × 12 cm). All hamsters were given a nestlet (in the enriched cages, 1 nestlet per hamster) and 1 cm of corncob bedding. Animal rooms were held at constant temperature and humidity (21 ± 4 °C and 50 ± 10%, respectively). All procedures were conducted in accordance with the National Institute of Health (1986) Guide for the Care and Use of Laboratory Animals and the Ohio State University Institutional Animal Care and Use Committees.

2.2. Induction of DTH

After 10 weeks of photoperiod and environmental treatment, hamster cages were brought to a procedure room one at a time between 1100 and 1300 EST (for all subsequent pinna measurements). Hamsters were lightly anesthetized with isoflurane vapors and a 2.5 × 2.5 cm patch of fur was shaved from the dorsum. To sensitize hamsters, 25 µl of 2,4, dinitro-l-flurobenzene (DNFB, Sigma) in a 0.5% solution (wt/vol) of 4:1 acetone to olive oil was applied to the dorsal skin in the same location on two consecutive days. DNFB was prepared fresh daily. During the first day of sensitization, 500 µl of blood was collected by a retroorbital sinus bleed. To obtain a baseline, pinna measurements were also taken on both days with a constant loading dial micrometer (Mitutoyo, America Corp., Aurora, IL). Hamsters were left undisturbed for 7 days, and again were anesthetized; pinnae thickness measured and challenged with 20 µl of 0.2% (wt/vol) DNFB in 4:1 acetone to olive oil on the surface of the right pinna. The left pinna was treated with the vehicle solution. Both

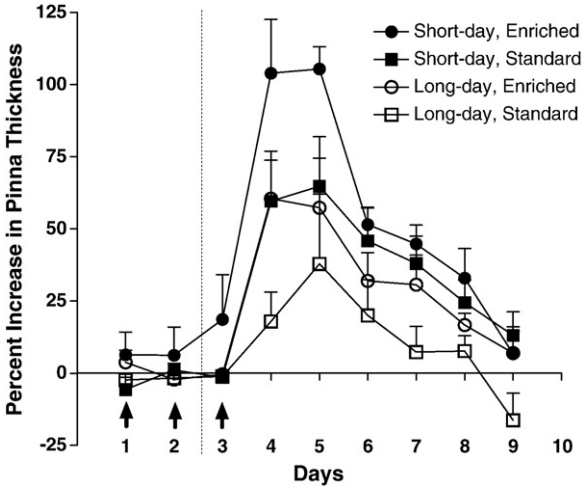

Fig. 1. Mean (±SEM) percent increase in pinna thickness. Short-day, enriched hamsters exhibited the most swelling, whereas long-day, standard-housed hamsters swelled the least. Of standard-housed hamsters, short days significantly increased swelling. Arrows represent DNFB application. On days 1 and 2, DNFB was applied to a shaved patch of skin on the dorsum (sensitization). On day 3 (challenge), DNFB was applied to the right pinna and vehicle was applied to the left. On 6 consecutive days thereafter, pinnae were measured at the same time of day. Note: days 1 and 2 and 3–9 are consecutive. Hamsters are allowed one week to build an immune response between sensitization and challenge.

pinnae were measured every 24 h for 7 days by the same investigator (J.L. Workman).

2.3. Necropsy

One week after the cessation of pinna measurements, isoflurane vapors were used to deeply anesthetize hamsters which were then rapidly decapitated. Testes, fat pads, epididymides, seminal vesicles, and spleens were removed; each organ was weighed (reproductive tissues are expressed as paired organ masses with the exception of testes mass which is expression in mg/g body mass). Trunk blood was also collected at this time. All blood samples were kept on ice until centrifugation at 6000 rpm (3.3 *g*) for 30 min at 4 °C. Plasma was drawn off and stored at −80 °C until the cortisol radioimmunoassay.

2.4. Cortisol radioimmunoassay

Plasma cortisol concentrations were determined in a single assay using a Diagnostic Systems Laboratories ^{125}I double antibody kit (Webster, TX). The assay was conducted based on the manufacturer's instructions. The sensitivity of this assay has been validated in this species [38]. The cross-reactivity of the antiserum in this kit for prednisolone, corticosterone, and other steroid hormones is 33.33%, 9.3% and <4%, respectively. The intraassay coefficient of variation was 13.8%.

2.5. Data analysis

Two hamsters in short photoperiod failed to regress their testes and were omitted from all analyses. The criterion for exclusion based on photoperiodic nonresponsiveness was paired testes mass must be within two standard deviations of the mean paired testes mass of long-day hamsters. One hamster failed to respond to DNFB (0% swelling on days typified by peak swelling: 4 and 5) and was removed from DTH analysis. Pinna thicknesses were transformed to % increase from left ear in swelling and these values were analyzed via a repeated measures ANOVA. Following a significant repeated measures ANOVA, 2×2 ANOVAs were performed for individual days to determine the effect of environment and photoperiod at peak swelling. Significant 2×2 (photoperiod×environment) ANOVAs were then followed by Fisher's PLSD to determine significant main effects as well as Student's *t* tests (based on *a priori* hypotheses) to determine simple main effects. ANOVAs (photoperiod×environment) were also performed for tissue masses and cortisol concentrations. Paired testes masses were divided by body mass and ANOVAs were conducted on these quotients. All data were analyzed in StatView software (v. 5.0.1, Cary, NC). Mean differences were considered statistically significant when $p \leq 0.05$.

3. Results

3.1. DTH responses

Both photoperiod and environment altered swelling responses across days ($F_{8,208}=2.828$, $p<0.01$; $F_{8,\ 208}=2.413$, $p<0.05$, respectively) but did not significantly interact with one another to alter pinna thickness ($F_{8,208}=0.939$, $p>0.05$; Fig. 1). One-way ANOVAs revealed that both photoperiod and environment altered swelling on day 4 ($F_{1,26}=6.981$, $p<0.05$; $F_{1,26}=7.255$, $p<0.05$, respectively). Fisher's PLSD revealed that both short days and environmental enrichment significantly increased swelling ($p<0.05$ in both cases). On day 5, photoperiod, but not housing environment, significantly altered swelling ($F_{1,26}=5.02$, $p<0.05$; $F_{1,26}=3.23$, $p>0.05$, respectively). Specifically, short days significantly increased swelling ($p<0.05$; Fig. 2A and B).

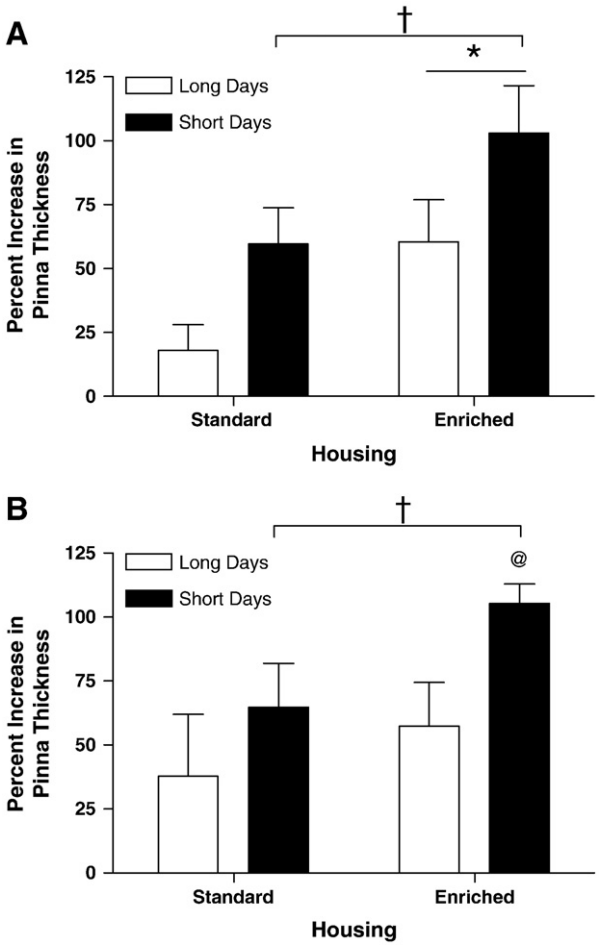

Fig. 2. Mean (±SEM) percent increase in pinna thickness. A) On day 4, short days significantly increased swelling and environmental enrichment also significantly increased swelling. B) On day 5, short days significantly increased swelling. Of enriched hamsters, short days significantly increased swelling. † indicates a main effect of photoperiod; * indicates a main effect of housing environment; @ indicates a simple main effect of photoperiod, $p<0.05$ in all cases. Note: Data replotted from Fig. 1.

3.2. Body mass and organ masses

There was a significant interaction between photoperiod and enrichment on body mass ($F_{1,26}=4.348$, $p<0.05$): short days significantly reduced body mass among standard-housed hamsters, but this difference was not present in enriched hamsters (Fig. 3).

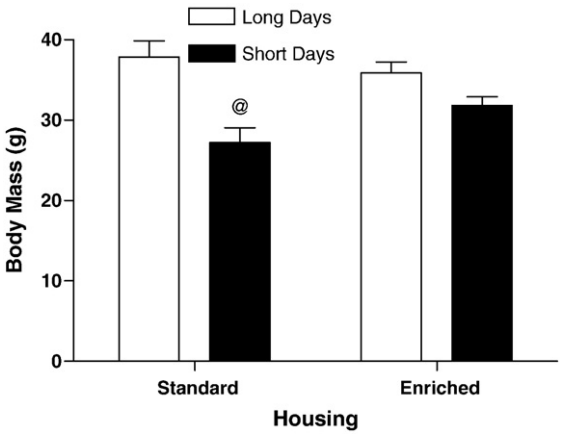

Fig. 3. Short days significantly reduced body mass in standard-housed, but not enriched hamsters. @ indicates simple main effect of photoperiod, $p<0.05$.

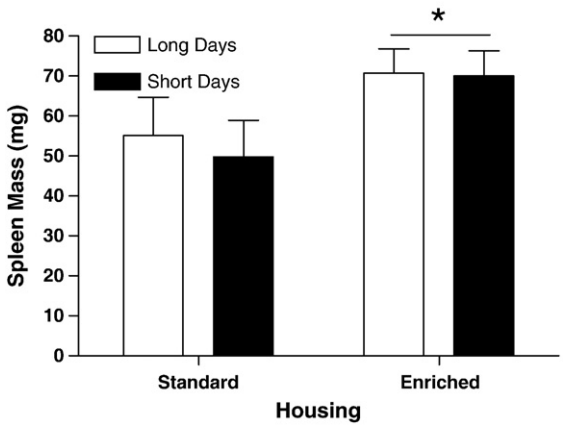

Fig. 4. Mean (±SEM) spleen mass in mg. Enrichment significantly elevated spleen mass in response to an LPS challenge 4 h earlier. * indicates a main effect of housing, $p<0.05$.

Enrichment, but not photoperiod, significantly altered spleen mass ($F_{1,27}=5.303$, $p<0.05$; $F_{1,27}=0.152$; $p>0.05$, respectively). PLSD revealed that enrichment significantly increased spleen mass ($p<0.05$; Fig. 4). Photoperiod and enrichment interacted to alter paired testes mass ($F_{1,27}=9.868$, $p<0.005$). As expected, short days significantly reduced testes mass regardless of housing environment ($p<0.0001$). Among long-day, but not short-day hamsters, environmental enrichment significantly increased relative paired testes mass (corrected for body mass, $t_{12}=2.803$, $p<0.05$). Photoperiod and enrichment interacted to alter epididymal mass ($F_{1,25}=4.121$, $p=0.05$). Short days significantly reduced epididymal mass regardless of housing condition ($p<0.0001$). Among long-day hamsters, enrichment significantly increased epididymal mass ($t_{12}=2.127$, $p=0.05$). Enrichment and photoperiod significantly interacted to alter fat pad mass ($F_{1,27}=13.575$, $p<0.005$). Among long-day hamsters, enrichment significantly reduced fat pad mass ($t_{12}=2.507$, $p<0.05$; Fig. 5A, B, C, and D). Short days significantly reduced seminal vesicle mass ($F_{1,26}=55.785$, $p<0.0001$), but enrichment had no effect ($p>0.05$).

3.3. Cortisol concentrations

Neither photoperiod nor enrichment significantly altered baseline cortisol concentrations ($p>0.05$ in both cases; Fig. 6).

4. Discussion

The goal of this study was to determine whether environmental enrichment in long photoperiods would enhance immune function or whether long-day investment in reproductive function precludes energy investment in immune function. These data suggest that long

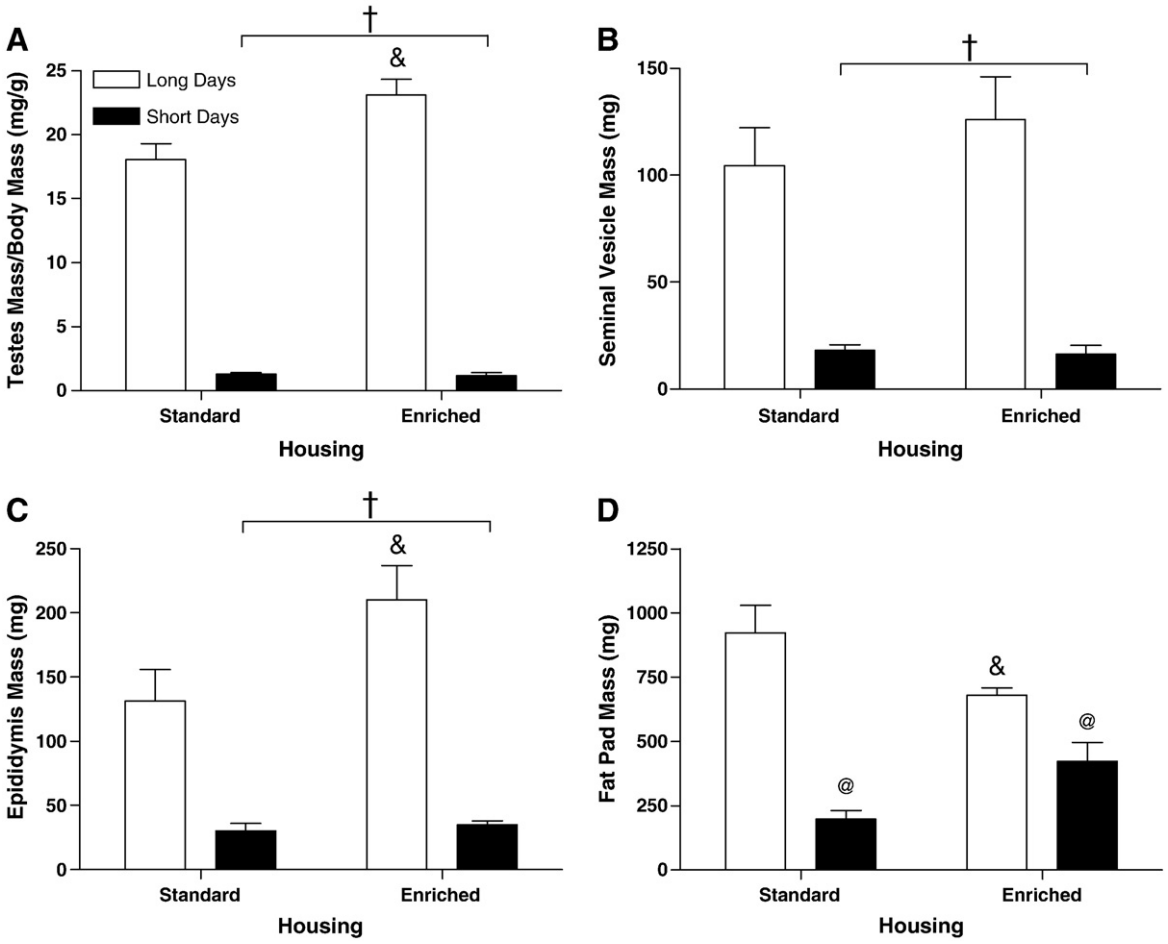

Fig. 5. Mean (±SEM) reproductive organ masses in mg; testes represented mg/g body mass. Open bars represent data from long-day hamsters and closed bars represent data from short-day hamsters. A) Short days significantly reduced relative testes mass irrespective of housing condition. Of long-day hamsters, enrichment significantly increased testes mass. B) Short days significantly decreased seminal vesicle mass. C) Short days significantly reduced epididymal mass. Of long-day hamsters, enrichment significantly increased epididymal mass. D) Photoperiod and enrichment interacted to alter fat pad mass. In both enriched and standard-housed hamsters, short days reduced fat pad mass. Enrichment reduced fat pad mass in long-day hamsters but increased fat pad mass in short-day hamsters. † indicates main effect of photoperiod; & indicates simple main effect of enrichment; @ indicates simple main effect of photoperiod, $p<0.05$ in all cases.

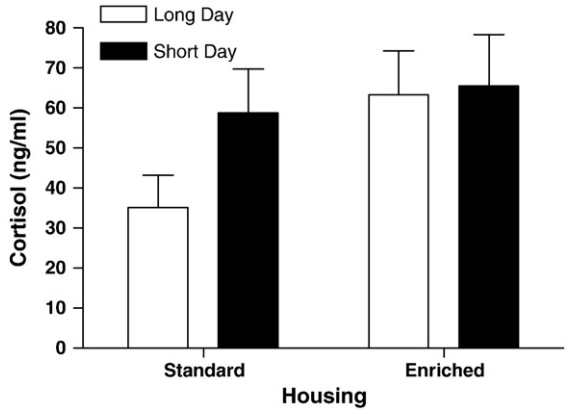

Fig. 6. Mean (±SEM) ng/ml cortisol concentrations from blood collected prior to DNFB exposure. Neither photoperiod nor housing environment altered cortisol concentrations ($p>0.05$).

days do not prevent investment of immune processes, allowing the immune system to respond to other environmental factors. These data disconfirm the hypothesis that organisms cannot simultaneously optimize reproductive and immune functions. We also tested the hypothesis that short days cap immune processes as short days may signal an energetic bottleneck. Our results suggest that environmental enrichment shifts the baseline of swelling responses. The results of our study also confirm and extend previous research indicating that environmental enrichment enhances some aspects of immune function [1,2] and that short days enhance cell-mediated immune function [36,43].

Our results suggest that the cost of maintaining reproductive function does not prevent the increased swelling responses. On the contrary, long-day enriched hamsters not only boosted DTH responses but also increased relative testes mass and absolute paired epididymides mass, which provides further evidence against the hypothesis that animals cannot simultaneously invest in reproductive and immune functions. Although short days may signal an energetic bottleneck, short days enhance skin immune responses in the laboratory [36,43]. This effect may represent an endogenous bolstering of immune function to compensate for chronic winter stressors (low food availability, low temperatures, and increased foraging) that may otherwise dampen immune function [26,27].

The proximate mechanisms governing short-day elevation in DTH responses are not entirely resolved. Melatonin may alter immune function both directly (through melatonin receptors on peripheral immune cells) and through downstream hormones (notably gonadal and adrenal steroids, prolactin, and leptin). However, short-day enhancement of skin immune responses is independent of testosterone as castration does not alter the effects of photoperiod on immune function [36]. In the present study, short days did not significantly increase cortisol concentrations. Baseline cortisol (the primary circulating glucocorticoid in Siberian hamsters) typically increases in individuals of this species maintained in short days [3,42] but see also [9]. It remains possible that the time at which blood was collected (1000 h EST) was at different points in the circadian cycle for short day hamsters (3 h after lights on) versus long-day hamsters (11 h after lights on). It should be noted, however, that the means of cortisol concentrations for the long-day standard and short day standard hamsters were in the expected direction. Chronic increases in cortisol (or corticosterone) typically suppress immune function [24], so it is also unlikely that cortisol mediates short-day enhancement in skin immune function. Other hormones that vary on a seasonal basis, such as leptin and prolactin, may also modulate immune function [9,18]. Several studies also suggest that melatonin may alter immune function directly, as they have reported melatonin binding sites on lymphatic tissue and circulating immune cells [34,35].

It is as yet unclear how environmental enrichment modulates changes in the immune system. However, exercise and social interactions are the most likely mediators as opposed to visual and tactile stimulation. Both exercise [4,12] and social interactions [7,14,17] can modulate immune processes as demonstrated in nonhuman animal studies. Positive social interactions may attenuate activity of the hypothalamic–pituitary–adrenal (HPA) axis and in turn, facilitate skin immune responses [8]. Research in humans also suggests these two variables are important modulators of immune function and disease outcome [11,15]. Our research indicates that photoperiod does not influence hamsters' responsiveness to enrichment in their skin immune responses. Although enrichment did not significantly affect cortisol concentrations, these data do not preclude a role for cortisol as one time point is insufficient to characterize how enrichment influences HPA axis activity and subsequently, DTH responses. It should also be noted that wheel running and social interactions can alter properties of circadian entrainment and this may impact swelling as well [25]. Environmental enrichment also increased spleen mass. Larger spleens may reflect cellular content and an influx of immune cells into the organ. However, this splenic swelling may also represent a pathological response in which larger spleens are not necessarily adaptive for the organism [29]. Regardless, this response remains an energetically expensive process, and our data suggest that enrichment shifts the baseline of spleen mass.

Short days reduced paired testes, epididymides, and seminal vesicle masses. In long-day hamsters, enrichment significantly increased paired testes and epididymides mass. Exercise can increase gonadotropin secretion and slow seasonal reproductive regression [32,33], which may explain why this group had large testes and epididymides. We previously reported that enrichment increased paired testes mass in white-footed mice [45]. In contrast, enrichment reduced paired gonad-associated fat pad mass in long-day hamsters. Rather than an indicator of reproductive investment, this gonadal fat pad mass may represent a reduction in overall body fat deposition as a result of increased voluntary exercise. Future research needs to address this hypothesis.

In conclusion, this research indicates that short days do not prevent further investment in immune function as evidenced by DTH and spleen mass. These results are also consistent with previous research investigating how photoperiod and exercise influences DTH responses [4]. The ultimate consequences of further enhancing immune function are unclear, but future research should be aimed at investigating the adaptive significance of maintaining flexibility of immune processes in short days.

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