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Early Life Experiences Affect Adult Delayed-Type Hypersensitivity in Short and Long Photoperiods

Laura K. Fonken, John S. Morris, and Randy J. Nelson

Departments of Neuroscience and Psychology, Institute for Behavioral Medicine Research, The Ohio State University, Columbus, Ohio, USA

Environmental experiences during development provide animals with important information about future conditions. Siberian hamsters are photoperiodic rodents that dramatically adjust their physiology and behavior to adapt to seasonal changes. For example, during short winter-like days, hamsters enhance some components of immune function putatively to cope with increasing environmental challenges. Furthermore, early life stress alters the developmental course of the immune system. Overall, immune function is typically suppressed in response to chronic stress, but responses vary depending on the type of stress and components of immune function assessed. This led us to hypothesize that delayed-type hypersensitivity (DTH), an antigen-specific, cell-mediated immune response, would be differentially modulated in hamsters that underwent early life maternal separation (MS) in either short or long photoperiods. At birth, hamsters were assigned to either short (SD; 8 h light/day) or long (LD; 16 h light/day) photoperiods and either daily 3 h MS, daily 15-min brief maternal separation (BMS), or no manipulation from postnatal day 2 through 14. In adulthood DTH was assessed. Hamsters reared in short days enhanced DTH responses. MS and BMS attenuated DTH responses in both short and long days. However, BMS long-day female hamsters did not suppress pinna swelling, suggesting a protective effect of female sex steroids on immune function. As is typical in short days, reproductive tissue was regressed. Reproductive tissue mass was also decreased in long-day MS female hamsters. Furthermore, MS altered photoperiod-induced changes in body mass. Taken together, these findings suggest that manipulations of early life mother-pup interactions in Siberian hamsters result in physiological changes and suppressed cell-mediated immunity. (Author correspondence: fonken.1@osu.edu).

Keywords: Delayed-type hypersensitivity, Immune, Maternal separation, *Phodopus sungorus*, Photoperiod, Sex differences

INTRODUCTION

Early life experiences can profoundly affect the growth and development of animals (McEwen, 2008). Adult disease and risk factors for poor health are embedded biologically during developmentally sensitive periods, during which time the brain is more reactive to wide-ranging positive and negative environmental signals (Johnson, 2005). Early experiences can prepare individuals for unstable, high-stress conditions; however, this can place individuals on a trajectory where the benefits of short-term survival may come at a significant cost to longer-term health (Shonkoff et al., 2009).

Because some seasonal stressors, such as low temperatures and food scarcities, are generally predictable, nontropical individuals have evolved mechanisms to determine time-of-year by attending to day length (photoperiod). Photoperiodic information from the lateral eyes is transduced into a physiological signal via the secretion of the nocturnal pineal hormone melatonin, with the duration of nighttime melatonin production being proportional to the duration of the night (Foster et al., 2007; Reiter, 1993). Siberian hamsters (*Phodopus sungorus*) are photoperiodic rodents that undergo dramatic physiological and behavioral changes in response to different seasons (Hoffmann, 1973; Pyter & Nelson, 2006). In the laboratory, season-specific characteristics of Siberian hamsters are recapitulated by manipulating photoperiod. In temperate and boreal regions, winter decreases energy availability, increases thermoregulatory demands, as well as increases mortality and the risk of infection and disease (Lee & McDonald, 1985). During summer when energy availability is comparatively abundant and environmental conditions are relatively mild, hamsters invest in reproduction, whereas during the challenging energetic conditions of fall and winter, hamsters shunt energy away from reproductive activities to

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Address correspondence to Laura K. Fonken, Department of Neuroscience, The Ohio State University, 460 West 12th Avenue, Columbus, OH 43210, USA. Tel.: 614-688-4674; Fax: 614-688-4733. E-mail: fonken.1@osu.edu

support survival adaptations (Goldman, 1999). Melatonin appears to serve as the physiological signal mediating this physiological trade-off between reproduction and survival (immune) mechanisms (Nelson & Drazen, 1999). Siberian hamsters significantly adjust immune function in preparation for the winter months. For example, adult Siberian hamsters housed in short-day lengths enhance delayed-type hypersensitivity (DTH; an index of cell-mediated immune activity), which suggests increased resistance to viruses, bacteria, and fungi during the winter (Bilbo et al., 2002a). Conversely, hamsters decrease febrile responses and phagocytosis (an index of the innate immune system) (Bilbo et al., 2002b; Yellon et al., 1999), which may protect against endotoxemia (Prendergast et al., 2003). Importantly, early life photoperiod also organizes adult immune function in Siberian hamsters (Weil et al., 2006).

In addition to photoperiod, other aspects of the environment during development provide important information about future conditions. In humans, early life stress alters the developmental course of the immune system. Childhood maltreatment predicts inflammatory bias in adults, possibly due to early adverse experiences programming stress-responses later in life (Danese et al., 2007). Alternatively, traumatic experiences are also associated with epigenetic changes in genes associated with immune function and may modulate immune function in adulthood independently of glucocorticoids (Uddin et al., 2010). Childhood socioeconomic status is similarly predictive of resistance to infection in adulthood (Miller et al., 2009).

Animal models have provided much insight into the differential phenotypic responsivity to stress early in development (Champagne, 2008; Meaney, 2001), with both rodent and primate models demonstrating how negative early life experiences produce long-lasting changes in stress-reactivity and immune function (Lewis et al., 2000). Brief and long-term separation of dams from their pups is a common method of manipulating early life experience in rodents (Anisman et al., 1998). Long-duration periods of maternal separation (MS) in the days directly after birth result in exaggerated stress-responses in adolescence and adulthood (Ladd et al., 2000), whereas brief periods of maternal separation (BMS or handling) during the same developmental epochs result in an opposing phenotype, with decreased physiological and behavioral responses to stress and more efficient hypothalamic-pituitary-adrenal (HPA)-axis functioning (Francis et al., 1999; Liu et al., 1997). Mice that undergo MS have altered host-response to viral infection, with increased cytokines and increased viral replication after exposure to influenza (Avitsur et al., 2006). Furthermore, MS mice display altered sickness behavior after injection with lipopolysaccharide (LPS), a bacterial cell wall component (Avitsur & Sheridan, 2009). BMS also alters immunological properties. For example, neonatal handling prevents stress-induced suppression of an immune measure in mice (Bhatnagar et al., 1996), whereas BMS exacerbates neurological damage and inflammatory properties after experimental stroke (Craft et al., 2006).

The differential phenotypes resulting from brief versus long-term maternal separation may relate to normal mother-pup interactions; in rodents, the dam is routinely away from the nest for periods of 20–30 min. However, protracted periods of separation result in loss of care. Indeed, naturally occurring maternal variations in licking and grooming behaviors produce differential phenotypes, much as those associated with brief and long maternal separation (Champagne, 2008); offspring of high-licking dams have more efficient HPA-axis functioning, whereas offspring from low-licking nests have decreased HPA-axis responses (Champagne, 2008). In addition to reducing the opportunity for grooming, long-duration, as opposed to brief, separation of pups from the dam increases depressive-like behaviors in the dam that correlate with decreased maternal care after the dam is reunited with the pups (Boccia et al., 2007). These alterations in mother-pup interactions may provide information to offspring about environmental conditions that cause changes in offspring development.

Photoperiod and maternal separation are both environmental manipulations that provide critical information to rodents early in life, information that may be used to adjust their developmental trajectory. Other rodent models have shown that maternal behavior may provide information to offspring about their future environment (McLeod et al., 2007). Therefore, we were interested in establishing whether a maternal signal for poor conditions has differential effects in short days compared to long days. In short days, hamsters are already preparing for harsh winter conditions by delaying growth and development of reproductive function. We examined the extent to which poor maternal behavior influences this winter preparation. More specifically, we evaluated how early life mother-pup relations interact with differential photoperiodic environments in developing the immune system. We hypothesized that MS and BMS would alter T cell–mediated immune function and that short days would increase pinna swelling responses in a delayed-type hypersensitivity test regardless of early life experience.

EXPERIMENTAL PROCEDURES

Animals

Siberian hamsters (*Phodopus sungorus*) used in this study were bred in our colony at the Ohio State University from a wild-bred stock obtained from Dr. K. Wynne-Edwards (Kingston, Ontario, Canada). Hamsters were housed in polypropylene cages (28 × 17 × 12 cm) with a nestlet and 1 cm of corncob bedding. All hamsters had ad libitum access to food (Harlan Teklad Rodent Diet 8640; Indianapolis, IN, USA) and filtered tap water. Animal rooms were held at constant temperature and

relative humidity (21°C ± 2°C and 50% ± 10%, respectively). All procedures were conducted in accordance with the US National Institute of Health (1986) Guide for the Care and Use of Laboratory Animals, the Ohio State University Institutional Animal Care and Use Committee, and the international ethical standards described in Portaluppi et al. (2010).

Early Life Experience

Breeding pairs were established in a long-day room (16 h light/day). Pairs were inspected daily for the presence of pups, and the day of birth was designated postnatal day 1 (PND 1). On PND 1, male breeders were removed from the breeding cage, and cages were either transferred to a short- (8 h light/day) or long-day room where they stayed for the remainder of the study. Litters were further assigned to receive daily BMS, MS, or no manipulation (animal facility reared; AFR) from PND 2 to PND 14. Each of the six groups was composed of four to five litters in order to minimize litter effects. The maternal separation procedure consisted of removing the hamster dam from the cage and placing her in a new cage (used throughout the 13 days of separation). Pups were then carefully removed from the cage, weighed, and whole litters were placed together in a separate cage inside of an incubator (30°C ± 1°C). MS pups remained in the incubator for 3 h and were then placed back in the home cage and covered in bedding before reintroducing the dam. BMS pups were treated in the same manner except their period of separation only lasted 15 min. Pups were weaned at 22 days of age and housed with one or two same-sex siblings until 6 wks of age when they were individually housed. Three litters with infanticide were dropped from the study. A subset of the offspring from the remaining 27 litters, totaling 82 pups, was used for subsequent testing in adulthood.

Delayed-Type Hypersensitivity

At 9 wks of age, hamsters were sensitized to 2,4-dinitro-1-flurobenzene (DNFB; Sigma, St. Louis, MO, USA) by a method and dose previously established to work with Siberian hamsters in our laboratory (Bilbo et al., 2002a). Hamsters were individually brought into a procedure room, lightly anesthetized with isoflurane vapors, weighed, and then a 1 × 2-cm patch of fur was shaved on their dorsum. Twenty-five microliters of DNFB in a 0.5% solution (*w/v*) of 4:1 acetone to olive oil (prepared fresh daily) was applied to the dorsal skin in the same location on two consecutive days. To obtain a baseline measurement, both right and left pinna were measured during sensitization with a constant-loading dial micrometer (Mitutoyo; America Corp., Aurora, IL, USA). Hamsters were then left undisturbed for 1 wk, after which they were again anesthetized, pinna thickness measured, and challenged with 20 μL of 0.2% (*w/v*) DNFB in 4:1 acetone to olive oil on the surface of the right pinna. The left pinna was treated with the vehicle solution, and both pinnae were measured every 24 h for 7 days by L. K. Fonken. Swelling values obtained on each day were expressed as a percentage of baseline thickness. The challenge was done at the same time as the measurements were on the subsequent days, between 07:30 and 09:00 h; this was well before lights-off, which occurred at 14:00 h, in the animal rooms. DTH is a measure of cell-mediated immune responses *in vivo* and is characterized by swelling at the site of the DNFB challenge, in this case the right pinna. This pinna swelling response is due to an infiltration of macrophages and lymphocytes into the epidermis and dermis (Vadas et al., 1975). Increase in pinna swelling has previously been positively correlated to the intensity of the immune reaction (Phanuphak et al., 1974). Although DTH does not reflect all aspects of immune function, it does provide information about the primary immune response to an invading pathogen.

Reproductive and Somatic Responses

One week after cessation of pinna measurements, hamsters were deeply anesthetized with isoflurane vapors, weighed, and rapidly decapitated. Reproductive tissues were removed (females: uteri, ovaries, and ovarian fat pads; males: testes, epididymal fat pads, seminal vesicles, and epididymides), cleaned of connective tissue, and weighed to the nearest 0.1 mg. All short-day-housed hamsters whose uteri or testes fell within 2 standard deviations of the long-day mean were considered photoperiodically nonresponsive. One short-day AFR male was reproductively nonresponsive and was excluded from further analyses.

Statistical Analyses

Reproductive tissue and body mass were analyzed by a 3 × 2 analysis of variance (ANOVA) (early life experience × photoperiod) separately for each sex. DTH reactions were analyzed separately for each sex by a repeated-measures ANOVA, with early life experience and photoperiod as the between-subjects factors and day as the within-subjects factor. Following a significant repeated-measures ANOVA, 3 × 2 ANOVAs (early life experience × photoperiod) were performed for individual days to determine the effect of early life experience and photoperiod on peak pinna swelling. After attaining significant F scores, multiple comparisons were conducted with Tukey's honestly significant differences test (Keppel & Wickens, 2004). All data were analyzed in StatView software (v. 5.0.1; Cary, NC, USA). In all cases, mean differences were considered statistically significant when $p \leq .05$.

RESULTS

Somatic Measures

At the end of the study, short-day female hamsters weighed significantly less than those housed in long days ($F_{1,38} = 58.944$, $p < .0001$; Figure 1A). There was

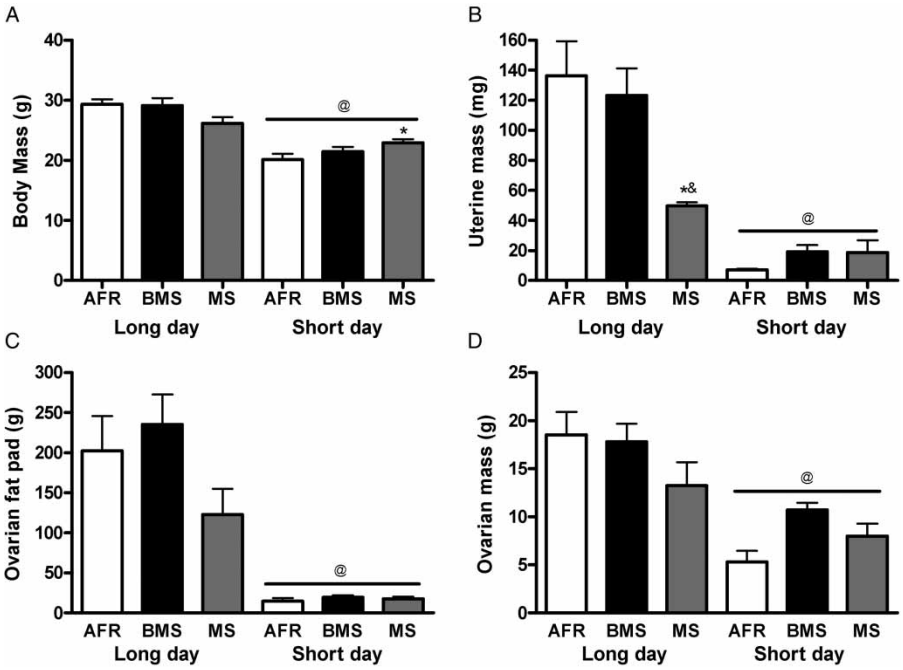

FIGURE 1. (A) Body mass is decreased in female short-day Siberian hamsters. However, maternally separated hamsters increased body mass in short days. (B) Short-day hamsters and long-day maternally separated hamsters decreased uterine mass. (C, D) Female hamsters reduced (C) ovarian fat pad mass and (D) ovarian mass in short days. Data expressed as mean (±standard error of the mean, SEM). $^{*}p < .05$ between MS and AFR; $^{\&}p < .05$ between BMS and MS; $^{@}p < .05$ between short-day and long-day hamsters.

also an interaction between rearing and photoperiod ($F_{2,38} = 3.736$, $p < .05$) such that among short-day female hamsters, MS significantly increased body mass as compared to AFR (post hoc, $p < .05$). Short days reduced uterine mass ($F_{1,35} = 51.873$, $p < .0001$; Figure 1B). Furthermore, there was an interaction between photoperiod and rearing; MS long-day hamsters had reduced uterine mass as compared to both AFR and BMS conspecifics (post hoc, $p < .005$). Short days also reduced ovarian fat pad mass ($F_{1,35} = 49.683$, $p < .05$; Figure 1C) and ovarian mass ($F_{1,35} = 34.474$, $p < .05$; Figure 1D).

Similar to females, male short-day hamsters weighed significantly less than those housed in long days at the conclusion of the study ($F_{1,30} = 57.855$, $p < .0001$; Figure 2A). Furthermore, there was an interaction between rearing and photoperiod ($F_{2,30} = 3.974$, $p < .05$); among long-day male hamsters, those that underwent MS as infants weighed significantly less than those that experienced BMS (post hoc, $p < .001$). Short days reduced testes mass ($F_{1,30} = 435.070$, $p < .0001$; Figure 2B), epididymal fat pad mass ($F_{1,30} = 84.222$; $p < .0001$; Figure 2C), seminal vesicle mass ($F_{1,30} = 34.739$, $p < .0001$), and epididymal mass ($F_{1,30} = 27.260$, $p < .0001$; Figure 2D). There was also an interaction between photoperiod and rearing with respect to epididymal fat pad mass ($F_{1,30} = 3.515$, $p < .05$); BMS long-day hamsters had elevated epididymal fat pad mass as compared to both AFR and MS conspecifics (post hoc, $p < .05$).

Delayed-Type Hypersensitivity Response

Vehicle-treated (left) pinna showed no swelling as compared to the baseline measurement during the experiment (data not shown). In contrast, DNFB elicited a strong swelling response on the right pinna in both female ($F_{5,180} = 38.861$, $p < .0001$; Figure 3A, B) and male Siberian hamsters ($F_{5,155} = 83.374$, $p < .0001$; Figure 3C, D). Among female hamsters, there was a significant effect of photoperiod ($F_{5,180} = 6.839$; $p < .001$; Figure 3A, B) and rearing ($F_{10,180} = 3.338$, $p < .001$) on DTH response. There was a main effect of photoperiod on days 1 ($F_{1,36} = 12.661$, $p < .005$) and 2 ($F_{1,36} = 13.027$, $p < .005$); short-day hamsters had elevated pinna swelling (post hoc, $p < .005$). There was a main effect of rearing conditions on postchallenge days 2 ($F_{2,36} = 5.124$, $p < .05$), 3 ($F_{2,36} = 9.763$, $p < .05$), 4 ($F_{2,36} = 7.132$, $p < .05$), and 5 ($F_{2,36} = 4.979$, $p < .05$), such that BMS and MS hamsters had decreased pinna swelling on days 2, 3, and 4, and MS hamsters also had decreased pinna swelling on day 5 as compared to AFR hamsters (post hoc, $p < .05$). Post hoc comparison revealed decreased pinna swelling in the BMS group was limited to short-day females.

Male hamsters also significantly differed in DTH response by photoperiod ($F_{5,155} = 2.977$, $p < .05$; Figure 3C, D) and rearing ($F_{10,155} = 3.181$, $p < .01$). There was a main effect of photoperiod on postchallenge days 1 ($F_{1,30} = 11.028$, $p < .05$) and 2 ($F_{1,30} = 7.278$, $p < .05$), such that short-day hamsters had an elevated pinna

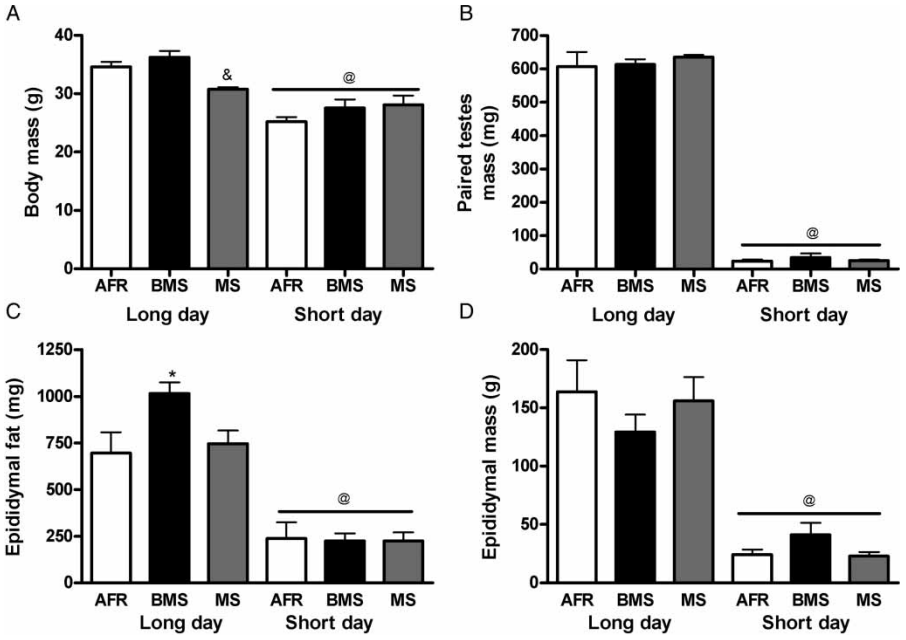

FIGURE 2. (A) Male Siberian hamsters decreased body mass in short days, and maternal separation decreased body mass in long days. (B–D) Male short-day hamsters reduced (B) testes mass, (C) epididymal fat pad mass, and (D) epididymal mass as compared to long-day conspecifics. Data expressed as mean (±standard error of the mean, SEM). $^{*}p < .05$ between BMS and AFR; $^{\&}p < .05$ between BMS and MS; $^{@}p < .05$ between short-day and long-day hamsters.

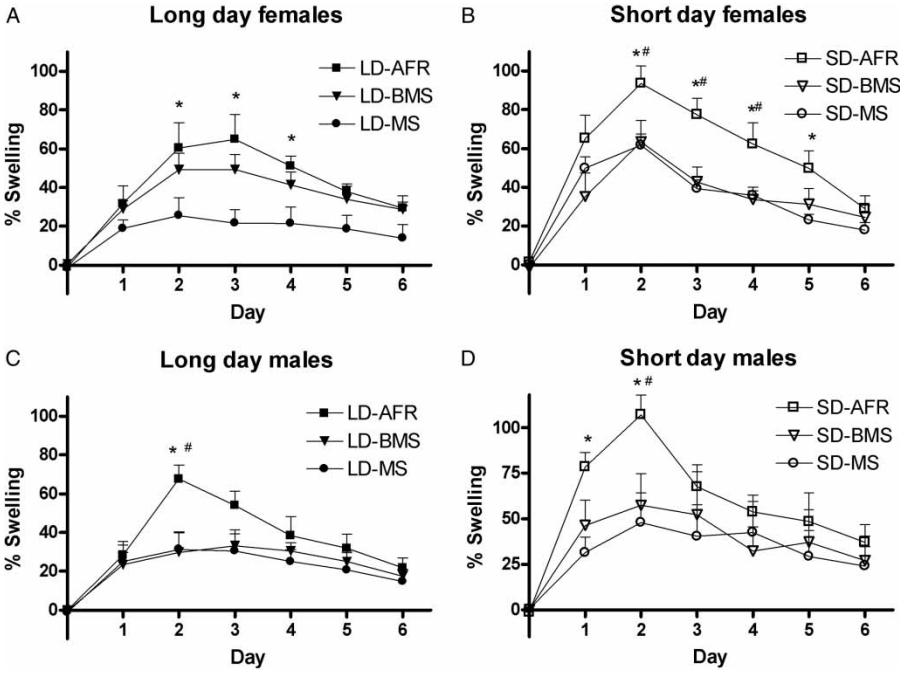

FIGURE 3. Delayed-type hypersensitivity responses in (A) long-day female, (B) short-day female, (C) long-day male, and (D) short-day male Siberian hamsters that underwent maternal separation, brief maternal separation, or control conditions early in life. Data expressed as mean (±SEM). $^{*}p < .05$ between MS and AFR hamsters; $^{\#}p < .05$ between BMS and AFR hamsters.

swelling response as compared to long-day hamsters (post hoc, $p < .001$). There was also a main effect of rearing on days 1 ($F_{2,30} = 3.633$, $p < .05$) and 2 ($F_{2,30} = 8.617$, $p < .05$), with AFR hamsters showing more robust pinna swelling than either MS or BMS hamsters.

DISCUSSION

The goals of this study were twofold: (1) to investigate the effects of early life mother-pup interactions on cell-mediated immune function and (2) to assess whether early life photoperiod differentially interacts with

maternal manipulations. Our results confirm and extend previous reports in rats and mice (Avitsur & Sheridan, 2009; Bhatnagar et al., 1996) by indicating that early life maternal separation decreases a cell-mediated immune response in adult Siberian hamsters regardless of photoperiod. Contrary to our prediction, most of the groups that underwent brief maternal separation also had impaired DTH responses with comparable pinna swelling to MS hamsters. Consistent with previous findings, both short-day male and female hamsters exhibited enhanced DTH responses. Furthermore, long-days may buffer against the deleterious effects of BMS on the immune system in female hamsters, as this group did not display the same decrease in pinna swelling as other hamsters that underwent early life manipulations. Reproductive tissue was regressed in short-day male and female hamsters, and MS significantly reduced reproductive tissue weights in long-day female hamsters. Both male and female hamsters that underwent MS altered photoperiod-induced adjustments in body mass.

Short-day male and female hamsters displayed more robust pinna swelling after the DTH challenge than long-day hamsters (Bilbo et al., 2002a). One limitation of this study is that hamsters born into long days would not normally experience an immediate shift to short days in the wild, although this manipulation likely targets similar physiological processes as the more gradual light transitions. In situations of acute stress, blood leukocytes redistribute to the skin, mucosal lining of the gastrointestinal and urinary-genital tracts, lung, liver, and lymph nodes—key areas in preventing immune defenses from being breached (Dhabhar, 2002; Dhabhar & McEwen, 1999). The mechanisms underlying the short-day increase in skin immune function have not been fully elucidated, but complex variation in androgens, melatonin, and other hormones are likely involved in seasonal fluctuations in immune function (Drazen et al., 2001; Kelley et al., 2007; Maestroni, 1993; Nelson & Drazen, 1999). For example, seasonal changes in immune function in Siberian hamsters follow a similar time course to seasonal changes in the reproductive system. Whereas short-term extension of melatonin rhythms is insufficient to cause immunoenhancement or reproductive regression (Drazen et al., 2002), long-term nightly extension of melatonin results in the short-day pattern of immune responses (Bilbo & Nelson, 2002; Bilbo et al., 2002a). However, melatonin also has direct immunomodulatory effects acting on immunocytes (for review see Srinivasan et al., 2008).

Both BMS and MS early in life suppressed DTH response in adulthood. Because of the opposite HPA-axis activity associated with MS and BMS, we expected cortisol to differentially modulate DTH in MS and BMS hamsters. For example, acute stress increases DTH, whereas chronic stress suppresses DTH reactions, demonstrating changes in DTH are in some cases related to altered glucocorticoid concentrations (Dhabhar & McEwen, 1999). Decreased pinna swelling response in the MS groups is congruous with previous models of DTH and chronic stress. Conversely, the decreased pinna swelling in BMS hamsters is unlikely mediated by changes in glucocorticoids, alone, because increased pinna swelling would be more congruous with the more efficient HPA-axis functioning previously described in BMS models. Alternatively, changes in DTH reactivity that occur in both MS and BMS groups may occur by a similar mechanism, independently of adulthood HPA axis activity. Stress can cause epigenetic changes in the immune system (Uddin et al., 2010), suggesting that immune cells may be programmed during early life to respond differentially to an immune challenge. Moreover, maternal separation may cause increased inflammation in infancy (Hennessy et al., 2007), and inflammation during early development impacts immune function in adulthood (Kentner et al., 2010).

The long-day females that underwent BMS did not show similar suppression in DTH response as the other BMS groups. This may indicate protective effects of female sex steroids, because short-day female hamsters have very low concentrations of estrogen and decreased pinna swelling similar to the BMS male hamsters. Estrogens directly modulate immune function, enhancing both cell-mediated and humoral immune responses (Klein, 2000). Furthermore, estrogens may indirectly alter immune function by interacting with early life stress conditions (Darnall & Suarez, 2009).

Short days produced the expected regression of reproductive tissue in all groups. MS long-day female hamsters also had reduced uterine mass, which may indicate impaired reproductive capabilities. Consistent with previous findings, short days decreased body mass in both male and female hamsters. There were, however, differences among MS hamsters with respect to photoperiod-induced changes in body mass. In short days, MS female hamsters increased body mass as compared to AFR hamsters. Furthermore, male MS hamsters housed in long days had reduced body mass. This demonstrates that MS hamsters may not respond effectively to photoperiod, which could be disadvantageous in the wild. Siberian hamsters putatively reduce body mass in the winter months to save energy by having less mass to support metabolically.

In conclusion, both photoperiod and maternal separation provide critical information to rodents early in life that impacts physiological and immunological developmental trajectory. Our data provide evidence that exposure to brief or long periods of maternal separation early in life alters primary host-immune responses as measured by delayed-type hypersensitivity in adulthood. This effect is differentially modulated by photoperiod as long-day female BMS hamsters did not compromise immune function as the other MS and BMS groups. Furthermore, we show that long periods of maternal separation may cause maladaptive alterations in body mass and reproductive tissue in adult hamsters. These results add to the growing body of literature demonstrating the

importance of early life experiences, including season of birth, on the developing immune system.

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