Published in final edited form as:

Physiol Behav. 2007 February 28; 90(2-3): 459–465.

Neonatal exposure to short days and low temperatures blunts stress response and yields low fluctuating asymmetry in Siberian hamsters

Zeynep Benderlioglu^{*}, Eliot Dow, and Leah M. Pyter

Departments of Psychology and Neuroscience, The Ohio State University, Columbus, OH 43210, USA

Abstract

Fluctuating asymmetry (FA) refers to small, non-directional deviations from perfect bilateral symmetry in morphological characters. Individuals with low FA presumably either developed in a relatively stable environment and/or were better able to buffer against developmental stressors. The present study investigated the effects of seasonal factors measured by day length and ambient temperature manipulations on the development of bilateral characters and concomitant changes in stress responses. Siberian hamsters were exposed to either long days (16 h of light per day) or short days (8 h of light per day) combined with either standard temperatures ($21 \pm 2^\circ\text{C}$) or low temperatures ($8 \pm 2^\circ\text{C}$) on the day of birth until weaning. Cortisol concentrations at baseline and following acute restraint stress, and FA values were measured in adulthood. Females reared in winter-like conditions with short day lengths and low temperatures had low FA and low cortisol concentrations following restraint stress compared to other females. Females reared in long day lengths and standard temperatures had the highest rate of increase in cortisol concentrations after restraint among other female groups. No group effects were observed in males regarding day length and temperature manipulations. Baseline and post-restraint cortisol concentrations were higher in females than males for all groups except in animals reared in short day lengths and low temperatures. Our results suggest that winter-like conditions during neonatal period evoke hyposensitivity to stress in adult females and this blunted response to stress is a key factor in achieving ideal growth patterns.

Keywords

stressors; neonatal development; reproduction; developmental instability; cortisol; photoperiod

1. Introduction

Fluctuating asymmetry (FA), small, non-directional deviations from perfect symmetry in bilateral characters is widely used as a proxy to quantify and explore the effects of developmental insults on individuals' health, fitness, and behavior. Individuals with symmetrical traits presumably either developed in a relatively stable environment and/or were better able to buffer against developmental stressors [1–4]. In several species, genetic

Address Correspondence To: Zeynep Benderlioglu, Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, 300 Aronoff Laboratory, 318 W. 12th Ave., Columbus, OH 43210, Phone: 614-292-8088, Fax: 614-292-2030, E-mail: benderlioglu.1@osu.edu.

^{*}Current Address: Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, Columbus OH 43210

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

perturbations, such as homozygosity [5], inbreeding [6–8], and harmful mutations [9] increase deviations from perfect symmetry and yield high FA in the offspring. Similarly, exposure to chemical pollutants [10–12], parasites [13], extreme temperatures [12,14], and food shortages [15] during ontogeny elevates FA.

Restricted food, extreme temperatures, and the prevalence of infectious agents vary with seasons, thus the offspring born in unfavorable months marked by the existence of such seasonal stressors should be at risk for asymmetrical developmental trajectory. Indeed, people born in winter and spring have higher FA compared to those born in fall [16]. The etiology of the birth season and FA relationship is poorly understood, however. Because of the difficulty in determining the exact timing and nature of the seasonal stressors in human studies, controlled experiments with non-human animals may illuminate this etiology. Accordingly, the current study aims to investigate specific mechanisms underlying seasonality in the development of bilateral morphological traits.

Two of the most salient seasonal indicators are day length (i.e. photoperiod) and ambient temperatures. The present study used Siberian hamsters, *Phodopus sungorus*, because these animals breed during long days of spring and summer when increasing ambient temperatures and food availability are favorable for reproduction. Seasonal reproduction in Siberian hamsters presumably evolved to conserve energy during challenging winter months and enhance the chances of offspring survival [17,18]. Because the onset of winter can be predicted by changes in the environment (e.g., photoperiod and ambient temperatures) by responding to predictive environmental cues, animals cease reproduction and allocate appropriate energy to other important physiological functions during winter, such as enhanced immune function [19–22]. We hypothesized that maintaining an ideal developmental trajectory may thus be energetically costly during winter months, which is characterized by short day lengths and low temperatures. Consequently, animals exposed to short days and low temperatures during neonatal development should display high FA. Our experimental manipulation was restricted to the neonatal period only, because previous studies indicated that gestating female rodents and other nonhuman mammals exposed to low temperatures frequently abort [23–25]. Also, exposure to stressors during postnatal development alone is sufficient to induce high FA in the offspring in rodents [26].

In this study, physiological response to stressors in adulthood and sex differences were also investigated to determine whether the expected morphological changes were accompanied by physiological changes as measured by cortisol concentrations. Previous reports indicated that hypothalamic-pituitary-adrenal (HPA) axis regulation and glucocorticoid secretion in response to stressors are sexually dimorphic in humans and nonhuman animals [see 27 for a review]. Although human studies yielded inconsistent results, animal studies generally show that glucocorticoid concentrations after HPA axis stimulation are higher in females than males [27]. Also, photoperiod appears to moderate sexually dimorphic responses to acute stress in Siberian hamsters [21]. Because FA is used as an enduring marker of stress in early development, it is expected that animals with high FA in adulthood would also have elevated cortisol concentrations both before and after exposure to a stressful stimulus and there will be marked sex differences.

2. Materials and methods

2.1. Animals and the initial mating procedure

Twenty-two female hamsters obtained from our breeding colony at the Ohio State University were randomly assigned to four groups of temperature and day length manipulations (see details below). They were then individually paired with males. Throughout gestation, all animals were maintained in polypropylene cages (27.8 x 7.5 x 13 cm) at $21 \pm 2^\circ$ C temperature

and $60 \pm 10\%$ relative humidity with 16 h of light per day (LD 16:8; lights illuminated at 22:00 h Eastern Standard Time [EST]), *ad libitum* access to food (Harlan Tekland 8640 rodent diet, Indianapolis, IN), and filtered tap water. On Day 16 of pairing, males were removed from the females' cages. On Day 17 and onwards, females were monitored for the presence of pups.

2.2. Temperature and day length manipulations

On the day of birth, the progeny (N=86) along with the dams were switched to their pre-assigned experimental groups as follows:

1. Animals maintained in long photoperiods (16 h of light per day) and low temperatures [$8 \pm 2^\circ\text{C}$] throughout lactation (21 days) (Group LD: 8°C , n=13),
2. Animals remained in long photoperiods and standard temperatures [$21 \pm 2^\circ\text{C}$] throughout lactation (Group LD: 21°C , n=31),
3. Animals switched to short photoperiods (8 h of light per day) and low temperatures during lactation (Group SD: 8°C , n=16)
4. Animals switched to short photoperiods and standard temperatures during lactation (Group SD: 21°C , n=26).

After weaning at 21 days of age, all progeny were individually housed and maintained in long photoperiods and standard temperatures until the completion of the study. The lower sample size in low temperature groups compared to standard temperature ones may in part be attributable to infanticidal dams. Whereas LD: 21°C and SD: 21°C dams did not cannibalize any pups, some mothers in LD: 8°C and SD: 8°C groups did so. This observation confirms previous reports on infanticide in Syrian hamsters due to exposure of low temperatures during lactation [23].

2.3. Restraint procedures

In order to assess whether the expected morphological changes due to temperature and photoperiod manipulations were accompanied by concomitant physiological changes in adulthood, animals were exposed to acute restraint, which is known to evoke a physiological stress response as measured by the elevated cortisol concentrations [21,28]. In this procedure, all 4 groups of hamsters at 75 days of age were restrained for 1h. Before exposure to stress, all hamsters were deeply anesthetized with isoflurane vapors and ~150- μl of blood was collected from the retro-orbital sinus to measure baseline cortisol concentrations. Following baseline blood collection, hamsters were placed in well-ventilated, plexiglass tubes without compression between 09:00–10:00 hrs EST. Immediately (<2 min) following restraint, the second blood sample (~150- μl) was collected using the alternate eye under previously described anesthesia. Blood samples were stored on ice until further processing. After these procedures, all animals were returned to their cages. It should be noted that retro-orbital bleeding is likely to cause undue stress in the animals [29]. However, the speed with which we collected two blood samples (<2 min between animal handling and blood collection) is in accordance with previous reports (e.g. ref [21]), therefore, represents the differences between pre- and post-restraint cortisol concentrations (see also the results below). Other studies also indicated that retro-orbital blood sampling method is one of the most accurate procedures for studying physiological responses to stress in rodents [30,31].

2.4. Radioimmunoassay

Blood samples were centrifuged at 4°C at 2500 r.p.m for 30 min for plasma collection. Plasma was stored at -70°C until assayed for cortisol concentrations. Cortisol was measured in duplicate using ^{125}I radioimmunoassay kit (Diagnostics Systems Laboratory, Webster, TX)

as directed by the manufacturer, except 25- μ l of plasma was used instead of 50- μ l. The intra-assay coefficient of variation was <10%. The detection limit of the assay was 5 ng/ml.

2.5. FA measurements

A week after the restraint and blood collection procedures, all animals were euthanized. Immediately after euthanasia, arm and leg bones were extracted. In order to remove the tissues from the bones, the specimens were put in 25mL glass scintillation vial with Batson's #17 Anatomical Corrosion liquid (Polysciences Inc., Washington, PA, USA). The vials were then placed in a mechanical convection oven at 50°C for 30 min. After 30 min in the oven, the vials were drained, washed with distilled water, and the bones retrieved from each animal were marked for the subsequent FA measurements.

The length of humerus, tibia, ulna, femur, and radius (bone) was measured with a digital caliper to the nearest 0.01mm. Previous studies on limb FA in rodents constituted the basis for trait selection [32,33]. Each trait was measured twice by the same investigator (ED). Second measurements were taken on a different day without reference to the prior data.

2.6. Data analysis

The difference between two sides of a paired trait is very small and may be confounded by measurement error. Therefore, we assessed this measurement error by employing two-way (side X animal), mixed ANOVA procedures with repeated measurements on each side [4]. Results revealed that the traits were correctly measured with negligible error, i.e. side X animal interaction effects were significant across all traits (radius: $F_{[1,81]} = 10.26$; $P < 0.0001$; ulna: $F_{[1,74]} = 17.04$; $P < 0.0001$; humerus: $F_{[1,84]} = 1.88$; $P = 0.0003$; tibia: $F_{[1,84]} = 2.33$; $P < 0.0001$; femur: $F_{[1,85]} = 2$; $P < 0.0001$). We then averaged the two replicate values for each trait to further reduce the measurement error [34]. In order to assess whether the traits were size-dependent or not, we performed additional analyses by regressing absolute symmetry ($|R-L|$) of each trait on the average size of that particular trait $[(R+L)/2]$ [27] controlling for sex. Radius showed significant size dependency regardless of the sex of the animal ($Beta = 0.19$; $P = 0.004$). Accordingly, size-corrected FA values were calculated as $|R-L|/0.5(R+L)$ [35] on each trait and a composite FA score for each individual was prepared by summing and averaging these size-corrected values. All FA analyses were conducted on the composite FA score by ANCOVAs with temperature and day length groups as between subject factor and weight as a covariate. Because of the size difference between males and females, these analyses were performed separately for each sex. Baseline and post-restraint cortisol concentrations were analyzed separately with one-way ANOVAs within each group of temperature and day length manipulations with sex as between subject factor. *Post hoc* multiple comparisons among groups were tested by Tukey-Kramer method for adjusted least square means within each sex and all differences were considered statistically significant if $P \leq 0.05$. All statistical tests were performed by SAS software, version 9.1 (SAS Institute, Cary, NC).

3. Results

3.1. Physiological measures

Both baseline and post-restraint cortisol concentrations displayed significant sex differences in all temperature and day length groups, except in SD: 8°C (*basal cortisol*: $P < 0.005$ in LD: 8°C; $P < 0.04$ in LD: 21°C; $P \leq 0.02$ in SD: 21°C; $P > 0.05$ in SD: 8°C; *post-restraint cortisol*: $P < 0.04$ in LD: 8°C; $P < 0.008$ in LD: 21°C; $P \leq 0.01$ in SD: 21°C; $P > 0.05$ in SD: 8°C) (Fig. 1A&B). Cortisol concentrations in each manipulation were then compared among groups within each sex. Females in the LD: 8°C group had higher baseline cortisol concentrations compared to those in LD: 21°C ($P \leq 0.01$) and SD: 8°C ($P \leq 0.02$) (Figure.1A). In addition, female hamsters in the SD: 8°C condition had lower post-restraint cortisol concentrations

compared to any other group ($P \leq 0.01$ for the LD: 8°C comparison; $P < 0.03$ for LD: 21°C; $P \leq 0.05$ for SD: 21°C) (Figure.1B). LD: 8°C, LD: 21°C, and SD: 21°C females did not differ from each other in this measure ($P > 0.05$). No group differences were observed in males in pre- and post-restraint manipulations (Fig.1A&B). On the other hand, overall percentage change in cortisol concentrations following restraint was higher in males compared to those of females (males: 244%, SEM=±20.51; females: 168%, SEM=±19.98, $P \leq 0.01$). This effect did not depend on the temperature and photoperiod groups the animals were assigned (i.e., the interaction effect of sex X group was not significant, $P > 0.05$). Overall, cortisol concentrations significantly increased for each sex following restraint (baseline mean_{females}: 38.62, SEM=±3.1; post-restraint mean_{females} = 87.17, SEM= ±3, $P < 0.0001$; baseline mean_{males}: 22.53, SEM=±3.1; post-restraint mean_{males} = 68.71, SEM= ±2.9, $P < 0.0001$). *Post hoc* multiple comparisons among groups within each sex revealed that percentage change in cortisol concentrations following restraint was highest in LD: 21°C females compared to any other group ($P_s < 0.05$). LD: 8°C, SD: 8°C, and SD: 21°C females did not differ from each other in this measure ($P_s > 0.05$) (Figure.2A). No group differences in males were observed ($P_s > 0.05$) (Figure.2B).

3.2. FA

There were no sex differences in FA in any of the groups ($P > 0.05$). However, significant group differences were observed in the female sample. SD: 8°C females had significantly lower composite FA compared to any other group ($P \leq 0.009$ for the LD: 8°C comparison; $P \leq 0.03$ for LD: 21°C; $P \leq 0.005$ for SD: 21°C;). LD: 8°C, LD: 21°C, and SD: 21°C females were not different from each other in this measure ($P_s > 0.05$) (Figure.3A). FA in males did not show any differences among groups ($P_s > 0.05$) (Figure.3B).

4. Discussion

The present study investigated the effects of seasonal indicators, namely, day length and ambient temperatures during neonatal development on FA of bilateral characters and concomitant changes in physiology in adult Siberian hamsters. It was predicted that winter-like conditions characterized by short photoperiods and low temperatures during neonatal development would yield high FA and evoke high cortisol concentrations in response to a stressful stimulus. The tradeoffs between allocating necessary energy resources to ideal growth and other important functions, such as immune responses during energetically challenging winter conditions provided the basis of these predictions [i.e., 22]. Contrary to our predictions, however, animals reared in winter-like conditions with short photoperiods and low ambient temperatures had low FA and low cortisol concentrations following acute restraint stress in adulthood. These effects were observed for females only; males did not differ in any of these measures across temperature and photoperiod groups. Moreover, significant differences between males and females were noted regarding basal and post-restraint cortisol concentrations. A detailed discussion for the group effects is presented below.

4.1. Sex differences

Both baseline and post-restraint cortisol concentrations were higher in females than males except for the SD: 8°C animals. Nonhuman female animals generally have higher glucocorticoids than their male counterparts in response to hypothalamic-pituitary-adrenal axis stimulation [21,27]. Also, cortisol concentrations both at baseline and after acute restraint stress do not differ between mature male and female Siberian hamsters maintained in short photoperiods in adulthood [21]. Because SD: 8°C females did not differ from SD: 8°C males, and, SD: 8°C females had the lowest post-restraint cortisol concentrations, our results are generally in congruence with previous studies. On the other hand, percentage change in cortisol concentrations following restraint was higher in males compared to females regardless of the

photoperiod and temperature treatment. This may be due to the relatively high levels of baseline cortisol in females compared to males. Because females have high cortisol concentrations to begin with, a ceiling effect may occur to prevent these high values from further increases.

As for the FA values, no sex differences were observed among groups. It should be noted, however, that SD: 8°C females had lower FA compared to SD: 8°C males, although this effect was not significant ($P=0.06$, data not shown).

4.2. Group differences

4.2.1. Day length and temperature manipulations—Females in LD: 8 °C appeared to have high baseline cortisol concentrations regarding other groups of females. On the other hand, SD: 8°C females had the lowest post-restraint cortisol compared to other females. LD: 21°C females had the highest percentage increase in cortisol concentrations following restraint compared to other females. These group differences are elaborated upon below. No group effects were observed in males.

4.2.2. FA—SD: 8°C females had the lowest FA values compared to any other group. Males did not differ from each other with respect to temperature and day length manipulations. To reiterate, SD: 8°C females had marginally low FA compared to SD: 8°C males, although this effect was not statistically significant. Because LD: 8°C and LD: 21°C females did not differ from other females, except SD: 8°C ones in composite FA, a blunted stress response must be the key factor in achieving low FA. That is, the presence of high glucocorticoids and a high responsiveness to restraint stress as observed in LD: 8°C and LD: 21°C females may not necessarily result in high FA. This is also supported by the lack of significant group effects in FA values and concomitant stress responses in male groups, although males generally showed higher rates of increase in cortisol concentrations following restraint compared to females. Apparently, winter-like conditions, during neonatal development evoke hyposensitivity to stress in adult females and enhance ideal growth patterns. Winter conditions are often stressful [22,36]. An elevated stress response marked by increased glucocorticoids in winter months may compromise immune function, growth, and other important functions that require significant energy reserves [37]. Therefore, energetic adaptations during winter may attenuate physiological stress response as energy shortages in this period may limit the animals' ability to cope with stress [i.e., 22]. Our results are consistent with these propositions.

Should the animals be exposed to environmental stressors (e.g., low temperatures) during gestation, lactation, or a combination of both, the results might have been different. Although previous studies indicated that all of the three conditions above independently induce FA in rodents [see 26; sex differences remain unspecified], prolonged exposure to low temperatures coupled with short day lengths during both gestation and lactation are likely to cause deleterious effects. Prenatal stress disturbs the hormonal milieu in pregnant dams and disrupts the HPA axis function and its response to stress in both dams and their offspring [38]. Moreover, earlier reports indicated that reproductive parameters, including litter size, maternal responsiveness to the young, and offspring survival were adversely affected in adult female rodents exposed to low temperatures during both gestation and lactation [39]. When female rodents were kept in low temperatures during lactation only, their reproductive performance in adulthood was less impaired than that of the animals maintained in low temperatures during both pre- and postnatal, or prenatal development alone [39]. Future studies will benefit from employing experiments with large sample sizes to also account for litter losses due to aborting or infanticidal dams, prevalent in low temperature studies. Consequently, the effects of different photoperiod and temperature manipulations during pre- or postnatal development, or a combination of the two can be adequately tested. To our knowledge, no study has

experimentally compared these different periods during ontogeny to test whether environmental stressors would yield varying levels of FA for each specific period [but see 26].

Also, this is the first study that employed photoperiod manipulations as seasonal factors in the development of bilateral characters. In doing so Siberian hamsters, a reproductively responsive species to seasonal changes, were used to test the hypotheses. We previously showed that humans born in winter and spring have elevated FA levels [16]. Contrary to the current results, these data were consistent with previous research on seasonal variation in neurological disorders and concomitant high FA in humans [see 16 for a review]. Perhaps because seasonal reproduction is blunted in humans, energetic adaptations observed in nonhuman animals that are reproductively sensitive to temperature and day length changes may not be observed in humans.

It should also be noted that our data differ from most experimental studies [e.g., 26,40] in that presumably stressful conditions during neonatal development did not necessarily induce high FA, but a blunted response to these stressors did induce low FA. FA is assumed to reflect the degree of developmental stress and decreased buffering capacity against environmental and genetic perturbations [1–3,41–43]. Individuals are presumably buffered against such developmental insults by employing homeostatic mechanisms to produce the ideal phenotype. In this specific study, seasonally reproductive females were better able to buffer against winter-like conditions by displaying blunted response to stressors and concomitantly achieving low FA. Perhaps because mammalian reproduction in females in terms of energy metabolism is costlier than that of in males [44], seasonally reproducing females may be more sensitive to stressful winter conditions and display a more plastic buffering capacity than males against stressors. The lack of significant findings among male groups and the low cortisol concentrations as well as low FA in SD: 8°C females support this proposition.

Finally, apart from glucorticoids, sex steroid hormones, such as estradiol and testosterone might have played a role in obtaining the current FA results. Sex steroids are in part responsible for the observed sex differences in HPA axis response to stress [see 27 for a review]. These hormones also show variations with respect to photoperiod [21]. Moreover, testosterone and estradiol are implicated in FA [45,46]. Our study did not measure sex steroids, therefore, we cannot conclusively state that our results were independent of these hormones. However, most experimental studies on developmental stress, physiology, and FA have focused on glucorticoids. These experimental studies directly linked prenatal exposure to high glucorticoids and high FA [e.g., 47,48]. On the other hand, experimental administration of androgen disrupting chemicals [49], testosterone [50], and estrogenic or antiestrogenic substances [51] did not yield any significant results in FA measurements. Future studies will benefit from exploring the direct relationships among sex steroids, glucorticoids, and environmental stress during both prenatal and postnatal development and FA in adulthood.

Acknowledgements

We thank Amanda Trainor for assistance with the experimental procedures, Trish Uhor and her staff for animal care, and two anonymous reviewers for their helpful comments on a previous version of the manuscript. This research was supported by NIH grants MH 57760 and P30NS045758 to Rand J. Nelson.

References

1. Van Valen L. A study of fluctuating asymmetry. *Evolution* 1962;16:125–142.
2. Livshits G, Davidi L, Kobylanski E, Ben Amitai D, Levi Y, Merlob P. Decreased developmental stability as assessed by fluctuating asymmetry of morphometric traits in preterm infants. *Am J Med Genet* 1988;29:793–805. [PubMed: 3400724]
3. Vollestad LA, Hindar K, Moller AP. A meta-analysis of fluctuating asymmetry in relation to heterozygosity. *Heredity* 1999;83:206–218. [PubMed: 10469209]

4. Palmer, AR. Fluctuating asymmetry analyses: a primer. In: Markow, TA., editor. Developmental instability: its origins and evolutionary implications. Kluwer; Dordrecht: 1994. p. 335-364.
5. Møller, AP.; Swaddle, JP. Asymmetry, developmental stability, and evolution. Oxford, England: Oxford University Press; 1997. 1997
6. Mather K. Genetic control of stability in development. *Heredity* 1953;7:297–336.
7. Thoday JM. Homeostasis in a selection experiment. *Heredity* 1958;12:401–415.
8. Markow TA, Martin JF. Inbreeding and developmental stability in a small human population. *Ann Hum Biol* 1993;20:389–394. [PubMed: 8346899]1993
9. McKenzie JA, Clarke GM. Diazonian resistance, fluctuating asymmetry, and fitness in the Australian sheep blowfly. *Genetics* 1988;120:213–220. [PubMed: 17246476]
10. Hoffman AA, Parsons PA. Selection for dessication resistance in *Drosophila melanogaster*: additive genetic control and correlated responses for other stresses. *Genetics* 1989;122:837–845. [PubMed: 2503423]
11. Valentine DW, Soulé ME, Samollow P. Asymmetry in fishes: a possible statistical indicator of environmental stress. *Fish Bull* 1973;71:357–370.
12. Parsons PA. Fluctuating asymmetry: an epigenetic measure of stress. *Biol Rev* 1990;65:131–145. [PubMed: 2190634]
13. Bailit HL, Workman PL, Niswander JD, MacLean CJ. Dental asymmetry as an indicator of genetic and environmental conditions in human populations. *Hum Biol* 1970;42:626–38. [PubMed: 5509692]
14. Parsons PA. Fluctuating asymmetry: a biological monitor of environmental and genomic stress. *Heredity* 1992;68:361–364. [PubMed: 1563968]
15. Nosil P, Reimchen TE. Tarsal asymmetry, nutritional condition, and survival in water boatmen (*Callicorixa vulnerata*). *Evolution* 2001;55:712–20. [PubMed: 11392389]
16. Benderlioglu Z, Nelson RJ. Season of birth and fluctuating asymmetry. *Am J Hum Biol* 2004;16:298–310. [PubMed: 15101055]
17. Heideman PD, Bronson FH. A pseudoseasonal reproductive strategy in a tropical rodent, *Peromyscus nudipes*. *Reprod Fertil* 1992;95:57–67.
18. Nelson RJ, Demas GE, Klein SL. Photoperiodic mediation of seasonal breeding and immune function in rodents: a multi-factorial approach. *Am Zool* 1998;38:226–237.
19. Nelson, RJ.; Demas, GE.; Klein, SL.; Kriegsfeld, LJ. Seasonal patterns of stress, immune function, and disease. Cambridge University Press; New York: 2002.
20. Bilbo SD, Drazen DL, Quan N, He L, Nelson RJ. Short day lengths attenuate the symptoms of infection in Siberian hamsters. *Proc Biol Sci* 2002;269(7):447–54. [PubMed: 11886635]
21. Bilbo SD, Nelson RJ. Sex differences in photoperiodic and stress-induced enhancement of immune function in Siberian hamsters. *Brain Behav Immun* 2003;17:462–72. [PubMed: 14583238]
22. Prendergast BJ, Wynne-Edwards KE, Yellon SM, Nelson RJ. Photorefractoriness of immune function in male Siberian hamsters (*Phodopus sungorus*). *J Neuroendocrinol* 2002;14:318–29. [PubMed: 11963829]
23. Schneider JE, Wade GN. Effects of ambient temperature and body fat content on maternal litter reduction in Syrian hamsters. *Physiol Behav* 1991;49:135–9. [PubMed: 2017467]
24. Day DE, Mintz EM, Bartness JM. Diet choice exaggerates food hoarding, intake and pup survival across reproduction. *Physiol Behav* 2002;75:143–157. [PubMed: 11890963]
25. Benderlioglu, Z.; Nelson, RJ. Unpublished data. The Ohio State University; 2005.
26. Siegel MI, Doyle WJ. The differential effects of prenatal and postnatal audiogenic stress on fluctuating dental asymmetry. *J Exp Zool* 1975;191:211–4. [PubMed: 1113068]
27. Kudielka BM, Kirschbaum C. Sex differences in HPA axis responses to stress: a review. *Biol Psychol* 2005;69:113–32. [PubMed: 15740829]
28. Dhabhar FS, Miller AH, McEwen BS, Spencer RL. Stress-induced changes in blood leukocyte distribution. Role of adrenal steroid hormones. *J Immunol* 1996;157:1638–44. [PubMed: 8759750]
29. Balcombe JP, Barnard ND, Sandusky C. Laboratory routines cause animal stress. *Contemporary Topics in Laboratory Animal Science* 2004;43:42–51. [PubMed: 15669134]

30. Grouzmann E, Cavadas C, Grand D, Moratel M, Aubert J, Brunner HR, Mazzolai L. Blood sampling methodology is crucial for precise measurement of plasma catecholamines concentrations in mice. *European Journal of Physiology* 2003;447:254–258. [PubMed: 12905032]
31. Pfersdorff C, Beck L, Monier M, Rouaud A, Penacchio E, Cahard D, Lacheretz F. Clinical biochemistry, hematology, and hormone samples in the rat under isoflurane anesthesia. *Toxicol Lett* 1998;95(Supp 1):191.
32. Siegel MI, Doyle WJ, Kelly C. Heat, stress, fluctuating asymmetry, and prenatal selection in the laboratory rat. *Am J Phys Anthropol* 1977;46:121–126. [PubMed: 556888]
33. Doyle WJ, Kelley C, Siegel MI. The effects of audiogenic stress on the growth of long bones in the laboratory rat (*Rattus norvegicus*). *Growth* 1977;41:183–9. [PubMed: 914053]
34. Graham JH, Shimizu K, Emlen JM, Freeman DC, Merkel J. Growth models and the expected distribution of fluctuating asymmetry. *Biol J Linn Soc* 2003;80:57–65.
35. Palmer AR, Strobeck C. Fluctuating asymmetry: measurement, analysis, patterns. *Annu Rev Ecol Syst* 1986;17:391–421.
36. Monjan, AA. Stress and immunologic competence: studies in animals. In: Ader, R., editor. *Psychoneuroimmunology*. Academic Press; New York: 1981. p. 185-228.
37. Sapolsky RM, Romero LM, Munck Au. How do glucorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev* 2000;21:55–89. [PubMed: 10696570]
38. Francis DD, Caldji C, Champagne F, Plotsky PM, Meaney MJ. The role of corticotropin-releasing factor--norepinephrine systems in mediating the effects of early experience on the development of behavioral and endocrine responses to stress. *Biol Psychol* 1999;46:1153–1166.
39. Benderlioglu Z, Eish J, Weil Z, Nelson RJ. Low temperatures during early development influence subsequent maternal and reproductive function in adult female mice. *Physiol Behav* 2006;87:416–423. [PubMed: 16343561]
40. Siegel MI, Doyle WJ. The effects of cold stress on fluctuating asymmetry in the dentition of the mouse. *J Exp Zool* 1975;193:385–389. [PubMed: 1176912]
41. Adams M, Niswander JD. Developmental “noise” and congenital malformations. *Genet Res* 1967;10:313–317. [PubMed: 5587946]
42. Leung B, Forbes MR. Fluctuating asymmetry in relation to stress and fitness: effect of trait type as revealed by meta-analysis. *Ecoscience* 1996;3:400–413.
43. Klingenberg, CP. A developmental perspective on developmental instability: theory models and mechanisms. In: Polak, M., editor. *Developmental instability: causes and consequences*. Oxford University Press; Oxford: 2001.
44. Wade GN, Schneider JE. Metabolic fuels and reproduction in female mammals. *Neurosci Biobehav Rev* 1992;16:235–272. [PubMed: 1630733]
45. Cunningham MR. Adaptive flexibility, testosterone, and mating fitness: are low FA individuals the pinnacle of evolution? *Behav Brain Sci* 2000;23:599.
46. Manning JT, Scutt D, Whitehouse GH, Leinster SJ. Breast asymmetry and phenotypic quality in women. *Evol Hum Beh* 1997;18:223–236.
47. Eriksen MS, Haug A, Torjesen PA, Bakken M. Prenatal exposure to corticosterone impairs embryonic development and increases fluctuating asymmetry in chickens (*Gallus gallus domesticus*). *Br Poult Sci* 2003;44:690–697. [PubMed: 14965089]
48. Satterlee DG, Cadd GG, Jones RB. Developmental instability in Japanese quail genetically selected for contrasting adrenocortical responsiveness. *Poult Sci* 2000;79:1710–1714. [PubMed: 11194031]
49. Quinn MJ, Summitt CL, Burrell K, Ottinger MA. Fluctuating asymmetry and growth as biomarkers for exposure to androgen disrupting chemicals in Japanese quail. *Ecotoxicology* 2005;14:637–643. [PubMed: 16215698]
50. Veiga JP, Salvador A, Martin J, Lopez P. Testosterone stress does not increase asymmetry of a hormonally mediated sexual ornament in a lizard. *Behav Ecol Sociobiol* 1997;41:171–176.
51. Leamy LJ, Doster MJ, Huet-Hudson YM. Effects of methoxychlor on directional and fluctuating asymmetry of mandible characters in mice. *Ecotoxicology* 1999;8:63–71.

FIGURE 1.

FIGURE.1A Mean ($\pm$ SEM) baseline cortisol concentrations in photoperiod and temperature groups across sexes. # significantly different from males ($P \leq 0.05$). Female group means sharing the same letter are not significantly different from each other ($P_s > 0.05$). No significant differences observed among male groups ($P_s > 0.05$). LD: 8°C = animals maintained in long photoperiods and low temperatures [$8 \pm 2^\circ\text{C}$] throughout lactation; LD: 21°C= animals maintained in long photoperiods and standard temperatures [$21 \pm 2^\circ\text{C}$] throughout lactation; SD: 8°C =animals maintained in short photoperiods and low temperatures during lactation; SD: 21°C =animals maintained in short photoperiods and standard temperatures during lactation.

FIGURE.1B Mean ($\pm$ SEM) post-restraint cortisol concentrations in photoperiod and temperature groups across sexes. No significant differences observed among male groups ($P_s > 0.05$). Other symbols and conventions are as in Figure.1A.

FIGURE 2.

FIGURE.2A Percentage change in cortisol ($\pm$ SEM) following restraint in females. Group means sharing the same letter are not significantly different from each other ($P>0.05$). Other symbols and conventions are as in Figure.1A.

FIGURE.2B Percentage change in cortisol ($\pm$ SEM) following restraint in males. Group means sharing the same letter are not significantly different from each other ($P>0.05$). Other symbols and conventions are as in Figure.1A.

FIGURE 3.

FIGURE.3A Mean composite FA ($\pm$ SEM) in females. Group means sharing the same letter are not significantly different from each other ($P>0.05$). Other symbols and conventions are as in Figure.1A.

FIGURE.3B Mean composite FA ($\pm$ SEM) in males. Group means sharing the same letter are not significantly different from each other ($P>0.05$). Other symbols and conventions are as in Figure.1A.