Hormones and Behavior 117 (2020) 104608

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Melatonin mediates seasonal transitions in aggressive behavior and circulating androgen profiles in male Siberian hamsters

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ARTICLE INFO

Keywords:
Aggression
Biological rhythms
DHEA
Photoperiod
Pineal
Seasonality
Territoriality
Testosterone

ABSTRACT

Some seasonally-breeding animals are more aggressive during the short, "winter-like" days (SD) of the non-breeding season, despite gonadal regression and reduced circulating androgen levels. While the mechanisms underlying SD increases in aggression are not well understood, previous work from our lab suggests that pineal melatonin (MEL) and the adrenal androgen dehydroepiandrosterone (DHEA) are important in facilitating non-breeding aggression in Siberian hamsters (*Phodopus sungorus*). To characterize the role of MEL in modulating seasonal transitions in aggressive behavior, we housed male hamsters in long days (LD) or SD, treated them with timed MEL (M) or saline injections, and measured aggression after 3, 6, and 9 weeks. Furthermore, to assess whether MEL mediates seasonal shifts in gonadal and adrenal androgen synthesis, serum testosterone (T) and DHEA concentrations were quantified 36 h before and immediately following an aggressive encounter. LD-M and SD males exhibited similar physiological and behavioral responses to treatment. Specifically, both LD-M and SD males displayed higher levels of aggression than LD males and reduced circulating DHEA and T in response to an aggressive encounter, whereas LD males elevated circulating androgens. Interestingly, LD and SD males exhibited distinct relationships between circulating androgens and aggressive behavior, in which changes in serum T following an aggressive interaction (ΔT) were negatively correlated with aggression in LD males, while ΔDHEA was positively correlated with aggression in SD males. Collectively, these findings suggest that SD males transition from synthesis to metabolism of circulating androgens following an aggressive encounter, a mechanism that is modulated by MEL.

1. Introduction

Because animals undergo substantial fluctuations in their environment across the seasons, many species restrict breeding to times of the year when conditions are favorable (Bronson, 1989; reviewed in Kumar et al., 2010; Whittier and Crews, 1987). Although a suite of biotic and abiotic environmental factors vary on a seasonal basis, photoperiod (i.e., day length) provides a reliable, error-free cue from which animals can coordinate seasonal shifts in morphology, physiology, and behavior (reviewed in Goldman, 2001; reviewed in Prendergast et al., 2003; reviewed in Walton et al., 2011). Consequently, animals have evolved the ability to utilize photoperiodic information to coordinate these seasonal adaptations with the appropriate time of the year. More specifically, seasonal changes in physiology and behavior are produced via a complex neural circuit, which begins with the perception of environmental light via retinal ganglion cells and culminates in the transduction of information about day length into a neuroendocrine signal within the pineal gland. The pineal gland secretes melatonin, a hormone that plays a prominent role in establishing and maintaining biological rhythms (Butler et al., 2010; reviewed in Dardente, 2012), into circulation in response to photoperiodic information. Because melatonin secretion tends to be high at night and low during the day, changes in photoperiod alter the pattern and duration of melatonin secretion, which conveys information about day length to the central nervous system (reviewed in Bartness et al., 1993; reviewed in Goldman, 2001).

While the role of melatonin in mediating seasonal reproduction and its underlying physiological processes has been well-characterized across a suite of species (reviewed in Bartness and Goldman, 1989; reviewed in Dawson et al., 2001; Whittier and Crews, 1987), the mechanisms that regulate seasonal shifts in social behaviors that are essential to reproductive success, such as aggression, are relatively understudied. Aggression is a behavior that is universally exhibited across vertebrates in a variety of social contexts (Jalabert et al., 2018; Nelson, 2006). Aggressive behavior is typically displayed when two or more individuals compete for a critical limited resource; such as mates,

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https://doi.org/10.1016/j.yhbeh.2019.104608
Received 7 August 2019; Received in revised form 30 September 2019; Accepted 3 October 2019

territories, or food; that, if attained, increases survival and/or reproductive fitness. Because aggressive encounters are a costly investment with respect to energy, predation risk, physical injury, and time, individuals must evaluate the costs and benefits of competing for resources and make a decision that results in maximal fitness payoffs (e.g., to fight back or give up). Thus, in solitary or territorial species, aggression typically determines access to the exclusive use of a resource (Kaufmann, 1983).

Traditionally, much of the research conducted on the neuroendocrine mechanisms underlying seasonal aggression has focused extensively on gonadally-derived testosterone (T) in males (reviewed in Soma, 2006; reviewed in Cunningham et al., 2012). However, numerous studies in males and females across animal taxa have indicated that aggression is not exclusively regulated by gonadal steroids. There is now strong evidence that the brain is capable of metabolizing circulating gonadal steroids and their precursors and synthesizing these hormones *de novo* from cholesterol (Do Rego et al., 2012; Tsutsui et al., 2000). Recent work suggests that additional steroidal and non-steroidal hormones also play a role in mediating aggression; including glucocorticoids (Haller and Kruk, 2003; Van Duyse et al., 2004; Wommack and Delville, 2003), the steroidogenic enzyme aromatase (Heimovics et al., 2013; Matsumoto et al., 2003; Schlinger and Callard, 1990), and the androgen precursor dehydroepiandrosterone (DHEA; Rendon and Demas, 2016; Nicolas et al., 2001; Soma et al., 2002; Wacker et al., 2016). Of these hormones, DHEA appears to be particularly important in mediating non-breeding aggression, especially in animals that maintain high levels of aggressive behavior year-round or increase aggression during the non-breeding season (reviewed in Soma et al., 2008; reviewed in Soma et al., 2015).

DHEA is an adrenal androgen that also serves as a prohormone for androgens and estrogens, including T and estradiol (E_2). Although DHEA is synthesized peripherally, circulating DHEA is capable of passing through the blood-brain-barrier and can be metabolized to active androgens and estrogens, such as T and E_2, via a multi-step conversion in brain regions that express the appropriate steroidogenic enzymes (reviewed in Beck and Handa, 2004; reviewed in Labrie et al., 2005). The role of adrenal DHEA in modulating aggressive behavior has mostly been studied in animals that exhibit year-round aggression, particularly birds and rodents (reviewed in Munley et al., 2018; reviewed in Soma et al., 2015). Both males and females of these species exhibit territorial aggression outside of the breeding season, despite gonadal regression and reduced circulating gonadal steroid levels. In fact, the level of aggressive behavior observed in some non-breeding songbirds, such as song sparrows (*Melospiza melodia*) and spotted antbirds (*Hylophylax naevioides*), is qualitatively and quantitatively similar to that of breeding individuals (Hau et al., 2004; Soma and Wingfield, 1999). Furthermore, some seasonally-breeding rodents; including beach mice (*Peromyscus polionotus*), deer mice (*Peromyscus maniculatus*), and Syrian (*Mesocricetus auratus*) and Siberian hamsters (*Phodopus sungorus*); exhibit higher levels of aggression towards conspecifics when they are not in breeding condition (Jasnow et al., 2000, 2002; Scotti et al., 2007; Trainor et al., 2006). Many of these species elevate circulating DHEA levels outside of the breeding season, and this increase in circulating DHEA levels has been associated with increased aggression (Demas et al., 2004; Gutzler et al., 2009; Newman et al., 2008; Rendon et al., 2015; Soma and Wingfield, 2001). Thus, these species are ideal for examining alternative, non-gonadal mechanisms of aggression because they display high levels of aggression that occur independently of reproduction, allowing us to study aggression independent of gonadal steroids (Crews, 1984; Crews and Moore, 1986).

Siberian hamsters are a particularly useful system for studying the evolutionary adaptations of seasonally breeding animals because they exhibit robust changes in morphology, physiology, and behavior on a seasonal basis. In the wild, Siberian hamsters are solitary and actively defend their territories year-round (Wynne-Edwards, 2003). These animals breed during the summer and undergo gonadal regression, a reduction in body mass, and changes in thermoregulation during the winter (Heldmaier and Steinlechner, 1981). Notably, Siberian hamsters primarily use photoperiod as an environmental cue to coordinate reproduction and its associated social behaviors with the breeding season. Therefore, these natural seasonal adaptations can consistently be elicited in the laboratory by housing animals in light cycles that mimic the photoperiods of the breeding and non-breeding seasons (Garrett and Campbell, 1980; Jasnow et al., 2000). For example, animals exposed to short, "winter-like" days (SDs; i.e., < 12.5 h of light/day) in the laboratory, which mimic the photoperiodic conditions of the non-breeding season, exhibit gonadal regression, a reduction in body mass, and a change in pelage color from brown to white (Bartness and Wade, 1985; Jasnow et al., 2000). These characteristic changes in physiology are associated with increased levels of aggression in both male and female hamsters (Demas et al., 2004; Rendon et al., 2015; Scotti et al., 2008; Scotti et al., 2007).

Previous work from our lab suggests that Siberian hamsters undergo a "seasonal switch" from gonadal regulation of aggression during the breeding season (i.e., long-day photoperiods, LDs) to adrenal regulation of aggression during the non-breeding season (i.e., SDs). More specifically, our data suggest that circulating gonadal steroids are essential in modulating breeding aggression, while adrenal DHEA primarily mediates non-breeding aggression by serving as an important source of T and E_2 when circulating gonadal steroid levels are low (reviewed in Munley et al., 2018). In addition, there is some evidence that melatonin mediates this neuroendocrine circuit. We have demonstrated that exogenous melatonin administration increases aggressive behavior in LD males (Demas et al., 2004; Jasnow et al., 2002). We have also found that timed melatonin injections, which mimic SD patterns of melatonin secretion, elevate aggression in LD females, whereas "mis-timed" melatonin injections do not (Rendon et al., 2015). Moreover, we determined that administering melatonin *in vitro* to cultured adrenal glands elevates DHEA output in SD, but not LD females; whereas melatonin treatment increases DHEA output from cultured ovaries in LD, but not SD females (Rendon et al., 2015). While these findings suggest that melatonin modulates seasonal aggression by altering peripheral steroidogenesis in females, little is known about the mechanisms by which melatonin modulates seasonal aggression in males.

The goal of this study was to characterize the role of melatonin in mediating seasonal transitions in aggressive behavior and circulating androgen profiles in male Siberian hamsters. Adult male hamsters were housed in LDs or SDs and treated with either timed melatonin or saline injections, and aggressive behavior was quantified after 3, 6, and 9 weeks of treatment. Furthermore, to assess whether melatonin mediates seasonal shifts in gonadal and adrenal androgen synthesis, serum T and DHEA concentrations were measured 36 h before and immediately following an aggressive encounter. We predicted that LD males administered timed melatonin injections (LD-M) will reduce body and reproductive tissue mass and increase aggressive behavior in response to treatment, as observed in SD males. Furthermore, we hypothesized that LD-M and SD males will elevate basal (i.e., pre-aggression) serum DHEA levels and reduce serum T levels in response to treatment. Finally, we predicted that LD-M and SD males will exhibit similar changes in circulating androgen levels following an aggressive encounter and that this response will differ from that of LD males.

2. Materials and methods

2.1. Experimental animals

Adult male Siberian hamsters (*Phodopus sungorus*, > 60 d of age) were reared and maintained in a breeding colony under long days (light:dark, 16 h:8 h; lights off at 1600 h Eastern Standard Time, EST). Animals were group-housed at weaning (post-natal day 18) in

polypropylene cages (28 × 17 × 12 cm) and remained in these housing conditions for at least 60 d prior to experimental testing. Sani-chip bedding (Tekland, Laboratory Grade; Envigo, Madison, WI, USA) was used in each cage, and hamsters were given *ad libitum* access to tap water and standard laboratory rodent chow (Tekland global 18% protein diet; Envigo, Madison, WI, USA). Ambient temperature was maintained at 20 ± 2 °C, and relative humidity was maintained at 55 ± 5%. All procedures were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Bloomington Institutional Animal Care and Use Committee (BIACUC) at Indiana University (protocol #17-001).

2.2. Photoperiodic manipulations

Prior to the start of photoperiodic manipulations, experimental (resident) hamsters ($n = 131$; see Section 2.1) were individually-housed for a two-week acclimation period on a long-day (LD) light cycle. Following acclimation, experimental animals were either transferred to a room on a short-day (SD) light cycle (light:dark, 8 h:16 h; lights on at 0800 h EST) or were relocated to a new room on a LD light cycle. Hamsters remained in photoperiodic treatment for 3 weeks (LD: $n = 19$, SD: $n = 22$), 6 weeks, (LD: $n = 23$, SD: $n = 22$), or 9 weeks (LD: $n = 22$, SD: $n = 23$) in order to assess changes in reproductive physiology, aggressive behavior, and circulating androgen profiles during the transition period between breeding (LD) and non-breeding (SD) condition.

2.3. In vivo melatonin administration

Melatonin profiles were manipulated in a subset of LD hamsters (LD-M, $n = 35$; Week 3: $n = 10$, Week 6: $n = 12$, Week 9: $n = 13$), which were administered timed subcutaneous injections (0.3 mL) of melatonin [15 μg/day (*M5250*; Sigma Aldrich, St. Louis, MO, USA) dissolved in 1:10 ethanol: saline solution] for the duration of the study, as described previously (Jasnow et al., 2002; Rendon et al., 2015; Stetson and Tay, 1983). All remaining animals in the study ($n = 96$; Week 3: $n = 31$; Week 6: $n = 33$; Week 9: $n = 32$) received daily injections (0.3 mL) of a control (1:10 ethanol:saline) solution. Injections were administered 2 h prior to lights out to extend the LD pattern of endogenous melatonin secretion, which mimics that of SD animals (Stetson and Tay, 1983). Thus, this protocol allowed us to examine the role of melatonin in modulating seasonal changes in adrenal and gonadal steroid secretion and aggressive behavior.

2.4. Seasonal phenotypes

Seasonal phenotypes of SD animals were determined based on *a priori* criteria that have been previously described for this species (Jasnow et al., 2000; Rendon et al., 2015; Scotti et al., 2007). These phenotypes were only classified in animals subjected to 9 weeks of photoperiodic treatment, since characteristic changes in body weight, reproductive tissue mass, and pelage color are only discernable after 8–10 weeks of treatment (Garrett and Campbell, 1980; Gaston and Menaker, 1967; Heldmaier and Steinlechner, 1981; Jasnow et al., 2000). Body mass was measured weekly for the duration of the study, and paired testes were collected and weighed and coat color was recorded following 9 weeks of treatment. LD hamsters had functional testes, displayed no significant change in body mass (< 4%), and had brown pelage. In contrast, SD animals had regressed testes, lost ≥ 4% of their body weight, and had white pelage ($n = 16$).

In many seasonally-breeding rodents, including Siberian hamsters, there is a subset of individuals (10–50%) that are non-responsive to SD photoperiods (Gorman and Zucker, 1997; Greives et al., 2010; Puchalski and Lynch, 1986; Rendon et al., 2017). These "non-responders" do not undergo gonadal regression or reduce body mass in response to SDs and generally respond physiologically and behaviorally like LD animals (Goldman, 2001; Gorman and Zucker, 1995; Lynch et al., 1989). In this study, SD individuals were classified as "non-responders" ($n = 7$, 30.4% of SD animals) if they lost < 4% of their body weight or gained weight over the course of the study, had functional testes, and had brown pelage. This group of animals had insufficient numbers for statistical analysis and was excluded from the study.

2.5. Behavioral testing

Territorial aggression was quantified within the first 3 h of the dark phase (1630–1900 h EST) using a 5 min same-sex resident-intruder paradigm, as described previously (Rendon et al., 2017; Rendon et al., 2015). Staged male dyads were created, which were composed of an experimental animal (i.e., resident) and a stimulus animal (i.e., intruder) of approximately the same age and body mass (± 5%) and from different parents as the experimental animal with which they were paired. The intruder was introduced into the resident's home cage, which had not been changed for 7 d prior to behavioral testing to allow the resident animal to establish its territory (Brain, 1975; Brain and Poole, 1974; Rendon et al., 2015). All trials were performed under low red light illumination, and intruders had small, shaved patches on their dorsum for the purpose of identification. Each intruder was used only once per testing period and no more than twice per day. To minimize aggression in intruder animals, these hamsters ($n = 20$) were individually-housed, handled minimally (e.g., only during weekly cage changes and for use in behavioral trials), and maintained in LDs prior to behavioral testing and throughout the study.

Behavioral interactions were video recorded, and aggressive (i.e., latency to first attack, number and duration of attacks, number and duration of chases) and non-aggressive social behaviors (i.e., number and duration of nose-to-nose investigations, anogenital investigations, scent marking events, and grooming events) were scored for each experimental (resident) animal by three trained, unbiased observers using ODLog™ software (Macropod, Eden Prairie, MN, USA). Measures of aggression, investigation, scent marking, and grooming were defined according to previous studies on same-sex aggression in male and female Siberian hamsters (Supplementary Material, Table S1; Jasnow et al., 2000; Scotti et al., 2015).

2.6. Blood sampling and tissue collection

To assess seasonal changes in circulating androgen levels both prior and in response to an aggressive encounter, a pre-aggression (i.e., pre-behavior) blood sample and a post-aggression (i.e., post-behavior) blood sample was collected from each experimental animal after 3, 6, or 9 weeks of treatment. Pre-aggression samples were drawn 36 h prior to behavioral testing (1030–1130 h EST), and post-aggression blood samples were drawn immediately (≤ 5 min) after behavioral trials. Animals were lightly anesthetized using isoflurane (Isothesia; Henry Schein Animal Health, Melville, NY, USA), and blood was drawn from the retro-orbital sinus into microcapillary tubes within 1 min. Handling was kept consistent and at a minimum; typically, < 3 min elapsed between removal and return to the animal's home cage. Blood samples were allowed to clot for either 1 h at room temperature (pre-aggression samples) or overnight at 4 °C (post-aggression samples), the clots were removed, and the samples were centrifuged at 4 °C for 25 min at 2500 rpm. Serum was aspirated and stored in sealable polypropylene microcentrifuge tubes at −20 °C until further analysis. Immediately following the collection of post-behavior blood samples, animals were euthanized with a lethal intraperitoneal injection (0.3 mL) of ketamine (150 mg/kg) and xylazine (30 mg/kg) cocktail in 0.9% saline. Paired testes and epididymal white adipose tissue (EWAT) were removed and weighed individually to the nearest mg.

2.7. Quantification of circulating steroid hormone levels

Serum DHEA concentrations were quantified using a commercially-available enzyme immunoassay (EIA) kit (DHEA ELISA kit ADI-901-093; Enzo Life Sciences, Farmingdale, NY, USA; assay sensitivity = 2.90 pg/mL). The validity of this assay was determined by comparing male Siberian hamster serum samples of varying dilutions with a standard curve generated using reference standards provided by the kit. This assay has some cross-reactivity with sulfated DHEA (DHEA-S, 30%); low cross-reactivity with androstenedione (0.73%), androsterone (0.29%), pregnenolone (0.28%), and T (0.10%); and negligible or undetectable cross-reactivity with other steroid hormones (< 0.06%). Serum samples were run neat or diluted 1:2, 1:4, or 1:8 with assay buffer to ensure 20–80% binding on a 4-parameter logistic standard curve (Microplate Manager 6 version 6.2; Bio-Rad Laboratories, Hercules, CA, USA). Samples were run in duplicate according to the manufacturer's instructions, and all samples contained levels of DHEA that were above the limit of detection for the assay. Pre-aggression and post-aggression serum samples from individual animals were run on the same plate, and samples from different treatments were counterbalanced across 10 plates of the same kit lot number (lot number: 10041705). Samples with a coefficient of variability (CV) > 10% and a maximum binding < 20% or > 80% were re-analyzed. The average intra-assay variability was 4.99%, and the inter-assay variability was 5.69%.

In addition, serum T concentrations were measured via a commercially-available EIA kit (Testosterone ELISA kit ADI-901-065; Enzo Life Sciences, Farmingdale, NY, USA; assay sensitivity = 5.67 pg/mL) that has been previously validated for use in Siberian hamsters (Long et al., 2018; Scotti et al., 2008). This EIA is highly specific for T and has low cross-reactivity with 19-hydroxytestosterone (14.6%), androstenedione (7.2%), DHEA (0.7%), and E_2 (0.4%) and has negligible or undetectable cross-reactivity with other steroid hormones (< 0.001%). Serum samples were diluted 1:40 or 1:80 with assay buffer to ensure 20–80% binding on a 4-parameter logistic standard curve (Microplate Manager 6 version 6.2; Bio-Rad Laboratories, Hercules, CA, USA). Samples were run in duplicate according to the manufacturer's instructions, including the use of a 1:120 dilution of steroid displacement reagent to inhibit T binding to proteins, and all samples contained levels of T that were above the limit of detection for the assay. Pre-aggression and post-aggression serum samples from individual animals were run on the same plate, and samples from different treatments were counterbalanced across 12 plates of the same kit lot number (lot number: 12211606). Samples with a CV > 10% and a maximum binding < 20% or > 80% were re-analyzed. The average intra-assay variability was 5.25%, and the inter-assay variability was 6.38%.

2.8. Statistical analyses

All data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using R version 3.3.3 (R Core Team, 2017), and statistical significance was attributed at $p < 0.05$. Normality and homogeneity of variances were assessed using Shapiro-Wilk tests and Levene's tests, respectively. Because all data violated normality and/or homogeneity of variances following transformation, non-parametric Kruskal-Wallis one-way ANOVA on ranks tests were used to compare body and reproductive tissue masses, aggressive and non-aggressive social behaviors, basal (i.e., pre-aggression) DHEA and T levels, and differences in pre-aggression and post-aggression serum androgen concentrations (ΔDHEA and ΔT) both between treatment groups at each time point tested (i.e., after 3, 6, or 9 weeks of treatment) and within treatment groups across time points. If a statistical test reported a significant effect of either time or treatment, Dunn's post-hoc tests for multiple comparisons were run to examine pairwise comparisons. Effect sizes were calculated for all Kruskal Wallis one-way ANOVA on ranks tests and Dunn post-hoc tests and are presented in the text. Effect sizes are expressed as transformed η^2 for Kruskal-Wallis tests (Cohen, 1988; Rosenthal, 1994) and Hedge's g for Dunn post-hoc tests to account for unequal sample sizes between treatment groups (Ellis, 2010).

To visualize differences in aggressive behavior and circulating androgen profiles across seasonal phenotypes, a principal component analysis (PCA) was conducted using aggression variables (latency to first attack, number and duration of attacks, number and duration of chases) and serum androgen concentrations (basal DHEA and T levels, ΔDHEA, ΔT) at the week 9 time point. These variables were evaluated for suitability of factor analysis using the Kaiser-Meyer-Olkin measure for sampling adequacy and Bartlett's test of sphericity (Williams et al., 2010). For the PCA, data were standardized using Z-scores to account for differences in scaling between variables (Jolliffe and Cadima, 2016). Data were used from at least 7 individuals per treatment group (LD: $n = 7$, LD-M: $n = 11$, SD: $n = 15$), and only PCs with an eigenvalue > 1 were retained for analysis. The first and third principal components (PC1 and PC3) of this analysis were used to compare aggression and circulating androgens across seasonal phenotypes, since both PC1 and the second principal component (PC2) were strongly loaded by aggression variables (attack latency, attack duration, and number and duration of chases) and PC2 and PC3 accounted for similar proportions of the total variance (PC2 = 21.3%, PC3 = 15.0%). Collectively, PC1 and PC3 accounted for 44.7% of the total variance (PC1 = 29.7%, PC3 = 15.0%; Supplementary Material, Fig. S1 and Table S2). In addition, to quantitatively compare relationships between aggressive behavior and circulating androgen profiles across seasonal phenotypes, Spearman's rank correlations were conducted by treatment group at the week 9 time point.

3. Results

3.1. Seasonal phenotypes differed across photoperiods and in response to melatonin treatment

Male hamsters that received timed melatonin injections or were housed in SDs reduced body mass in response to treatment and over the course of the study (Fig. 1A). Specifically, LD-M and SD males had significantly lower percent changes in body mass than LD males after 6 weeks ($H = 10.058$, d.f. = 2, $p = 0.007$, $\eta^2 = 0.201$) and 9 weeks of treatment ($H = 18.977$, d.f. = 2, $p < 0.001$, $\eta^2 = 0.485$). However, SD males showed a more pronounced reduction in body mass than LD-M males following 9 weeks of treatment ($H = 18.977$, d.f. = 2, $p = 0.001$, $g = -1.382$). Furthermore, SD males exhibited a significant decrease in percent change in body mass over time ($H = 32.814$, d.f. = 2, $p < 0.001$, $\eta^2 = 0.560$). In contrast, there was no effect of time on percent change in body mass in LD-M males ($H = 2.295$, d.f. = 2, $p = 0.318$, $\eta^2 = 0.009$).

LD-M and SD males also displayed significantly altered reproductive morphology in response to treatment and over time (Fig. 1B–C). After 6 weeks and 9 weeks of treatment, both LD-M and SD males had significantly lower paired testes masses (6 weeks: $H = 22.916$, d.f. = 2, $p < 0.001$, $\eta^2 = 0.523$; 9 weeks: $H = 30.564$, d.f. = 2, $p < 0.001$, $\eta^2 = 0.816$) than LD males. Furthermore, LD-M and SD males exhibited significant reductions in EWAT mass following 9 weeks of treatment ($H = 15.010$, d.f. = 2, $p < 0.001$, $\eta^2 = 0.372$). Notably, as observed for body mass, SD males showed greater reductions in paired testes mass ($H = 30.564$, d.f. = 2, $p < 0.001$, $g = -2.015$) and EWAT mass ($H = 15.010$, d.f. = 2, $p = 0.022$, $g = -0.979$) than LD-M males after 9 weeks of treatment. In addition, LD-M and SD males exhibited significant decreases in paired testes mass over time (LD-M: $H = 15.014$, d.f. = 2, $p = 0.001$, $\eta^2 = 0.407$; SD: $H = 41.505$, d.f. = 2, $p < 0.001$, $\eta^2 = 0.718$; Fig. 1B). Interestingly, while SD males significantly reduced EWAT mass over the course of the study ($H = 7.442$, d.f. = 2, $p = 0.024$, $\eta^2 = 0.099$), there was no effect of time on EWAT mass in LD-M males ($H = 0.311$, d.f. = 2, $p = 0.856$, $\eta^2 = 0.053$; Fig. 1C).

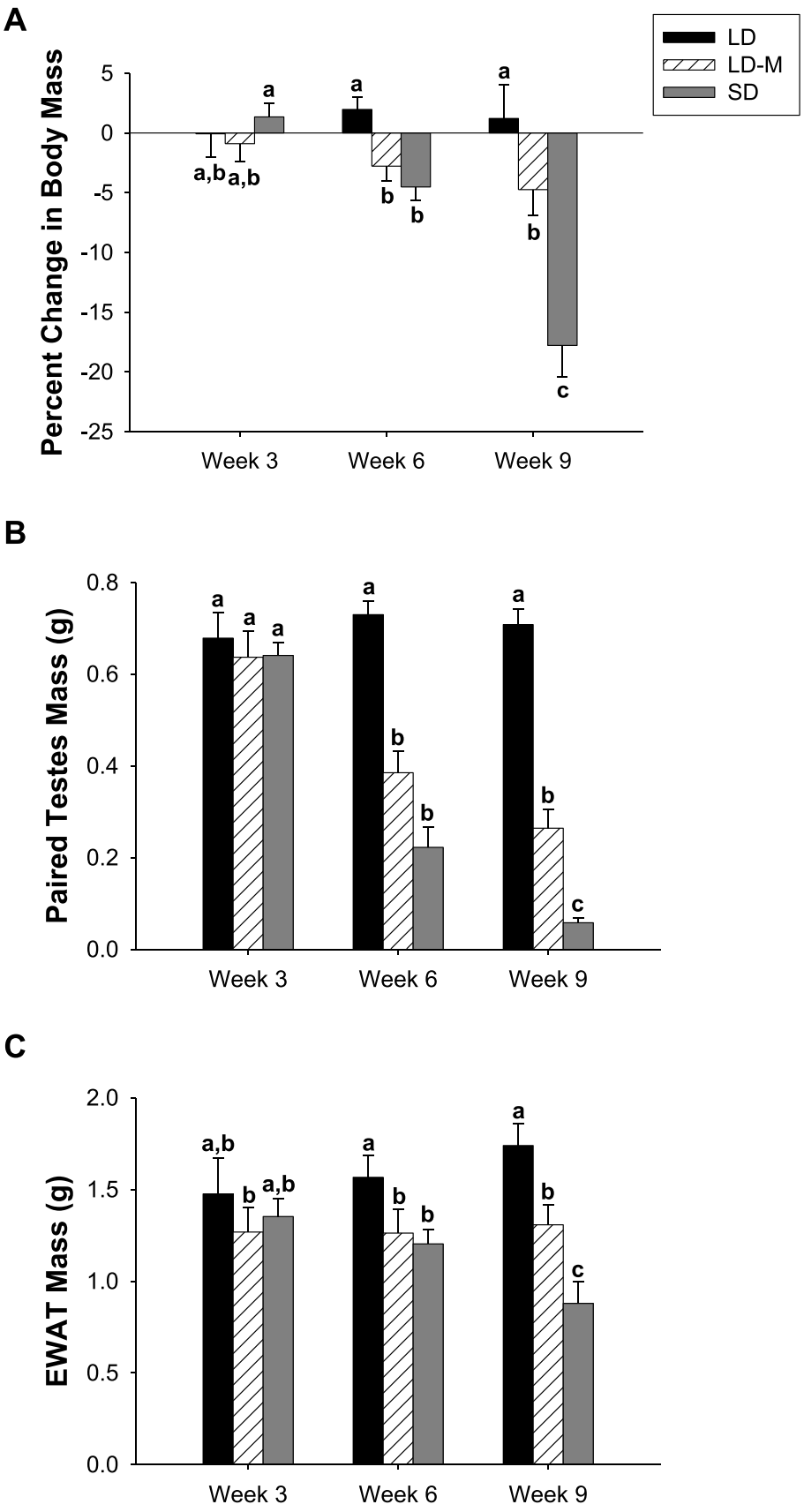

Fig. 1. Melatonin (M) administration and exposure to short-day (SD) photoperiods reduced body and reproductive tissue mass. Percent change in body mass (A), paired testes mass (B), and epididymal white adipose tissue (EWAT) mass (C) in long day hamsters (LD; black bars), LD hamsters administered timed melatonin injections (LD-M; white striped bars), and SD hamsters (gray bars) following 3 weeks, 6 weeks, and 9 weeks of treatment. Data are presented as mean ± SEM (LD: n = 9–11, LD-M: n = 10–13, SD: n = 16–22). Bars with different letters indicate a significant difference between groups ($p < 0.05$).

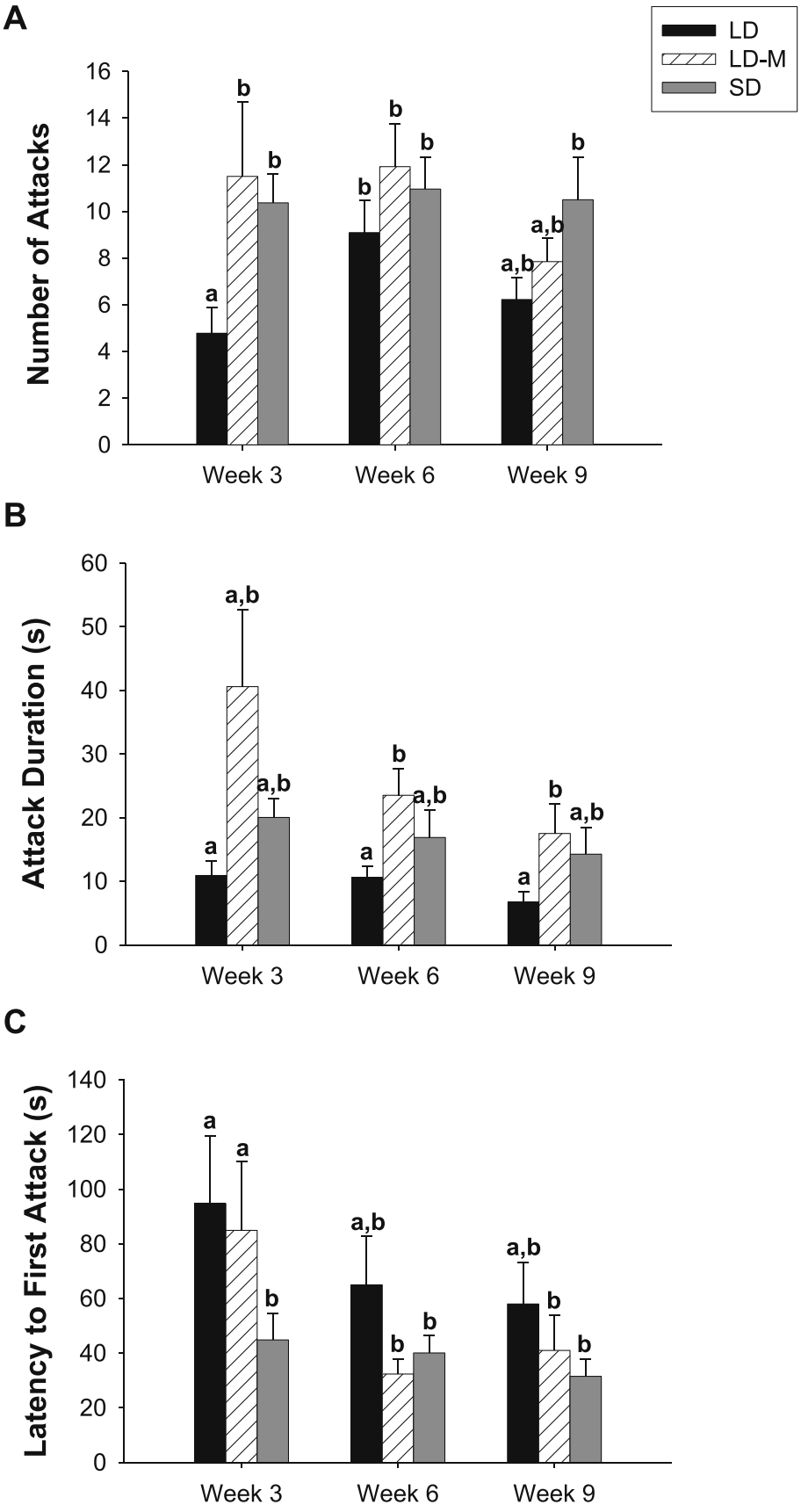

Fig. 2. Timed melatonin (M) injections and exposure to short-day (SD) photoperiods elevated aggressive behavior. Number of attacks (A), attack duration (B), and latency to first attack (C) in long day hamsters (LD; black bars), LD hamsters administered timed melatonin injections (LD-M; white striped bars), and SD hamsters (gray bars) following 3 weeks, 6 weeks, and 9 weeks of treatment. Data are presented as mean ± SEM (LD: n = 9–11, LD-M: n = 10–13, SD: n = 16–22). Bars with different letters indicate a significant difference between groups ($p < 0.05$).

3.2. Melatonin administration and short-day photoperiods increased aggressive behavior

Male hamsters that were administered timed melatonin injections exhibited SD-like levels of aggression in response to treatment and over time (Fig. 2). LD-M males displayed a greater number of attacks following 3 weeks and 6 weeks of treatment ($H = 7.236$, d.f. = 2, $p = 0.027$, $\eta^2 = 0.187$) and had a shorter attack latency following 6 weeks and 9 weeks of treatment ($H = 9.939$, d.f. = 2, $p = 0.007$, $\eta^2 = 0.256$) compared to LD males at the week 3 time point. Moreover, LD-M males exhibited a significantly higher attack duration than LD males after 6 weeks and 9 weeks of treatment (6 weeks: $H = 7.438$, d.f. = 2, $p = 0.011$, $g = 1.155$; 9 weeks: $H = 5.435$, d.f. = 2, $p = 0.011$, $g = 0.800$). Similarly, SD males displayed a higher number of attacks ($H = 9.368$, d.f. = 3, $p = 0.025$, $\eta^2 = 0.101$) and a shorter attack latency ($H = 11.824$, d.f. = 3, $p = 0.008$, $\eta^2 = 0.140$) at all three time points relative to week 3 LD males. There was no effect of time on number of attacks or attack duration in LD-M (number of attacks: $H = 2.499$, d.f. = 2, $p = 0.287$, $\eta^2 = 0.016$; attack duration: $H = 1.622$, d.f. = 2, $p = 0.445$, $\eta^2 = 0.012$) or SD males (number of attacks: $H = 0.143$, d.f. = 2, $p = 0.931$, $\eta^2 = 0.034$; attack duration: $H = 3.615$, d.f. = 2, $p = 0.164$, $\eta^2 = 0.029$; Fig. 2A–B). However, LD-M, but not SD males exhibited a reduction in attack latency over time (LD-M: $H = 6.213$, d.f. = 2, $p = 0.045$, $\eta^2 = 0.132$; SD: $H = 2.271$, d.f. = 2, $p = 0.321$, $\eta^2 = 0.005$; Fig. 2C). LD-M and SD males displayed little chasing behavior over the course of the study, and there was no effect of treatment or time on number of chases ($H = 13.852$, d.f. = 8, $p = 0.128$, $\eta^2 = 0.051$) or chase duration ($H = 14.043$, d.f. = 8, $p = 0.121$, $\eta^2 = 0.053$; Table 1).

3.3. Melatonin treatment and short-day photoperiods affected some non-aggressive social behaviors

Melatonin administration and exposure to SDs caused changes in some non-aggressive social behaviors (Tables 1–2). As observed for chasing behavior, LD-M and SD males displayed few scent marking events during behavioral testing (Table 1). Yet, LD-M males exhibited significant increases in scent marking frequency ($H = 3.975$, d.f. = 2, $p = 0.035$, $g = 0.940$) and duration ($H = 3.704$, d.f. = 2, $p = 0.046$, $g = 0.817$) relative to SD males after 3 weeks of treatment. In addition, SD males exhibited a higher scent marking frequency ($H = 3.788$, d.f. = 2, $p = 0.038$, $g = 0.615$) and duration ($H = 3.548$, d.f. = 2, $p = 0.040$, $g = 0.536$) than LD males following 9 weeks of treatment. SD males also trended towards significant increases in scent marking frequency and duration over time (SM frequency: $H = 5.277$, d.f. = 2, $p = 0.072$ $\eta^2 = 0.060$; SM duration: $H = 5.054$, d.f. = 2, $p = 0.080$, $\eta^2 = 0.056$). However, there was no effect of time on scent marking frequency or duration in LD-M males (SM frequency: $H = 1.927$, d.f. = 2, $p = 0.382$, $\eta^2 = 0.002$; SM duration: $H = 1.562$, d.f. = 2, $p = 0.458$, $\eta^2 = 0.014$; Table 1).

Furthermore, LD-M and SD males decreased investigative behaviors in response to treatment (Table 2). Specifically, LD-M males displayed significant reductions in anogenital investigation frequency and duration and nose-to-nose investigation frequency and duration after 6 weeks (AGI frequency: $H = 5.403$, d.f. = 2, $p = 0.028$, $g = -0.845$; AGI duration: $H = 5.030$, d.f. = 2, $p = 0.036$, $g = -0.844$; NTN frequency: $H = 11.925$, d.f. = 2, $p = 0.001$, $g = -1.379$; NTN duration: $H = 13.254$, d.f. = 2, $p = 0.001$, $g = -1.245$) and 9 weeks of treatment (AGI frequency: $p = 0.008$, $g = -0.724$; AGI duration: $p = 0.009$, $g = -0.795$; NTN frequency: $p < 0.001$, $g = -1.897$; NTN duration: $p < 0.001$, $g = -2.087$) relative to LD males at the week 3 time point. Moreover, SD males exhibited significant decreases in anogenital investigation frequency and duration following 3 weeks (AGI frequency: $H = 4.842$, d.f. = 2, $p = 0.018$, $g = -1.148$; AGI duration: $H = 4.123$, d.f. = 2, $p = 0.037$, $g = -1.063$) and 6 weeks of treatment (AGI frequency: $p = 0.023$, $g = -0.590$; AGI duration: $p = 0.021$, $g = -0.700$) compared to week 3 LD males. SD males also displayed reductions in nose-to-nose investigation frequency and duration relative to week 3 LD males at all three time points (NTN frequency: $H = 11.332$, d.f. = 3, $p = 0.010$, $\eta^2 = 0.132$; NTN duration: $H = 14.632$, d.f. = 3, $p = 0.002$, $\eta^2 = 0.185$). Interestingly, while LD-M males exhibited a significant decrease in nose-to-nose investigation duration over time ($H = 6.894$, d.f. = 2, $p = 0.032$, $\eta^2 = 0.153$), there was no effect of time on nose-to-nose investigation duration in SD males ($H = 2.907$, d.f. = 2, $p = 0.234$, $\eta^2 = 0.016$). In addition, there was no effect of time on nose-to-nose investigation frequency, anogenital investigation frequency, or anogenital investigation duration in LD-M (NTN frequency: $H = 4.333$, d.f. = 2, $p = 0.115$, $\eta^2 = 0.073$; AGI frequency: $H = 1.239$, d.f. = 2, $p = 0.538$, $\eta^2 = 0.024$; AGI duration: $H = 1.261$, d.f. = 2, $p = 0.532$, $\eta^2 = 0.023$) or SD males (NTN frequency: $H = 0.451$, d.f. = 2, $p = 0.798$, $\eta^2 = 0.028$; AGI frequency: $H = 0.560$, d.f. = 2, $p = 0.756$, $\eta^2 = 0.026$; AGI duration: $H = 0.607$, d.f. = 2, $p = 0.738$, $\eta^2 = 0.025$).

Conversely, LD-M and SD males showed few changes in grooming behavior over the course of the study (Table 2). Week 6 SD males trended towards a significant increase in grooming duration relative to week 3 LD-M and SD males ($H = 5.736$, d.f. = 2, $p = 0.057$, $\eta^2 = 0.076$). However, there was no effect of time on grooming frequency or grooming duration in LD-M (GR frequency: $H = 2.645$, d.f. = 2, $p = 0.267$, $\eta^2 = 0.020$; GR duration: $H = 0.685$, d.f. = 2, $p = 0.710$, $\eta^2 = 0.041$) or SD males (GR frequency: $H = 1.639$, d.f. = 2, $p = 0.441$, $\eta^2 = 0.007$; GR duration: $H = 5.336$, d.f. = 2, $p = 0.070$, $\eta^2 = 0.061$).

3.4. Basal serum androgen levels differed across photoperiods and in response to timed melatonin injections

Timed melatonin injections resulted in elevated basal (i.e., pre-aggression) circulating DHEA levels and produced SD-like changes in basal T levels (Fig. 3A, C). LD-M males displayed significant increases in serum DHEA concentration following 6 weeks and 9 weeks of treatment relative to week 3 LD males ($H = 12.441$, d.f. = 2, $p = 0.002$, $\eta^2 = 0.337$). Surprisingly, similar changes were not observed in SD males; SD males trended towards a significant increase in serum DHEA following 3 weeks of treatment ($H = 2.185$, d.f. = 2, $p = 0.086$,

Table 1
Mean ± SEM (LD: $n = 9$–11, LD-M: $n = 10$–13, SD: $n = 16$–22) of chasing and scent marking (SM) behaviors exhibited by long day hamsters (LD), LD hamsters administered timed melatonin injections (LD-M), and short day hamsters (SD) following 3 weeks, 6 weeks, and 9 weeks of treatment. Different letters indicate a significant difference between groups for a given behavior ($p < 0.05$).

Behavior	Week 3			Week 6			Week 9		
	LD	LD-M	SD	LD	LD-M	SD	LD	LD-M	SD
Chase frequency	$0.8 \pm 0.5^{a,b}$	3.6 ± 1.5^{a}	0.5 ± 0.3^{b}	0.3 ± 0.1^{b}	1.2 ± 0.8^{b}	0.4 ± 0.3^{b}	$0.0 \pm 0.0^{a,b}$	0.4 ± 0.2^{b}	0.5 ± 0.2^{b}
Chase duration (s)	$0.5 \pm 0.3^{a,b}$	3.1 ± 1.4^{a}	0.4 ± 0.2^{b}	0.2 ± 0.1^{b}	1.2 ± 0.8^{b}	0.2 ± 0.2^{b}	$0.0 \pm 0.0^{a,b}$	0.2 ± 0.1^{b}	0.2 ± 0.1^{b}
SM frequency	$0.3 \pm 0.2^{a,b}$	0.3 ± 0.2^{a}	0.1 ± 0.1^{b}	0.0 ± 0.0^{b}	$0.2 \pm 0.1^{a,b}$	0.2 ± 0.2^{b}	$0.0 \pm 0.0^{a,b}$	$0.1 \pm 0.1^{a,b}$	0.6 ± 0.3^{a}
SM duration (s)	$0.5 \pm 0.4^{a,b}$	0.2 ± 0.1^{a}	0.1 ± 0.1^{b}	0.0 ± 0.0^{b}	$0.1 \pm 0.1^{a,b}$	0.8 ± 0.8^{b}	$0.0 \pm 0.0^{a,b}$	$0.1 \pm 0.1^{a,b}$	0.7 ± 0.4^{a}

Table 2
Mean ± SEM (LD: n = 9–11, LD-M: n = 10–13, SD: n = 16–22) of investigation (anogenital, AGI; nose-to-nose, NTN) and grooming (GR) behaviors exhibited by long day hamsters (LD), LD hamsters administered timed melatonin injections (LD-M), and short day hamsters (SD) following 3 weeks, 6 weeks, and 9 weeks of treatment. Different letters indicate a significant difference between groups for a given behavior ($p < 0.05$).

Behavior	Week 3			Week 6			Week 9		
	LD	LD-M	SD	LD	LD-M	SD	LD	LD-M	SD
AGI frequency	9.1 ± 2.2^{a}	6.4 ± 2.7^{a,b}	3.8 ± 0.8^{b}	5.8 ± 1.7^{a,b}	4.3 ± 1.4^{b}	5.4 ± 1.3^{b}	2.8 ± 0.7^{b}	4.2 ± 1.9^{b}	5.3 ± 1.3^{a,b}
AGI duration (s)	19.6 ± 6.1^{a}	12.4 ± 5.1^{a,b}	7.2 ± 1.6^{b}	12.5 ± 4.8^{a,b}	7.5 ± 3.0^{b}	9.5 ± 2.6^{b}	4.0 ± 1.7^{b}	7.1 ± 3.8^{b}	13.5 ± 4.4^{a,b}
NTN frequency	16.8 ± 3.3^{a}	8.3 ± 2.4^{a,b}	7.8 ± 2.1^{b,c}	7.8 ± 2.1^{b,c}	5.3 ± 2.0^{c}	5.3 ± 1.2^{b,c}	4.4 ± 1.2^{c}	3.8 ± 0.9^{c}	4.7 ± 0.7^{b,c}
NTN duration (s)	32.7 ± 7.1^{a}	17.3 ± 5.6^{a,b}	18.4 ± 6.0^{b}	12.8 ± 5.0^{b,c}	9.9 ± 4.5^{c}	8.3 ± 2.3^{b,c}	6.5 ± 2.6^{c}	3.8 ± 1.0^{c}	5.3 ± 1.1^{b,c}
GR frequency	7.0 ± 1.3^{a}	6.5 ± 1.8^{a}	7.5 ± 1.5^{a}	7.2 ± 1.3^{a}	10.2 ± 1.6^{a}	8.0 ± 1.0^{a}	8.8 ± 1.9^{a}	8.6 ± 1.6^{a}	9.8 ± 1.7^{a}
GR duration (s)	11.5 ± 3.3^{a,b}	9.8 ± 2.6^{a}	10.3 ± 1.9^{a}	11.3 ± 1.9^{a,b}	14.8 ± 3.5^{a,b}	22.8 ± 4.5^{b}	14.8 ± 4.6^{a,b}	20.0 ± 8.9^{a,b}	13.5 ± 2.5^{a,b}

$g = 0.478$), yet showed no significant difference in serum DHEA concentration following 6 weeks ($H = 5.963$, d.f. = 2, $p = 0.424$, $g = -0.194$) and 9 weeks of treatment ($H = 6.738$, d.f. = 2, $p = 0.461$, $g = -0.070$). Likewise, while LD-M males displayed an increase in serum DHEA levels over time ($H = 13.982$, d.f. = 2, $p < 0.001$, $\eta^2 = 0.374$), there was no effect of time on serum DHEA levels in SD males ($H = 0.648$, d.f. = 2, $p = 0.723$, $\eta^2 = 0.025$; Fig. 3A).

In contrast, both LD-M and SD males exhibited significant decreases in circulating T levels in response to treatment (Fig. 3C). LD-M and SD males showed reductions in serum T concentration following 9 weeks of treatment ($H = 19.492$, d.f. = 2, $p < 0.001$, $\eta^2 = 0.500$). Interestingly, while this reduction in serum T was more pronounced in SD males than LD-M males after 9 weeks of treatment ($H = 19.492$, d.f. = 2, $p = 0.019$, $g = -0.648$), this response occurred more rapidly in LD-M males than in SD males. Specifically, LD-M males exhibited significantly lower serum T concentrations at all three time points relative to week 3 LD males ($H = 8.722$, d.f. = 3, $p = 0.033$, $\eta^2 = 0.160$), whereas SD males did not display a significant reduction in serum T until the week 6 time point ($H = 18.296$, d.f. = 3, $p = 0.029$, $g = -0.804$). However, SD males exhibited decreases in serum T levels over time ($H = 14.634$, d.f. = 2, $p = 0.001$, $\eta^2 = 0.308$), whereas LD-M males showed no significant difference in serum T concentration over the course of the study ($H = 2.400$, d.f. = 2, $p = 0.301$, $\eta^2 = 0.012$).

3.5. Melatonin administration and short-day photoperiods reduced circulating androgen levels following an aggressive encounter

Melatonin administration and exposure to SD photoperiods caused a decrease in post-aggression androgen levels (i.e., decreases in ΔDHEA and ΔT; Fig. 3B, D). SD males displayed significant decreases in ΔDHEA following 6 weeks ($H = 2.872$, d.f. = 2, $p = 0.045$, $g = -0.572$) and 9 weeks of treatment ($H = 9.196$, d.f. = 2, $p = 0.009$, $g = -1.478$) compared to LD males. LD-M males also exhibited a significant decrease in ΔDHEA after 9 weeks of treatment ($H = 9.196$, d.f. = 2, $p = 0.002$, $g = -1.498$). Moreover, LD-M males showed a reduction in ΔDHEA over time ($H = 11.674$, d.f. = 2, $p = 0.003$, $\eta^2 = 0.322$), and SD males trended towards a decrease in ΔDHEA over the course of the study ($H = 4.982$, d.f. = 2, $p = 0.083$, $\eta^2 = 0.093$; Fig. 3B).

Similarly, both LD-M and SD males showed significant decreases in ΔT following 9 weeks of treatment ($H = 19.034$, d.f. = 2, $p < 0.001$,

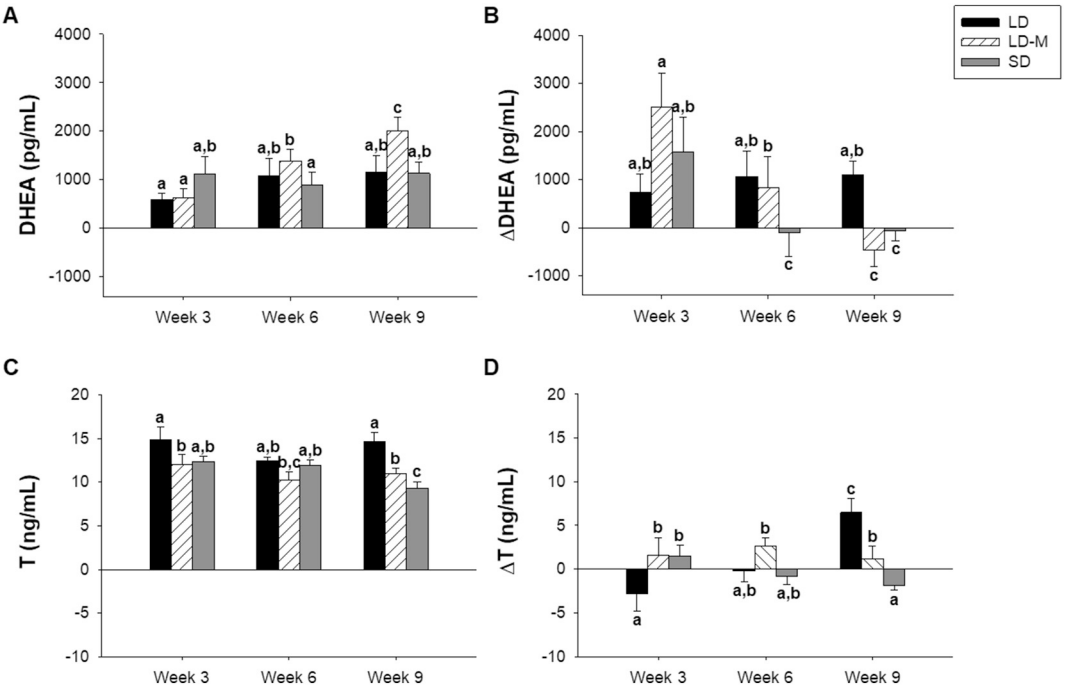

Fig. 3. Melatonin (M) administration and exposure to short-day (SD) photoperiods reduced serum testosterone (T) levels and changes in circulating androgen levels following behavioral testing. Basal dehydroepiandrosterone (DHEA) levels (A), ΔDHEA (B), basal T levels (C), and ΔT in long day hamsters (LD; black bars), LD hamsters administered timed melatonin injections (LD-M; white striped bars), and SD hamsters (gray bars) following 3 weeks, 6 weeks, and 9 weeks of treatment. ΔDHEA and ΔT represent the difference between pre-aggression and post-aggression serum DHEA levels and serum T levels, respectively. Data are presented as mean ± SEM (LD: n = 7–11, LD-M: n = 9–12, SD: n = 10–16). Bars with different letters indicate a significant difference between groups ($p < 0.05$).

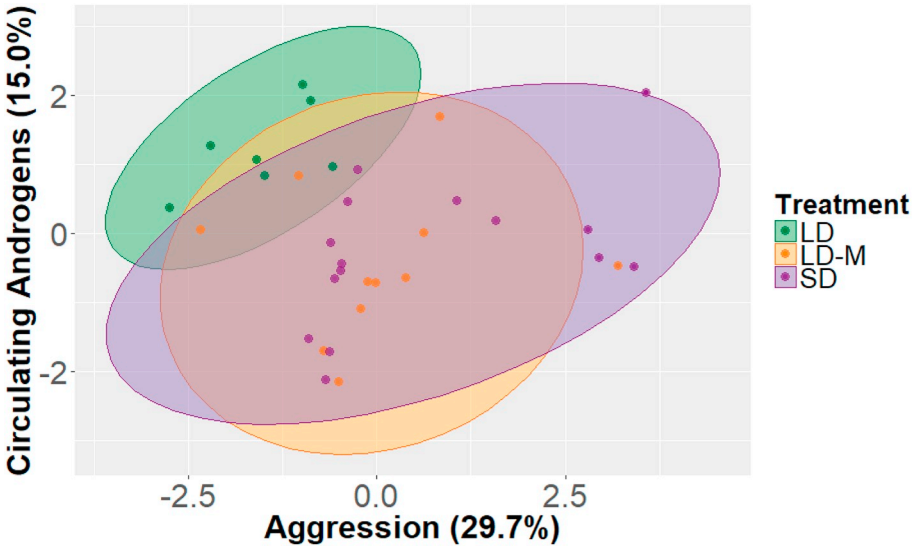

Fig. 4. Hamsters administered timed melatonin (M) injections or exposed to short-day (SD) photoperiods showed similar physiological and behavioral responses to treatment. Principal component analysis (PCA) was conducted using aggression variables and serum androgen concentrations in long day hamsters (LD; green), LD hamsters administered timed melatonin injections (LD-M; orange), and SD hamsters (purple) following 9 weeks of treatment. Normal 95% confidence ellipses are shown around individuals associated with each treatment group (LD: $n = 7$, LD-M: $n = 11$, SD: $n = 15$). For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

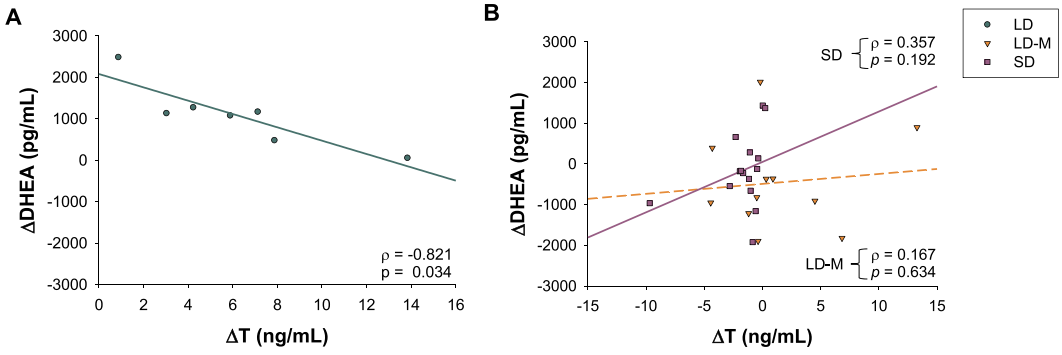

Fig. 5. Animals administered timed melatonin (M) injections or exposed to short-day (SD) photoperiods showed similar relationships between circulating androgen profiles following an aggressive encounter. (A) Changes in serum testosterone levels following behavioral testing (ΔT) were negatively correlated with changes in serum dehydroepiandrosterone levels (ΔDHEA) in long day hamsters (LD; green circles) following 9 weeks of treatment. (B) ΔT was positively correlated with ΔDHEA in LD hamsters administered timed melatonin injections (LD-M; orange triangles) and SD hamsters (purple squares) following 9 weeks of treatment. Regression lines were generated from Spearman's rank correlations within treatment groups (LD: $n = 7$, LD-M: $n = 11$, SD: $n = 15$). For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

$\eta^2 = 0.501$) relative to LD males. However, this reduction in ΔT was more pronounced in SD males than in LD-M males ($H = 19.034$, d.f. = 2, $p = 0.021$, $g = -0.822$). SD males also exhibited a significantly lower ΔT value than LD-M males after 6 weeks of treatment ($H = 10.183$, d.f. = 2, $p = 0.002$, $g = -0.974$). In addition, SD males displayed a reduction in ΔT over time ($H = 8.588$, d.f. = 2, $p = 0.014$, $\eta^2 = 0.161$). Surprisingly, LD males exhibited an increase in ΔT over the course of the study ($H = 12.264$, d.f. = 2, $p = 0.002$, $\eta^2 = 0.411$). However, there was no effect of time on ΔT in LD-M males ($H = 1.783$, d.f. = 2, $p = 0.410$, $\eta^2 = 0.007$; Fig. 3D).

3.6. Treatment with melatonin injections or short-day photoperiods resulted in similar physiological and behavioral responses

A PCA was used to examine differences in aggressive behavior and circulating androgen profiles across seasonal phenotypes following 9 weeks of treatment (Fig. 4). This analysis yielded a set of three principal components (PCs) that drove variability in the data (≥15.00% of cumulative variance explained for each PC; Kaiser-Meyer-Olkin measure of sampling adequacy = 0.570; Bartlett's test of sphericity: $\chi^2 = 589.372$, d.f. = 36, $p < 0.001$). PC1 accounted for 29.72% of the total variance and was strongly loaded by number of attacks, attack duration, number of chases, chase duration, and ΔT. PC3 accounted for 15.00% of the total variance and was strongly loaded by basal (i.e., pre-aggression) DHEA, basal T, ΔDHEA, and attack duration (Supplementary Material, Fig. S1 and Table S2). Based on the variables driving each PC, PC1 and PC3 likely represent aggressive behavior and circulating androgen profiles, respectively. LD-M and SD males grouped together along both PC1 ("Aggression") and PC3 ("Circulating Androgens"), and the confidence ellipses of these treatment groups almost completely overlapped. Conversely, LD males formed a distinct group along both PC1 and PC3, which was separated from that of LD-M and SD males. Furthermore, there was little overlap between the confidence ellipse of LD males and those of LD-M and SD males. Collectively, this analysis suggests that timed melatonin injections and exposure to SDs produce similar changes in aggression and circulating androgens in male hamsters after 9 weeks of treatment.

3.7. Melatonin administration and short-day photoperiods produce similar relationships between circulating DHEA and T following an aggressive encounter

Melatonin administration and exposure to SD photoperiods produced similar relationships between ΔT and ΔDHEA following 9 weeks of treatment (Fig. 5). ΔT was significantly negatively correlated with ΔDHEA in LD males ($r_s = -0.821$, $n = 7$, $p = 0.034$; Fig. 5A). In contrast, ΔT was positively correlated with ΔDHEA in both LD-M and SD males, although neither of these relationships reached significance (LD-

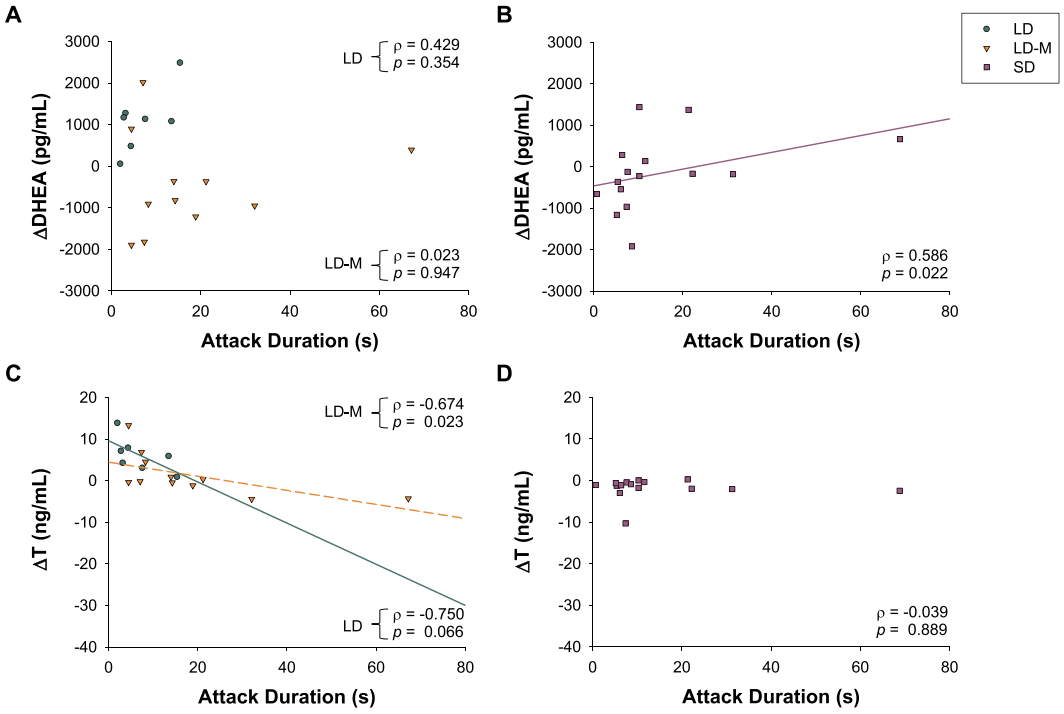

Fig. 6. Seasonal phenotypes exhibited distinct relationships between circulating androgen profiles and aggressive behavior after 9 weeks of treatment. (A) Attack duration was not correlated with changes in serum dehydroepiandrosterone levels following an aggressive encounter (ΔDHEA) in long day hamsters (LD; green circles) or LD hamsters administered timed melatonin injections (LD-M; orange triangles). (B) Attack duration was positively correlated with ΔDHEA in short day hamsters (SD; purple squares). (C) Attack duration trended towards a significant correlation with changes in serum testosterone levels following an aggressive encounter (ΔT) in LD hamsters and was negatively correlated with ΔT in LD-M hamsters. (D) Attack duration and ΔT were not correlated in SD hamsters. Regression lines were generated from Spearman's rank correlations within treatment groups (LD: $n = 7$, LD-M: $n = 11$, SD: $n = 15$). For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

M: $r_s = 0.164$, $n = 11$, $p = 0.634$; SD: $r_s = 0.357$, $n = 15$, $p = 0.192$; Fig. 5B). Taken together, these results suggest that LD-M and SD males exhibit similar associations between circulating DHEA and T profiles after an aggressive interaction, and that this relationship is distinct from that observed in LD males.

3.8. Seasonal phenotypes exhibited distinct associations between circulating androgen profiles and aggressive behavior

LD and SD males showed different relationships between circulating androgen profiles and aggressive behavior following 9 weeks of treatment (Fig. 6). Attack duration was significantly positively correlated with ΔDHEA in SD males ($r_s = 0.586$, $n = 15$, $p = 0.022$; Fig. 6A). Conversely, attack duration was not correlated with ΔDHEA in LD or LD-M males (LD: $r_s = 0.429$, $n = 7$, $p = 0.354$; LD-M: $r_s = 0.023$, $n = 11$, $p = 0.947$; Fig. 6B). Furthermore, LD males trended towards a negative association between attack duration and ΔT (LD: $r_s = -0.750$, $n = 7$, $p = 0.066$). Interestingly, attack duration was significantly negatively associated with ΔT in LD-M males ($r_s = -0.674$, $n = 11$, $p = 0.023$; Fig. 6C). However, attack duration was not correlated with ΔT in SD males (SD: $r_s = -0.039$, $n = 15$, $p = 0.889$; Fig. 6D). Together, these findings suggest that aggression in male hamsters during SDs is modulated by circulating DHEA, whereas aggression during LDs is modulated by circulating T.

4. Discussion

Previous work from our lab suggests that Siberian hamsters employ a "seasonal switch" from gonadal regulation of aggression during the breeding season (i.e., LDs) to adrenal regulation of aggression during the non-breeding season (i.e., SDs; reviewed in Munley et al., 2018; Rendon et al., 2015; reviewed in Soma et al., 2015; Scotti et al., 2008). While this shift in neuroendocrine mechanisms can be induced by changes in photoperiod, the role of the pineal hormone melatonin in facilitating these seasonal changes in steroidogenesis and aggressive behavior is unknown. For the first time, we show that providing a long-term, SD-like photoperiodic signal to LD males via timed melatonin injections causes gonadal regression and elevates aggressive behavior, changes that are characteristic of SD males. We also demonstrate that males administered timed melatonin injections or exposed to SDs decrease circulating androgens in response to an aggressive encounter and that male Siberian hamsters exhibit distinct associations between circulating androgen profiles and aggression across seasonal phenotypes. Taken together, these findings suggest that SD males transition from synthesis to metabolism of circulating androgens following an aggressive encounter, a mechanism that is mediated by melatonin and culminates in increased aggression during the non-breeding season.

4.1. Timed melatonin injections and short-day photoperiods altered reproductive physiology and behavior

As predicted, both LD-M and SD males decreased body and paired testes mass in response to treatment. Moreover, both melatonin administration and exposure to SD photoperiods resulted in a reduction in EWAT mass over the course of the study, suggesting that adipose stores, in addition to reproductive tissues, respond to seasonal changes in melatonin signaling. These findings are in agreement with previous work in Siberian hamsters, which has shown that males and females undergo gonadal regression and exhibit a significant reduction in body mass and adipose tissue mass following exposure to SDs (Carlton and Demas, 2015; Jasnow et al., 2000; Rendon et al., 2015). Collectively, these melatonin-induced decreases in body mass and adipose stores; along with other physiological adaptations, such as daily torpor and increased cold tolerance; allow these animals to survive the harsh

environmental conditions that are characteristic of Siberian winters (reviewed in Bartness et al., 2002; reviewed in Bartness and Wade, 1985).

Interestingly, we also found that SD males exhibited more pronounced reductions in body mass, paired testes mass, and EWAT mass than LD-M males at the end of the 9 week study. While it is possible that environmental cues other than photoperiod, such as ambient temperature, humidity, or food availability, may be necessary for Siberian hamsters to undergo a full transition from breeding to non-breeding condition, previous work suggests that this possibility is unlikely, since timed MEL injections cause a reduction in body mass in LD males (Wade and Bartness, 1984) and pinealectomy prevents gonadal regression when males are transferred from LDs to SDs (Hoffman and Reiter, 1965; Hoffmann, 1974). However, it is important to note that we examined seasonal transitions in body and reproductive tissue mass over a period of 9 weeks, whereas previous studies from our lab have typically examined these measures following 10–12 weeks of photoperiodic treatment (Jasnow et al., 2000; Rendon et al., 2015; Scotti et al., 2008). Therefore, it is possible that an additional 1–3 weeks of timed melatonin administration would have been sufficient to elicit decreases in body and reproductive tissue mass that were statistically equivalent to those of SD animals.

In addition, LD-M males exhibited SD-like levels of aggressive and investigative behaviors following 9 weeks of treatment. These findings support previous work from our lab, which has shown that SD male and female hamsters exhibit higher levels of aggressive behavior than LD hamsters (Jasnow et al., 2000; Rendon and Demas, 2016; Scotti et al., 2007), timed melatonin injections increase aggression in LD males and females (Demas et al., 2004; Rendon et al., 2015), and SD females display lower levels of social investigation than LD females (Rendon et al., 2015). Interestingly, despite the occurrence of few scent marking events during this study, LD-M and SD animals also displayed increases in scent marking behavior. Taken together, these data suggest that melatonin not only mediates seasonal transitions in aggressive behavior, but also modulates seasonal changes in investigative and scent marking behaviors. Siberian hamsters use a suite of chemical signaling mechanisms, including deposition of chemical constituents in urine and ventral gland secretions, to communicate with conspecifics (Burger et al., 2001; Soini et al., 2005). Of these chemical cues, ventral gland secretions are thought to be particularly important for territorial marking, and hamsters in the wild use scent marking to establish boundaries for their home territories (Wynne-Edwards, 2003; Wynne-Edwards et al., 1992). In turn, hamsters investigate conspecifics and these chemical cues to determine an appropriate behavioral response, such as fighting, fleeing, or mating. Thus, elevated aggression and scent marking behavior and reduced social investigation are consistent with increased territoriality during SDs.

4.2. Rising to the challenge: seasonal changes in aggression-induced androgen profiles and social priming

In the present study, we demonstrated that LD-M males exhibited changes in basal, pre-aggression levels of circulating androgens that were generally similar to those of SD males. Specifically, we found that LD-M animals exhibited an increase serum DHEA concentration and a decrease in serum T concentration in response to treatment. These findings are consistent with those of previous studies, which have shown that SD males and females elevate serum DHEA (Rendon and Demas, 2016; Rendon et al., 2015; Scotti et al., 2008) and LD females treated with exogenous melatonin increase circulating DHEA levels (Rendon et al., 2015). Moreover, these data support the hypothesis that melatonin signaling mediates a "seasonal switch" from gonadal regulation of aggression during LDs to adrenal regulation of aggression during SDs. Adrenal DHEA has been implicated as an important precursor of androgens and estrogens in some species of birds and mammals that exhibit territorial aggression year-round (Gutzler et al., 2009; Heimovics et al., 2016; Newman and Soma, 2009; Pradhan et al., 2008; Rendon et al., 2015; Soma et al., 2004). Circulating DHEA can cross the blood-brain-barrier and be converted to T and/or E_2 in brain regions that express the necessary steroidogenic enzymes (reviewed in Beck and Handa, 2004; reviewed in Labrie et al., 2005). Furthermore, because DHEA has minimal affinity for androgen and estrogen receptors (AR and ER, respectively; reviewed in Webb et al., 2006), it is likely that metabolic conversion of DHEA to T and/or E_2 is primarily responsible for modulating the neural circuits relevant to territorial aggression during the non-breeding season, allowing these species to maintain low levels of circulating androgens and estrogens while still exhibiting high levels of aggression (reviewed in Munley et al., 2018; Soma et al., 2015). The results of the current study build on our "seasonal switch" hypothesis and suggest that melatonin coordinates seasonal changes in aggression by altering peripheral steroidogenesis in male hamsters.

Furthermore, we found that LD-M and SD animals exhibited similar hormonal changes in response to an aggressive interaction. Both LD-M and SD animals reduced serum DHEA and T following an aggressive encounter, whereas LD hamsters increased serum DHEA and T levels. This divergent physiological response was further confirmed through correlational analyses, in which ΔT was significantly negatively associated with ΔDHEA in LD males, yet was positively associated with ΔDHEA in LD-M and SD males. Taken together, these data provide evidence that SD-like patterns of melatonin secretion facilitate a seasonal shift from elevated to reduced post-aggression circulating androgen levels. While we cannot rule out the potential effects of diurnal rhythms on adrenal and gonadal steroid secretion in these samples (Ottenweller et al., 1987; reviewed in Kalsbeek et al., 2012), these results are in agreement with those of previous studies, which have demonstrated that LD males decrease circulating DHEA following an aggressive interaction at night (e.g., in the presence of high levels of circulating melatonin; Scotti et al., 2009) and SD females reduce serum DHEA and T in response to an aggressive interaction (Rendon and Demas, 2016; N. M. Rendon, C. L. Petersen, K. M. Munley, and G. E. Demas, unpublished results). This rapid decrease in post-aggression circulating androgens suggests that a SD-like melatonin signal elevates DHEA and T metabolism to E_2 within circulation. While circulating E_2 was not measured in the present study, we have previously shown that SD females increase serum E_2 in response to an aggressive interaction, suggesting behaviorally-induced conversion of DHEA to E_2 (N.M. Rendon, C. L. Petersen, K. M. Munley, and G. E. Demas, unpublished results). Moreover, studies in other species that exhibit territorial aggression year-round have suggested a role for circulating E_2 in modulating non-breeding aggression. Administering E_2 systemically to non-breeding song sparrows (*Melospiza melodia*), white-throated sparrows (*Zonotrichia albicollis*), beach mice (*Peromyscus polionotus*), and California mice (*Peromyscus californicus*) elevates aggressive behavior (Heimovics et al., 2015a; Merritt et al., 2018; Trainor et al., 2008; Trainor et al., 2007a), suggesting that rapid conversion of E_2 precursors, such as DHEA and T, may allow these animals to maintain aggression during the non-breeding season.

While the classic genomic actions of E_2 on aggression have been well-studied (Adkins-Regan, 2005), there is emerging evidence that E_2 also exerts rapid effects on aggression via rapid, non-genomic signaling mechanisms (reviewed in Heimovics et al., 2015b; reviewed in Heimovics et al., 2018; reviewed in Laredo et al., 2014b). These alternative, non-genomic mechanisms are consistent with previous studies, which have shown effects of E_2 on aggression over time courses that are too rapid to be associated with changes in gene expression (Cornil et al., 2006; Wendler et al., 2010). During the non-breeding season, a surge in E_2 following an aggressive interaction may allow for rapid modulation of the neural circuits relevant to aggressive behavior. Indeed, recent studies suggest that local changes in steroid synthesis, metabolism, and/or steroid receptor abundance within the brain, which are mediated by the non-genomic actions of E_2, are responsible for elevating non-breeding aggression in some birds and rodents. Non-

breeding male song sparrows exhibit localized changes in neurosteroid levels and show increases in 3β-hydroxysteroid dehydrogenase/ Δ5-Δ4 isomerase activity, an enzyme that converts DHEA to androstenedione; and aromatase activity, an enzyme that converts T to E_2; in the brain following an aggressive interaction (Heimovics et al., 2016; Pradhan et al., 2010; Soma et al., 2003). Furthermore, SD male beach mice (*Peromyscus polionotus*), SD male deer mice (*Peromyscus maniculatus*), and SD male and female Siberian hamsters exhibit increases in ERα abundance in brain nuclei that regulate aggressive behavior (Kramer et al., 2008; Rendon et al., 2017; Trainor et al., 2007a; Trainor et al., 2007b). Collectively, post-aggression surges in E_2 may be adaptive and, thus, socially prime individuals for future aggressive encounters. Future studies will examine the specific physiological mechanisms that underlie this post-aggression decrease in serum androgen levels in SD male hamsters by measuring the conversion of DHEA to other biologically-active steroids, such as E_2, both within circulation and in the brain and by examining the potential genomic and non-genomic signaling mechanisms of E_2 on seasonal aggression.

4.3. Potential mechanisms underlying seasonal shifts in melatonin signaling and aggression

Overall, our data suggest that seasonal phenotypes exhibit distinct relationships between circulating androgens and aggression. Specifically, we found that SD, but not LD males exhibited a significant positive correlation between attack duration and ΔDHEA. Conversely, we determined that LD, but not SD males showed a significant negative correlation between attack duration and ΔT. These findings support our hypothesis that gonadal steroids, such as T, are essential in mediating aggression during the breeding season; whereas DHEA is responsible for modulating aggression during the non-breeding season.

To date, few studies have examined the potential mechanisms underlying melatonin-induced increases in non-breeding aggression. In male California mice, exogenous melatonin increases aggression during LDs, and this response is partially blocked by the non-selective melatonin 1a receptor (Mel1aR) and melatonin 1b receptor (Mel1bR) antagonist luzindole (Laredo et al., 2014a). Furthermore, our lab has shown that timed melatonin injections elevate aggression in LD male and female hamsters (Demas et al., 2004; Rendon et al., 2015) and *in vitro* melatonin administration stimulates adrenal DHEA release from SD, but not LD females (Rendon et al., 2015). While these findings suggest that melatonin signaling elevates non-breeding aggression in some species, each of these studies used techniques that either altered systemic levels of melatonin or manipulated systemic melatonin signaling by administering a pharmacological inhibitor that blocks both subtypes of melatonin receptor (Boutin et al., 2005; Dubocovich, 1988). Therefore, it is unclear how melatonin acts locally (e.g., peripherally and/or centrally) via its receptors to modulate seasonal changes in steroidogenesis and aggressive behavior.

Melatonin may modulate seasonal aggression via direct actions on neural substrates, such as the hypothalamus and pituitary gland; and/or via peripheral actions on the adrenal glands and gonads (reviewed in Borniger and Nelson, 2017; reviewed in Munley et al., 2018). After being secreted into circulation, melatonin can bind to one of three subtypes of membrane-bound G protein-coupled melatonin receptors (Dubocovich and Markowska, 2005; reviewed in von Gall et al., 2002; Witt-Enderby et al., 2003). Interestingly, melatonin exerts regulatory functions at all levels of the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-adrenal (HPA) axes in seasonally breeding animals by binding to Mel1aR located on the hypothalamus (Bae et al., 2014; reviewed in Wood and Loudon, 2014), anterior pituitary gland (reviewed in Chowdhury et al., 2013; Williams et al., 1997), gonads (Frungieri et al., 2005; McGuire et al., 2011), and adrenal glands (Richter et al., 2008; Skinner and Robinson, 1995). Thus, the presence of Mel1aR at different tiers of the HPA and HPG axes suggests that melatonin could alter gonadal and adrenal steroid synthesis on a seasonal basis via peripheral and/or central signaling mechanisms.

Siberian hamsters are an ideal system for examining how melatonin receptor signaling regulates seasonal aggression. While most mammals express two subtypes of melatonin receptor, Mel1aR and Mel1bR (Dubocovich and Markowska, 2005; reviewed in von Gall et al., 2002; Witt-Enderby et al., 2003), Siberian hamsters only possess one function melatonin receptor subtype, Mel1aR (Weaver et al., 1996). Thus, this species is particularly useful for determining whether peripheral and/or central melatonin signaling regulates seasonal changes in aggression, because we can be confident that, by locally manipulating Mel1aR expression or abundance, we are targeting all potential melatonin signaling pathways within a given tissue. Studies are currently underway to investigate the central and peripheral actions of melatonin signaling on seasonal aggression in male hamsters, specifically by targeting Mel1aR expression in the paraventricular hypothalamic nucleus or adrenal glands.

5. Conclusions

In the present study, we showed that providing a SD-like melatonin signal to LD male hamsters via timed injections causes physiological and behavioral changes similar to those of SD males. Specifically, we found that LD-M and SD males increased aggression and decreased post-aggression circulating androgen levels, a response that may allow these individuals to modulate aggressive behavior via rapid, non-genomic mechanisms and prepare them for future aggressive encounters. Collectively, these findings suggest that melatonin regulates seasonal changes in peripheral steroidogenesis and aggressive behavior in male hamsters, though additional studies are needed to examine whether peripheral and/or central melatonin signaling mechanisms are essential in modulating seasonal aggression. These results provide important insight into the neuroendocrine mechanisms by which seasonally breeding animals transition from breeding to non-breeding condition and how changes in these mechanisms can influence aggression, a behavior that is critical for reproductive success.

Data availability

Data from this study are available in Mendeley Data.

Acknowledgements

We thank Daniel Boyes, Sarah Henderson, Leanne Jossund, Cameron Logan, Dr. Kristyn Sylvia, and Michael Vu for assistance in animal procedures, behavioral filming, and necropsies; and Karen Rogers, D.V.M., Randalyn Shepard, D.V.M., and the staff of Laboratory Animal Resources at Indiana University for caring for and monitoring the health of the animals used in this study. We are also grateful to Dr. Kim Rosvall and two anonymous reviewers, who provided valuable insight and feedback on a previous version of this manuscript.

Declaration of competing interests

The authors declare no competing interests, financial or otherwise.

Author contributions

K.M.M., J.E.D., and G.E.D. designed the experiments. K.M.M. and J.E.D. performed photoperiodic treatments, administered timed melatonin injections, staged behavioral interactions, and collected blood samples. K.M.M., J.E.D., and C.C.R. performed necropsies and scored behavioral videos. K.M.M. determined reproductive phenotypes, ran hormone assays, and conducted statistical analyses. K.M.M. and G.E.D. interpreted the data and drafted the manuscript, with editorial contributions from J.E.D. and C.C.R.

Funding

This research was funded by National Institutes of Health Grant R21MH109942 (to G.E.D.) and Indiana University. K.M.M. was supported by a National Institutes of Health Predoctoral Fellowship (T32HD049336 – "Common Themes in Reproductive Diversity") through the Center for the Integrative Study of Animal Behavior at Indiana University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2019.104608.

References

Adkins-Regan, E., 2005. Hormones and Animal Social Behavior. Princeton University Press, Princeton, NJ.
Bae, S.E., Wright, I.K., Wyse, C., Samson-Desvignes, N., Le Blanc, P., Laroche, S., Hazlerigg, D.G., Johnston, J.D., 2014. Regulation of pituitary MT1 melatonin receptor expression by gonadotrophin-releasing hormone (GnRH) and early growth response factor-1 (Egr-1): *in vivo* and *in vitro* studies. PLoS One 9, e90056.
Bartness, T.J., Goldman, B.D., 1989. Mammalian pineal melatonin: a clock for all seasons. Experientia 45, 939–945.
Bartness, T.J., Wade, G.N., 1985. Photoperiodic control of seasonal body weight cycles in hamsters. Neurosci. Biobehav. Rev. 9, 599–612.
Bartness, T.J., Bradley, J., Hastings, M.H., Bittman, E.L., Goldman, B.D., 1993. The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses? J. Pineal Res. 15, 161–190.
Bartness, T.J., Demas, G.E., Song, C.K., 2002. Seasonal changes in adiposity: the roles of the photoperiod, melatonin and other hormones, and sympathetic nervous system. Exp. Biol. Med. 227, 363–376.
Beck, S.G., Handa, R.J., 2004. Dehydroepiandrosterone (DHEA): a misunderstood adrenal hormone and spine-tingling neurosteroid? Endocrinology 145 (3), 1039–1041.
Borniger, J.C., Nelson, R.J., 2017. Photoperiodic regulation of behavior: *Peromyscus* as a model system. Semin. Cell Dev. Biol. 61, 82–91.
Boutin, J.A., Audinot, V., Ferry, G., Delagrange, P., 2005. Molecular tools to study melatonin pathways and actions. Trends Pharmacol. Sci. 26, 412–419.
Brain, P., 1975. What does individual housing mean to a mouse? Life Sci. 16, 187–200.
Brain, P., Poole, A., 1974. Some studies on use of "standard opponents" in intermale aggression testing in TT albino mice. Behaviour 50, 100–110.
Bronson, F.H., 1989. Mammalian Reproductive Biology. University of Chicago Press, London.
Burger, B.V., Smit, D., Spies, H.S., Schmidt, C., Schmidt, U., Telitsina, A.Y., Grierson, G.R., 2001. Mammalian exocrine secretions XV. Constituents of secretion of ventral gland of male dwarf hamster, *Phodopus sungorus sungorus*. J. Chem. Ecol. 27, 1259–1276.
Butler, M.P., Turner, K.W., Park, J.H., Schoomer, E.E., Zucker, I., Gorman, M.R., 2010. Seasonal regulation of reproduction: altered role of melatonin under naturalistic conditions in hamsters. Proc. R. Soc. B Biol. Sci. 277, 2867–2874.
Carlton, E.D., Demas, G.E., 2015. Body mass affects seasonal variation in sickness intensity in a seasonally breeding rodent. J. Exp. Biol. 218, 1667–1676.
Chowdhury, V.S., Ubuka, T., Tsutsui, K., 2013. Review: melatonin stimulates the synthesis and release of gonadotropin-inhibitory hormone in birds. Gen. Comp. Endocrinol. 181, 175–178.
Cohen, J., 1988. Statistical Power Analysis for the Behavioral Sciences, 2 ed. Lawrence Erlbaum Associates, New York, NY.
Cornil, C.A., Ball, G.F., Balthazart, J., 2006. Functional significance of the rapid regulation of brain estrogen action: where do the estrogens come from? Brain Res. 1126, 2–26.
Crews, D., 1984. Gamete production, sex hormone secretion, and mating behavior uncoupled. Horm. Behav. 18, 22–28.
Crews, D., Moore, M.C., 1986. Evolution of mechanisms controlling mating behavior. Science 231, 121–125.
Cunningham, R.L., Lumia, A.R., McGinnis, M.Y., 2012. Androgen receptors, sex behavior, and aggression. Neuroendocrinology 96, 131–140.
Dardente, H., 2012. Melatonin-dependent timing of seasonal reproduction by the pars tuberalis: pivotal roles for long daylengths and thyroid hormones. J. Neuroendocrinol. 24, 249–266.
Dawson, A., King, V.M., Bentley, G.E., Ball, G.F., 2001. Photoperiodic control of seasonality in birds. J. Biol. Rhythm. 16, 365–380.
Demas, G.E., Polacek, K.M., Durazzo, A., Jasnow, A.M., 2004. Adrenal hormones mediate melatonin-induced increases in aggression in male Siberian hamsters (*Phodopus sungorus*). Horm. Behav. 46, 582–591.
Do Rego, J.L., Seong, J.Y., Burel, D., Leprince, J., Vaudry, D., Luu-The, V., Tonon, M.-C., Tsutsui, K., Pelletier, G., Vaudry, H., 2012. Regulation of neurosteroid biosynthesis by neurotransmitters and neuropeptides. Front. Endocrinol. 3, 4.
Dubocovich, M.L., 1988. Luzindole (N-0774): a novel melatonin receptor antagonist. J. Pharmacol. Exp. Ther. 246, 902–910.
Dubocovich, M.L., Markowska, M., 2005. Functional MT_1 and MT_2 melatonin receptors in mammals. Endocrine 27, 101–110.
Ellis, P.D., 2010. The Essential Guide to Effect Sizes: Statistical Power, Meta-Analysis, and the Interpretation of Research Results. Cambridge University Press. Cambridge University Press, Cambridge, UK.
Frungieri, M.B., Mayerhofer, A., Zitta, K., Pignataro, O.P., Calandra, R.S., Gonzalez-Calvar, S.I., 2005. Direct effect of melatonin on Syrian hamster testes: melatonin subtype 1a receptors inhibition of androgen production, and interaction with the local corticotropin-releasing hormone system. Endocrinology 146, 1541–1552.
Garrett, J.W., Campbell, C.S., 1980. Changes in social behavior of the male golden hamster accompanying photoperiodic changes in reproduction. Horm. Behav. 14, 303–319.
Gaston, S., Menaker, M., 1967. Photoperiodic control of the hamster testis. Science 158, 925–928.
Goldman, B.D., 2001. Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. J. Biol. Rhythm. 16, 283–301.
Gorman, M.R., Zucker, I., 1995. Testicular regression and recrudescence without subsequent photorefractoriness in Siberian hamsters. Am. J. Physiol. Regul. Integr. Comp. Physiol. 269, R800–R806.
Gorman, M.R., Zucker, I., 1997. Environmental induction of photon on responsiveness in the Siberian hamster, Phodopus sungorus. Am. J. Physiol. Regul. Integr. Comp. Physiol. 272, R887–R895.
Greives, T.J., French, S.S., Zysling, D.A., Garcia, N.W., Demas, G.E., 2010. The glutamate agonist NMDA blocks gonadal regression and enhances antibody response to an immune challenge in Siberian hamsters (*Phodopus sungorus*). J. Comp. Physiol. B: Biochem. Mol. Biol. 180, 267–277.
Gutzler, S.J., Karom, M., Erwin, W.D., Albers, H.E., 2009. Photoperiodic regulation of adrenal hormone secretion and aggression in female Syrian hamsters. Horm. Behav. 56, 481–489.
Haller, J., Kruk, M.R., 2003. Neuroendocrine stress responses and aggression. In: Mattson, M.P. (Ed.), Neurobiology of Aggression: Understanding and Preventing Violence. Humana Press, New Jersey, pp. 93–118.
Hau, M., Stoddard, S.T., Soma, K.K., 2004. Territorial aggression and hormones during the non-breeding season in a tropical bird. Horm. Behav. 45, 40–49.
Heimovics, S.A., Fokidis, H.B., Soma, K.K., 2013. Brain aromatase and territorial aggression across the seasons in male song sparrows. In: Balthazart, J., Ball, G.F. (Eds.), Brain, Aromatase, Estrogens and Behavior. Oxford University Press, Oxford, pp. 199–220.
Heimovics, S.A., Ferris, J.K., Soma, K.K., 2015a. Non-invasive administration of 17β-estradiol rapidly increases aggressive behavior in non-breeding, but not breeding, male song sparrows. Horm. Behav. 69, 31–38.
Heimovics, S.A., Trainor, B.C., Soma, K.K., 2015b. Rapid effects of estradiol on aggression in birds and mice: the fast and the furious. Integr. Comp. Biol. 55, 281–293.
Heimovics, S.A., Prior, N.H., Ma, C., Soma, K.K., 2016. Rapid effects of an aggressive interaction on dehydroepiandrosterone, testosterone and oestradiol levels in the male song sparrow brain: a seasonal comparison. J. Neuroendocrinol. 28, 12345.
Heimovics, S.A., Merritt, J.R., Jalabert, C., Ma, C., Maney, D.L., Soma, K.K., 2018. Rapid effects of 17β-estradiol on aggressive behavior in songbirds: environmental and genetic influences. Horm. Behav. 104, 41–51.
Heldmaier, G., Steinlechner, S., 1981. Seasonal control of energy requirements for thermoregulation in the djungarian hamster (*Phodopus sungorus*), living in natural photoperiod. J. Compar. Physiol. B Biochem. Mol. Biol. 142, 429–437.
Hoffman, R.A., Reiter, R.J., 1965. Pineal gland: influence on gonads of male hamsters. Science 148, 1609–1611.
Hoffmann, K., 1974. Testicular involution in short photoperiod inhibited by melatonin. Naturwissenschaften 61, 364–365.
Jalabert, C., Munley, K.M., Demas, G.E., Soma, K.K., 2018. Aggressive behavior. In: Skinner, M.K. (Ed.), Encyclopedia of Reproduction, 2 ed. Academic Press: Elsevier, pp. 242–247.
Jasnow, A.M., Huhman, K.L., Bartness, T.J., Demas, G.E., 2000. Short-day increases in aggression are inversely related to circulating testosterone concentrations in male Siberian hamsters (*Phodopus sungorus*). Horm. Behav. 38, 102–110.
Jasnow, A.M., Huhman, K.L., Bartness, T.J., Demas, G.E., 2002. Short days and exogenous melatonin increase aggression of male Syrian hamsters (*Mesocricetus auratus*). Horm. Behav. 42, 13–20.
Jolliffe, I.T., Cadima, J., 2016. Principal component analysis: a review and recent developments. Phil. Trans. R. Soc. A 374, 20150202.
Kalsbeek, A., van der Spek, R., Lei, J., Endert, E., Buijs, R.M., Fliers, E., 2012. Circadian rhythms in the hypothalamo–pituitary–adrenal (HPA) axis. Mol. Cell. Endocrinol. 349, 20–29.
Kaufmann, J.H., 1983. On the definitions and functions of dominance and territoriality. Biol. Rev. Camb. Philos. Soc. 58, 1–20.
Kramer, K.M., Simmons, J.L., Freeman, D.A., 2008. Photoperiod alters central distribution of estrogen receptor α in brain regions that regulate aggression. Horm. Behav. 53, 358–365.
Kumar, V., Wingfield, J.C., Dawson, A., Ramenofsky, M., Rani, S., Bartell, P., 2010. Biological clocks and regulation of seasonal reproduction and migration in birds. Physiol. Biochem. Zool. 83, 827–835.
Labrie, F., Luu-The, V., Bélanger, A., Lin, S.X., Simard, J., Pelletier, G., Labrie, C., 2005. Is dehydroepiandrosterone a hormone? J. Endocrinol. 187 (2), 169–196.
Laredo, S.A., Orr, V.N., McMackin, M.Z., Trainor, B.C., 2014a. The effects of exogenous melatonin and melatonin receptor blockade on aggression and estrogen-dependent gene expression in male California mice (*Peromyscus californicus*). Physiol. Behav. 128, 86–91.
Laredo, S.A., Villalon Landeros, R., Trainor, B.C., 2014b. Rapid effects of estrogens on behavior: environmental modulation and molecular mechanisms. Front. Neuroendocrinol. 35, 447–458.

Long, K.L.P., Bailey, A.M., Greives, T.J., Legan, S.J., Demas, G.E., 2018. Endotoxin rapidly desensitizes the gonads to kisspeptin-induced luteinizing hormone release in male Siberian hamsters (*Phodopus sungorus*). J. Exp. Biol. 221, jeb185504.
Lynch, G.R., Lynch, C.B., Kliman, R.M., 1989. Genetic analyses of photoresponsiveness in the Djungarian hamster, *Phodopus sungorus*. J. Compa. Physiol. A Neuroethol. Sens. Neur. Behav. Physiol. 164, 475–481.
Matsumoto, T., Honda, S., Harada, N., 2003. Alteration in sex-specific behaviors in male mice lacking the aromatase gene. Neuroendocrinology 77, 416–424.
McGuire, N.L., Kangas, K., Bentley, G.E., 2011. Effects of melatonin on peripheral reproductive function: regulation of testicular GnIH and testosterone. Endocrinology 152, 3461–3470.
Merritt, J.R., Davis, M.T., Jalabert, C., Libecap, T.J., Williams, D.R., Soma, K.K., Maney, D.L., 2018. Rapid effects of estradiol on aggression depend on genotype in a species with an estrogen receptor polymorphism. Horm. Behav. 98, 210–218.
Munley, K.M., Rendon, N.M., Demas, G.E., 2018. Neural androgen synthesis and aggression: insights from a seasonally breeding rodent. Front. Endocrinol. 9, 136.
Nelson, R.J., 2006. Biology of Aggression. Oxford University Press, New York.
Newman, A.E., Soma, K.K., 2009. Corticosterone and dehydroepiandrosterone in songbird plasma and brain: effects of season and acute stress. Eur. J. Neurosci. 29, 1905–1914.
Newman, A.E., Pradhan, D.S., Soma, K.K., 2008. Dehydroepiandrosterone and corticosterone are regulated by season and acute stress in a wild songbird: jugular versus brachial plasma. Endocrinology 149, 2537–2545.
Nicolas, L.B., Pinoteau, S., Papot, S., Routier, S., Guillaumet, G., Mortaud, S., 2001. Aggressive behavior induced by the steroid sulfatase inhibitor COUMATE and by DHEAS in CBA/H mice. Brain Res. 922, 216–222.
Ottenweller, J.E., Tapp, W.N., Pitman, D.L., Natelson, B.H., 1987. Adrenal, thyroid, and testicular hormone rhythms in male golden hamsters on long and short days. Am. J. Physiol. Reg. Integr. Compar. Physiol. 253, R321–R328.
Pradhan, D.S., Yu, Y., Soma, K.K., 2008. Rapid estrogen regulation of DHEA metabolism in the male and female songbird brain. J. Neurochem. 104, 244–253.
Pradhan, D.S., Newman, A.E.M., Wacker, D.W., Wingfield, J.C., Schlinger, B.A., Soma, K.K., 2010. Aggressive interactions rapidly increase androgen synthesis in the brain during the non-breeding season. Horm. Behav. 57, 381–389.
Prendergast, B.J., Nelson, R.J., Zucker, I., 2003. Mammalian seasonal rhythms: behavior and neuroendocrine substrates. In: Pfaff, D.W. (Ed.), Hormones, Brain and Behavior. Academic Press, New York, pp. 93–156.
Puchalski, W., Lynch, G.R., 1986. Evidence for differences in the circadian organization of hamsters exposed to short day photoperiod. J. Compar. Physiol. A Neuroethol. Sens. Neur. Behav. Physiol. 159, 7–11.
R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.
Rendon, N.M., Demas, G.E., 2016. Bi-directional actions of dehydroepiandrosterone and aggression in female Siberian hamsters. J. Exp. Zool. A Ecol. Genet. Physiol. 325, 116–121.
Rendon, N.M., Rudolph, L.M., Sengelaub, D.R., Demas, G.E., 2015. The agonistic adrenal: melatonin elicits female aggression via regulation of adrenal androgens. Proc. R. Soc. B Biol. Sci. 282.
Rendon, N.M., Amez, A.C., Proffitt, M.R., Bauserman, E.R., Demas, G.E., 2017. Aggressive behaviours track transitions in seasonal phenotypes of female Siberian hamsters. Funct. Ecol. 31, 1071–1081.
Richter, H.G., Torres-Farfan, C., Garcia-Sesnich, J., Abarzua-Catalan, L., Henriquez, M.G., Alvarez-Felmer, M., Gaete, F., Rehren, G.E., Seron-Ferre, M., 2008. Rhythmic expression of functional MT1 melatonin receptors in the rat adrenal gland. Endocrinology 149, 995–1003.
Rosenthal, R., 1994. Parametric measures of effect size. In: Cooper, H., Hedges, L.V. (Eds.), The Handbook of Research Synthesis. Russell Sage Foundation, New York, NY, pp. 231–244.
Schlinger, B.A., Callard, G.V., 1990. Aromatization mediates aggressive behavior in quail. Gen. Comp. Endocrinol. 79, 39–53.
Scotti, M.A., Place, N.J., Demas, G.E., 2007. Short-day increases in aggression are independent of circulating gonadal steroids in female Siberian hamsters (*Phodopus sungorus*). Horm. Behav. 52, 183–190.
Scotti, M.A., Belén, J., Jackson, J.E., Demas, G.E., 2008. The role of androgens in the mediation of seasonal territorial aggression in male Siberian hamsters (*Phodopus sungorus*). Physiol. Behav. 95, 633–640.
Scotti, M.A., Schmidt, K.L., Newman, A.E., Bonu, T.N., Soma, K.K., Demas, G.E., 2009. Aggressive encounters differentially affect serum dehydroepiandrosterone and testosterone concentrations in male Siberian hamsters (*Phodopus sungorus*). Horm. Behav. 56, 376–381.
Scotti, M.A., Rendon, N.M., Greives, T.J., Romeo, R.D., Demas, G.E., 2015. Short-day aggression is independent of changes in cortisol or glucocorticoid receptors in male Siberian hamsters (*Phodopus sungorus*). J. Exp. Zool. A Ecol. Genet. Physiol. 323, 331–341.
Skinner, D.C., Robinson, J.E., 1995. Melatonin-binding sites in the gonadotroph-enriched zona tuberalis of ewes. Journal of Reproductive Fertility 104, 243–250.
Soini, H.A., Wiesler, D., Apfelbeck, R., König, P., Vasilieva, N.Y., Novotny, M.V., 2005. Comparative investigation of the volatile urinary profiles in different *Phodopus* hamster species. J. Chem. Ecol. 31, 1125–1143.
Soma, K.K., 2006. Testosterone and aggression: Berthold, birds and beyond. J. Neuroendocrinol. 18, 543–551.
Soma, K.K., Wingfield, J.C., 1999. Endocrinology of aggression in the nonbreeding season. In: Adams, N.J., Slotow, R.H. (Eds.), 22nd International Ornithological Congress. BirdLife, South Africa, Johannesburg, pp. 1606–1620.
Soma, K.K., Wingfield, J.C., 2001. Dehydroepiandrosterone in songbird plasma: seasonal regulation and relationship to territorial aggression. Gen. Comp. Endocrinol. 123, 144–155.
Soma, K.K., Wissman, A.M., Brenowitz, E.A., Wingfield, J.C., 2002. Dehydroepiandrosterone (DHEA) increases territorial song and the size of an associated brain region in a male songbird. Horm. Behav. 41, 203–212.
Soma, K.K., Schlinger, B.A., Wingfield, J.C., Saldanha, C.J., 2003. Brain aromatase, 5α-reductase, and 5β-reductase change seasonally in wild male song sparrows: relationship to aggressive and sexual behavior. J. Neurobiol. 56, 209–221.
Soma, K.K., Alday, N.A., Hau, M., Schlinger, B.A., 2004. Dehydroepiandrosterone metabolism by 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase in adult zebra finch brain: sex difference and rapid effect of stress. Endocrinology 145, 1668–1677.
Soma, K.K., Scotti, M.A., Newman, A.E., Charlier, T.D., Demas, G.E., 2008. Novel mechanisms for neuroendocrine regulation of aggression. Front. Neuroendocrinol. 29, 476–489.
Soma, K.K., Rendon, N.M., Boonstra, R., Albers, H.E., Demas, G.E., 2015. DHEA effects on brain and behavior: insights from comparative studies of aggression. J. Steroid Biochem. Mol. Biol. 145, 261–272.
Stetson, M.H., Tay, D.E., 1983. Time course of sensitivity of golden hamsters to melatonin injections throughout the day. Biol. Reprod. 29, 432–438.
Trainor, B.C., Martin, L.B., Greiwe, K.M., Kulhman, J.R., Nelson, R.J., 2006. Social and photoperiod effects on reproduction in five species of Peromyscus. Gen. Comp. Endocrinol. 148, 252–259.
Trainor, B.C., Lin, S., Finy, M.S., Rowland, M.R., Nelson, R.J., 2007a. Photoperiod reverses the effects of estrogens on male aggression via genomic and nongenomic pathways. Proceedings of the National Academy of Sciences USA 104, 9840–9845.
Trainor, B.C., Rowland, M.R., Nelson, R.J., 2007b. Photoperiod affects estrogen receptor α, estrogen receptor β and aggressive behavior. Eur. J. Neurosci. 26, 207–218.
Trainor, B.C., Finy, M.S., Nelson, R.J., 2008. Rapid effects of estradiol on male aggression depend on photoperiod in reproductively non-responsive mice. Horm. Behav. 53, 192–199.
Tsutsui, K., Ukena, K., Usui, M., Sakamoto, H., Takase, M., 2000. Novel brain function: biosynthesis and actions of neurosteroids in neurons. Neurosci. Res. 36, 261–273.
Van Duyse, E., Pinxten, R., Darras, V.M., Arckens, L., Eens, M., 2004. Opposite changes in plasma testosterone and corticosterone levels following a simulated territorial challenge in male great tits. Behaviour 141, 451–467.
von Gall, C., Stehle, J.H., Weaver, D.R., 2002. Mammalian melatonin receptors: molecular biology and signal transduction. Cell Tissue Res. 309, 151–162.
Wacker, D.W., Khalaj, S., Jones, L.J., Champion, T.L., Davis, J.E., Meddle, S.L., Wingfield, J.C., 2016. Dehydroepiandrosterone heightens aggression and increases androgen receptor and aromatase mRNA expression in the brain of a male songbird. J. Neuroendocrinol. 28.
Wade, G.N., Bartness, T.J., 1984. Effects of photoperiod and gonadectomy on food intake, body weight and body composition in Siberian hamsters. Am. J. Physiol. Reg. Integr. Compar. Physiol. 246, R26–R30.
Walton, J.C., Weil, Z.M., Nelson, R.J., 2011. Influence of photoperiod on hormones, behavior, and immune function. Front. Neuroendocrinol. 32, 303–319.
Weaver, D.R., Liu, C., Reppert, S.M., 1996. Nature's knockout: the Mel1b receptor is not necessary for reproductive and circadian responses to melatonin in Siberian hamsters. Mol. Endocrinol. 10, 1478–1487.
Webb, S.J., Geoghegan, T.E., Prough, R.A., 2006. The biological actions of dehydroepiandrosterone involves multiple receptors. Drug Metab. Rev. 38, 89–116.
Wendler, A., Baldi, E., Harvey, B.J., Nadal, A., Wehling, M., 2010. Position paper: rapid responses to steroids: current status and future prospects. Eur. J. Endocrinol. 162, 825–830.
Whittier, J.M., Crews, D., 1987. Seasonal reproduction: patterns and control. In: Norris, D.O., Jones, R.E. (Eds.), Hormones and Reproduction in Fishes, Amphibians, and Reptiles. Springer, Boston, MA, pp. 385–409.
Williams, L.M., Lincoln, G.A., Mercer, J.G., Barrett, P., Morgan, P.J., Clarke, I.J., 1997. Melatonin receptors in the brain and pituitary gland of hypothalamo-pituitary disconnected Soay rams. J. Neuroendocrinol. 9, 639–643.
Williams, B., Onsman, A., Brown, T., 2010. Exploratory factor analysis: a five-step guide for novices. Australasian Journal of Paramedicine 8.
Witt-Enderby, P.A., Bennett, J., Jarzynka, M.J., Firestine, S., Melan, M.A., 2003. Melatonin receptors and their regulation: biochemical and structural mechanisms. Life Sci. 72, 2183–2198.
Wommack, J.C., Delville, Y., 2003. Repeated social stress and the development of agonistic behavior: individual differences in coping responses in male golden hamsters. Physiol. Behav. 80, 303–308.
Wood, S., Loudon, A., 2014. Clocks for all seasons: unwinding the roles and mechanisms of circadian and interval timers in the hypothalamus and pituitary. J. Endocrinol. 222, R39–R59.
Wynne-Edwards, K.E., 2003. From dwarf hamster to daddy: the intersection of ecology, evolution, and physiology that produces paternal behavior. Advances in the Study of Behavior 32, 207–261.
Wynne-Edwards, K.E., Surov, A.V., Telitzina, A.Y., 1992. Field studies of chemical signalling: direct observations of dwarf hamsters (Phodopus) in soviet Asia. In: Doty, R.L., Müller-Schwarze, D. (Eds.), Chemical Signals in Vertebrates. Springer, pp. 485–491.