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Short-day increases in aggression are independent of circulating gonadal steroids in female Siberian hamsters (*Phodopus sungorus*)

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Abstract

Among the suite of adaptations displayed by seasonally-breeding rodents, individuals of most species display reproductive regression and concomitant decreases in gonadal steroids during the winter. In addition, some species display increased aggression in short "winter-like" days compared with long "summer-like" day lengths. For example, male Syrian and Siberian hamsters held in short days express heightened levels of aggression that are independent of gonadal steroids. Virtually nothing is known, however, regarding seasonal aggression in female Siberian hamsters (*Phodopus sungorus*). Studies were undertaken to determine female levels of aggression in long and short days as well as the role of gonadal steroids in mediating this behavior. In Experiment 1, females were housed in long or short days for 10 weeks and resident–intruder aggression was assessed. Prior to testing, estrous cycle stages were determined by vaginal cytology and females were tested during both Diestrus I and Proestrus. In Experiment 2, hormone levels were experimentally manipulated; long-day females were ovariectomized (OVx) or given sham surgeries whereas short-day females were implanted with capsules containing 17β-estradiol (E_2) or Progesterone (P). In Experiment 3, both long- and short-day females were ovariectomized and implanted with either an exogenous E_2 or blank capsule, or given a sham surgery. Short-day hamsters displayed increased aggression relative to long-day females. Aggression was not affected by estrous stage. There was no difference in aggression between long-day OVx and sham animals. Furthermore, neither exogenous E_2 nor P had any significant effect on aggression. These results support previous findings of increased non-breeding aggression and suggest that short-day aggression is not likely mediated by circulating levels of gonadal steroids. These results also suggest that the endocrine regulation of seasonal aggression may be similar between the sexes.

Keywords: Seasonal; Estrogen; Progesterone; Gonadal steroids; Photoperiod

Introduction

One of the most important and intensely studied social behaviors exhibited by animals is aggression. Historically, studies of aggression in mammals, and particularly laboratory rodents (e.g., rats, mice), have focused on males, the sex that typically shows the most overt agonistic behavior (reviewed in Adkins-Regan, 2005). A majority of these studies have suggested a role for the gonadal steroid testosterone (T) in the mediation of aggressive behavior (Berthold, 1849; Uhrich, 1938). For example, marked sex differences in aggression exist across many species with males displaying more aggression than females; castration reduces or eliminates this sex difference in aggression whereas exogenous T restores the behavior (e.g., Beeman, 1947; Brain and Nowell, 1969; Leshner and Moyer, 1975; Wagner et al., 1979). Further, a positive correlation between serum T levels and aggression exists in both human and non-human animals (Mazur, 1983). Collectively, these studies have established the importance of gonadal steroids in the mediation of mammalian aggressive behavior.

Much less work has focused on female aggression. This is especially true for rodents, in which females have traditionally been thought to display significantly less aggressive behavior outside of the context of maternal aggression (but see Barfield, 1984; Floody, 1983). An exception to this generality is the Syrian hamster (*Mesocricetus auratus*). Females of this species are highly aggressive towards conspecifics of either sex and dominate males in agonistic encounters (Floody, 1983). Interestingly, although this species is a long-day breeder (i.e.,

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reproductively active during the long days of summer), both sexes exhibit increases in aggression during winter, non-breeding (i.e., short-day) photoperiods when the gonads are regressed and the production of sex steroids is diminished (Badura and Nunez, 1989; Elliot and Nunez, 1991; Fleming et al., 1988; Jasnow et al., 2002).

Work in female Syrian hamsters has sought to explore the role of ovarian steroids in the mediation of the seasonal aggression. For example, aggression was examined in gonadectomized long-day females and their behavior compared with intact long-day individuals as well as intact and ovariectomized short-day females (Fleming et al., 1988). Ovariectomy (OVx) had no significant effect on aggressive behavior, although long-day OVx animals showed a trend towards increased aggression relative to intact individuals (Fleming et al., 1988). This outcome suggests that short-day induced increases in aggression are independent, or perhaps inversely related to serum levels of ovarian hormones.

Male Siberian hamsters (*Phodopus sungorus*), like Syrian hamsters (Garrett and Campbell, 1980) display increased aggression during short days (Jasnow et al., 2000). Siberian hamsters also breed in long days but express significantly heightened aggression outside of breeding condition (i.e., short days) when gonads are regressed and circulating levels of gonadal steroids are low (Jasnow et al., 2000). In fact, short-day males implanted with T display significantly *reduced* aggression relative to short-day control males receiving empty capsules, suggesting short-day aggression may be inversely related to gonadal steroids (Jasnow et al., 2000).

Aggression in female Siberian hamsters, unlike Syrian hamsters, has not been well characterized. A single published report that has explored female aggression in this species suggests that female Siberian hamsters display appreciable long-day aggression that, in some cases, is comparable to that of males (Wynne-Edwards and Lisk, 1987). For example, analysis across sex found that female attack frequency did not significantly differ from that of males, although males did chase and bite significantly more than females (Wynne-Edwards and Lisk, 1987). To our knowledge, no study has examined seasonal fluctuations in aggressive behavior in female Siberian hamsters. Furthermore, nothing is known about the hormonal control of aggressive behavior in the females of this species.

The goals of the present study were: 1) to examine resident–intruder aggression in female Siberian hamsters, 2) to determine whether female aggressive behavior changes seasonally, and 3) to examine the role of ovarian hormones in mediating aggressive behavior. In Experiment 1 we took advantage of the natural fluctuations in ovarian steroid concentrations that occur across the rodent estrous cycle. In Experiments 2 and 3, circulating levels of ovarian steroids were experimentally manipulated by a combination of ovariectomy and administration of exogenous gonadal steroids. We predicted that females housed in short days would express significantly heightened levels of aggression compared with long-day housed individuals. Additionally, we hypothesized that aggressive behavior would be independent of circulating gonadal steroid concentrations as is the case in male Siberian hamsters.

Materials and methods

Animals and housing conditions

Adult (>60 days of age) Siberian hamsters (*P. sungorus*) were obtained from our breeding colony and were group-housed at weaning. One week before the start of the experiments, hamsters were housed individually in polypropylene cages (40×20×20 cm) in colony rooms with a 24 h light: dark 16:8 cycle (lights off 1800 h EST). Temperature was kept constant at 20±2 °C and relative humidity was maintained at 50±5%. Food (Purina Rat Chow) and tap water were available *ad libitum* throughout the experiment. Additional animals were used as non-aggressive intruders during behavioral testing and were group-housed (four animals per cage) in long days (light: dark 16:8) to keep aggression to a minimum (Brain, 1972). These animals were approximately 2 months younger than experimental animals and thus, weighed less. These animals were chosen to facilitate aggression from the resident (Jasnow et al., 2000). Non-aggressive intruders were used no more than twice per night. All animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and a protocol previously approved by the Indiana University Institutional Animal Care and Use Committee (IACUC).

Experiment 1

Twenty adult female Siberian hamsters (*P. sungorus*) were individually housed under either a light:dark 16:8 h (n=10) or a light:dark 8:16 h cycle (n=10) for 10 weeks at the beginning of the experiment. At the end of 10 weeks, estrous stages were determined in long-day hamsters via vaginal cytology, and females in Diestrus I and Proestrus were tested. These two stages were chosen because they represent stages of the estrous cycle where gonadal steroids are relatively low and high, respectively. Five animals were tested (see below) both during Diestrus I and Proestrus. All animals were tested using a resident–intruder model of aggression by introducing a non-aggressive intruder into the home cage of an experimental animal for 5 min to assess territorial aggression (Jasnow et al., 2000). Intruders were in the same estrus stage as residents. Behavior was tested 2 h prior to lights-off to control for circadian rhythmicity of behavior. All trials were performed under low illumination/red light conditions, which allowed sufficient light to allow for video recording and observation without disturbing the behavior of the hamsters. Intruders were identified by small patches of shaved fur on their dorsal surfaces. To increase territorial aggression, the bedding material of experimental animals remained unchanged for at least 1 week prior to behavioral testing (Jasnow et al., 2000). Hamsters were lightly anesthetized with ether and blood samples were drawn from the retro-orbital sinus. Samples were taken from each experimental animal at least 5 h prior to behavioral testing, with sera extracted following centrifugation. Behavioral interactions were videotaped and scored using ODlog™ software (Macropod) by an observer naïve to experimental conditions. Aggressive, social and non-social behaviors were characterized. Upon completion of behavioral trials, all animals were euthanized and their gonads and uterine horns were removed, cleaned of fat and connective tissues and weighed together and reported as a single reproductive mass.

In many seasonally breeding rodents, including Siberian hamsters, there is a small subset of individuals that are non-responsive to short-day photoperiods. These photoperiodic "non-responders" do not undergo gonadal regression and generally respond physiologically and behaviorally like long-day animals. There were no non-responders among the short-day hamsters in Experiments 1 and 3. One non-responder (i.e., reproductive mass >0.100 g) was present in Experiment 2; this individual was removed from all further data analyses.

Vaginal cytology

Daily vaginal cell samples were obtained by vaginal lavage from long-day females (n=10) between 0800 and 1000 h EST to determine the stage of the estrous cycle (Drazen et al., 1999). Samples were transferred to microscope slides, fixed, stained with methylene blue and evaluated for estrous stage under 100× magnification. Stages were defined as Diestrus 1 by the presence of polymorphonuclear leucocytes and keratinized epithelial cells and Proestrus by clumps of lightly staining nucleated epithelial cells in the smears (Drazen et al., 1999).

Experiment 2

Thirty adult female Siberian hamsters were individually housed at the beginning of the experiment. A subset ($n=12$) of animals were maintained in long days whereas the remaining animals ($n=18$) were transferred to short days as described in Experiment 1. Long-day animals were further subdivided into animals that received ovariectomies ($n=6$) with the intent of mimicking short-day levels of gonadal steroids. The remaining long-day hamsters ($n=6$) received sham surgeries. In contrast, short-day females were implanted with Silastic capsules containing 17β-estradiol (E_2) ($n=6$) or progesterone (P) ($n=6$); the remaining control animals received empty capsules ($n=6$). Thus, the goal of these manipulations was to approximate short- and long-day levels of E_2 and P separately in long- and short-day hamsters, respectively (Jasnow et al., 2000). Four weeks following surgeries, animals were tested using a resident intruder model of aggression, as described in Experiment 1, with the exception that blood samples were taken approximately 24 h prior to behavioral testing.

Experiment 3

In Experiment 2, we assumed that short-day females, like males, would have regressed gonads and have low circulating levels of sex steroids. Thus, we did not include a short-day/ovariectomized (SD/OVx) group. The unexpected results of comparable levels of E_2 in short-day housed females relative to long-day individuals (see Results) required us to include a SD/OVx group in order to examine the role of ovarian-derived E_2 in mediating seasonal aggression in Siberian hamster females. Thus, the goal of Experiment 3 was to replicate Experiment 2 with a larger sample size and to account for the E_2 profiles observed in both long- and short-day females and to further investigate the role of estrogen in mediating seasonal aggression. To accomplish this, 60 adult female Siberian hamsters were individually housed at the beginning of the experiment. Half of these animals ($n=30$) were maintained in long days and the other half ($n=30$) were transferred to short days as described above. After 4 weeks, the animals in each photoperiod were equally divided into three groups: ovariectomized+empty Silastic capsule (OVx), ovariectomized+capsule packed with 17β-estradiol (OVx+E_2), and sham+empty capsule (Sham). Four weeks following surgeries, animals were tested using a resident intruder model of aggression, as described in Experiment 1, except that blood samples were taken approximately 24 h prior to behavioral trials.

Ovariectomies

Ovariectomies in Experiments 2 and 3 were performed as described previously (Demas and Nelson, 1998). Briefly, hamsters were anesthetized with 0.05 cc of a ketamine (20mg/ml)/ xylazine (4mg/ml) cocktail in 0.09% saline. Small (~2 cm) bilateral incisions were made in the animals' dorsal surface, the ovaries removed, and the ovarian arteries tied off to prevent excessive bleeding. The abdominal wall was sutured closed and the skin incision was closed with 9 mm wound clips. The wounds were treated with nitrofurazone antibacterial powder (2% nitrofurazone; Ken Vet™). Hamsters undergoing sham ovariectomies received a similar procedure, except the ovaries were visualized only prior to closing. Upon completion of the behavior trials, all animals were euthanized and the gonads and uterine horns of intact animals were removed and weighed together (i.e., reproductive masses).

Hormonal manipulations

E_2 (Experiments 2 and 3) and P (Experiment 2) were administered via Silastic capsule implants. E_2 implants consisted of a 10 mm length of Silastic tubing (Dow Corning, 1.47 mm i.d.; 1.95 mm o.d.) sealed at both ends with medical grade Silastic sealant (Dow Corning) and filled with crystalline E_2 (Sigma #E8875). P implants consisted of a 25 mm length of Silastic tubing sealed at both ends with medical grade Silastic sealant and filled with crystalline P (Sigma #P0130). The implants were inserted subcutaneously via a small incision in the skin on the interscapular region of the hamsters' dorsum. The wound was closed using 9 mm wound clips and the wounds were treated with nitrofurazone antibacterial powder.

Behavioral testing and scoring

A resident–intruder model of aggression was used, consisting of the placement of a non-aggressive long-day female hamster into the home cage of a singly housed resident/experimental animal for 5 min (Jasnow et al., 2000). Intruder animals were adult females younger and smaller than residents/experimental animals. The following behaviors were scored over the 5 min testing period: aggressive behaviors (i.e., chasing and attacking), investigative behavior (i.e., ano-genital investigation) and maintenance behavior (i.e., grooming). We defined attack as physical contact between the resident and intruder that was initiated by the resident and resulted in biting and/or pinning. We considered an interaction a chase when the resident pursued a fleeing intruder eventually resulting in an attack. Ano-genital investigation was scored as the resident sniffing the ano-genital region of the intruder. In addition to the number of attacks, the latency to initial attack was also quantified (Jasnow et al., 2000).

Hormone assays

In Experiments 1 and 2 total serum E_2 (Experiments 1 and 2) was measured in duplicate samples using an EIA kit produced by Cayman Chemicals (Ann Arbor, MI). The minimum detection for this assay is 8 pg/ml. Serum samples were diluted 1:2 with assay buffer. Samples for the two studies were run on separate plates. The intra-assay variable for these assays was 3.49% and 5.26%. Due to limited availability of serum, we were unable to measure serum P.

Because E_2 concentrations measured in short-day females in Experiments 1 and 2 were counterintuitive (i.e., uterine size was significantly smaller in short-day than in long-day females, yet E_2 levels in short-days were comparable to or even higher than levels measured in long-days) we elected to measure the concentration of E_2 by an alternate method in Experiment 3. We sent samples to the Diagnostic Endocrinology Laboratory at the Animal Health Diagnostic Center, Cornell University, where radioimmunoassays (RIA) for E_2 are performed on a variety of species on a weekly basis. The RIA follows an extraction of steroids from serum samples with diethyl ether, which would serve to remove elements of the serum that may falsely elevate E_2 values in samples from short-day females. Prior to running samples from Experiment 3, we validated the RIA for Siberian hamsters. A pooled serum sample from five female hamsters was serial diluted in assay buffer (PBS-BSA) after spiking with an 1000 pg/ml standard at a 2:1 ratio (hamster serum: standard). The E_2 concentration of the spiked sample was only 6.6% greater than expected and the serial dilution was parallel to the standard curve, except for a modest deviation at the lowest end of the curve (<40 pg/ml).

The RIA used to measure E_2 concentrations in serum samples from Experiment 3 is a modified solid-phase ^{125}I kit (Diagnostic Products Corporation, Los Angeles, CA). Following the addition of ^{3}H estradiol (50 μl, ~1900 cpm) to determine extraction efficiencies, serum samples (165–300 μl) were extracted in diethyl ether, dried under N_2, and reconstituted in 335 μl of assay buffer. A 100 μl aliquot was counted on a scintillation counter to calculate recoveries for ^{3}H–E_2 and separate 100 μl aliquots were added to Coat-A-Count® tubes in duplicate for RIA. The cross-reactivity of the highly specific antibody to other estrogens is less than 2%, save for estrone (10%). A standard curve (15.6–1000 pg/ml) was made by serially diluting an E_2 stock solution in assay buffer. Tubes were incubated at room temperature (23 °C) for 3 h following the addition of ^{125}I estradiol tracer. Tubes were aspirated of their contents then counted in a gamma counter. Volume and percent extraction recovery specific to each sample were used to calculate concentrations interpolated from the standard curve. All samples were run in a single assay, which met all quality assurance criteria, and internal controls (bovine, equine, canine) were run at the beginning, middle and end of the assay. The intra-assay coefficient of variation was 7.8% and the minimum detectable limit of the assay was 18 pg/ml. Samples with E_2 values above or below the assay's limits were assigned the upper or lower limits for the assay.

To compare the E_2 values measured in the EIA to those from the RIA, we also ran eight different pooled serum samples in both assays. While the absolute values were generally higher in the EIA than the RIA, the correlation between methods was significant ($r=0.823$, $p=0.009$).

Statistical analyses

Comparisons between estrous stages (Experiment 1) were conducted using a linear mixed model to take into account individuals that were tested more than once. All other analyses was performed using a general linear model (GLM) followed by Tukey's post hoc analysis. Data were log transformed when the assumptions of equal variance or normality were violated. Differences in group means were considered significant if $p<0.05$.

Results

Experiment 1

Females housed in short days displayed significantly more attacks than did those housed in long days ($F_{1,19}=14.063$, $p=0.002$) (Fig. 1A). There was no effect of estrous stage on attack number ($F_{1,\ 6.933}=0.46$, $p=>0.05$) (Fig. 1A). There was no significant difference in latency to first attack, number of chases, ano-genital investigations, or grooming bouts between any of the groups ($p>0.05$) (Table 1).

Animals housed in short days had significantly reduced reproductive (i.e., paired ovaries and uterine horns) masses ($F_{1,19}=20.660$, $p<0.001$) (Fig. 1B). Females tested during late Proestrus had relatively higher levels of serum E_2 relative to females tested during Diestrus I ($F_{1,4.127}=5.573$, $p=0.076$) (Table 2). Unexpectedly, short day housed individuals had significantly higher levels of E_2 than did individuals housed in long days ($F_{1,19}=15.288$, $p=0.001$) (Table 2). A Tukey's post hoc test showed, however, that short-day individuals had higher levels of E_2 than long day individuals tested in Diestrus 1 ($p<0.001$) but not those housed in Proestrus ($p>0.05$).

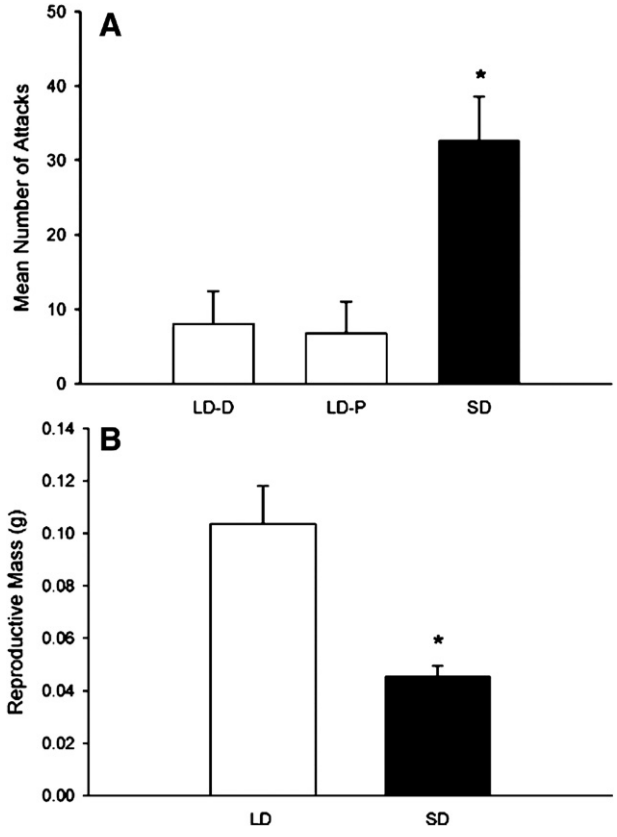

Fig. 1. Mean (+S.E.M.) number of attacks. (A) Long-day animals in Diestrus 1 (LD-D) and Proestrus (LD-P) and SD animals. (B) Reproductive mass (ovaries plus uterine horns) in grams. An asterisk (*) denotes statistically significant difference at $p<0.05$.

Table 1
Mean (±S.E.M.) social (chases, ano-genital investigations, attacks) and non-social (groom bouts) behaviors in long-day (LD) or short-day (SD) female Siberian hamsters that were in Diestrus I or Proestrus or that were ovariectomized (OVx) or received sham surgeries (Sham) and implanted with Silastic capsules containing 17β-estradiol (E_2) or progesterone (P)

	Treatment	Chases	Groom bouts	Ano-genital investigations	Latency to initial attack (s)
Experiment 1	LD/ Diestrus	0.2±0.2	5.0±1.2	7.7±2.0	121.30±29.0
	LD/ Proestrus	0	5.9±3.5	3.8±1.1	168.0±52.8
	SD	0.6±0.4	4.2±1.0	3.9±1.3	53.9±23.2
Experiment 2	LD/Sham	0	12.2±3.2	19.0±3.5	76.1±13.2
	LD/OVx	1.6±1.0	10.8±2.0	18.0±3.8	134.4±47.7
	SD	0.6±0.4	11.6±3.0	15.4±3.3	35.8±9.7
	SD+E_2	0.8±0.5	10.5±3.5	18.3±3.8	96.8±41.6
	SD+P	2.3±1.6	13.2±2.2	14.5±3.1	88.3±19.2
Experiment 3	LD/Sham	0.1±0.1	4.0±0.8	6.9±2.0	111.1±37.5
	LD/OVx	0.8±0.6	2.6±0.6	5.6±2.1	103.0±31.5
	LD/ OVx+E_2	0.1±0.1	5.0±1.6	8.6±1.6	121.3±35.8
	SD/Sham	5.2±3.3	4.3±1.8	6.4±2.9	110.4±38.1
	SD/OVx	4.0±1.9	3.1±0.9	7.0±2.6	68.1±20.7
	SD/ OVx+E_2	0.3±0.2	4.6±1.0	7.9±1.7	97.2±15.1

Experiment 2

There was a significant treatment effect on total attack number ($F_{4,23}=2.970$, $p=0.023$). Post hoc analysis showed that the only treatment groups that differed significantly in attack number were the long-day sham animals and the short-day housed animals that received empty Silastic capsules ($p=0.030$) (Fig. 2A). Interestingly, short-day housed females that received exogenous E_2 tended to be less aggressive than short-day controls ($p=0.061$). There was no significant effect of treatment or photoperiod on latency to first attack, chase number, chase duration, number of grooming bouts, duration of grooming, duration of ano-genital investigations or number of ano-genital

Table 2
Mean (±S.E.M.) serum E2 in long-day (LD) or short-day (SD) female Siberian hamsters that were in Diestrus I or Proestrus or that were ovariectomized (OVx) or received sham surgeries (Sham) and implanted with Silastic capsules containing 17β-estradiol (E2) or progesterone (P)

	Treatment	Serum 17β-estradiol (pg/ml)
Experiment 1	LD/Diestrus	49.1±8.17
	LD/Proestrus	82.2±20.6
	SD	118.0±21.1*
Experiment 2	LD/Sham	22.9±5.7
	LD/OVx	51.7±11.3
	SD	110.1±19.3**
	SD+E_2	198.2±37.6***
	SD+P	54.2±12.6

*=different from LD/Diestrus.
**=different from LD/Sham.
***=different from all except SD.

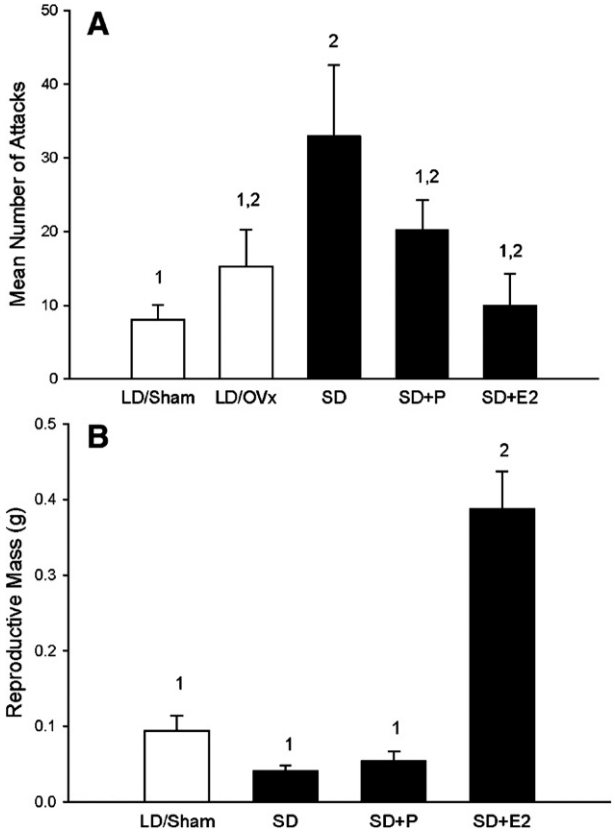

Fig. 2. Mean (+S.E.M.) (A) number of attacks for long-day sham (LD/Sham), long-day ovariectomized (LD/OVx), short-day control (SD), short-day plus progesterone (SD+P) and short-day plus 17β-estradiol (SD+E2). (B) Reproductive mass (ovaries plus uterine horns) in grams. Bars sharing at least one same number are statistically equivalent. Bars with different numbers are statistically different at $p<0.05$.

investigations (Table 1). There was a significant effect of treatment on reproductive organ weight ($F_{3,22}$= 19.865, $p<0.001$) (Fig. 2B). Post_hoc analysis found that estrogen treated animals had significantly heavier reproductive weights than short-day controls, short-day animals treated with P, and long-day sham animals ($p<0.001$ in all cases).

There was a main effect of treatment on serum E_2 concentrations ($F_{4,27}=9.623$, $p<0.001$). However, long-day females that were OVx did not have significantly lower levels of serum E_2 than did long-day housed animals that received sham surgeries ($p>0.05$) (Table 2). Long-day intact animals did not have significantly different levels than any of the other groups, save for short-day individuals that received exogenous E_2 and short-day control individuals ($p<0.001$ and $p=0.002$ respectively). Short-day females with E_2-implants had significantly higher levels of E_2 than all other groups except short-day control animals ($p>0.05$) (Table 2).

Experiment 3

Unlike Experiments 1 and 2 there was no effect of photoperiod on attack number ($F_{1,54}=2.374$, $p>0.05$), however, the mean number of attacks in SD and LD differed in the same direction and magnitude as in the previous experiments. There was no effect of treatment or photoperiod on ano-genital investigation, or grooming bouts ($p>0.05$) (Table 1). There was no effect of treatment on number of chases, however, there was a photoperiod effect. Short-day animals chased significantly more than long-day animals ($F_{1,56}=5.383$ $p=0.024$). There was a main effect of treatment on attack number ($F_{2,55}=3.643$, $p=0.034$). Overall, OVx individuals were significantly more aggressive than OVx+E_2 animals ($p=0.025$) (Fig. 3A).

There was a significant effect of photoperiod on reproductive weights, with long-day sham females having significantly higher reproductive weights than short-day sham females ($F_{1,18}=$ 25.072, $p<0.001$). In animals that received ovariectomies, there was both an effect of photoperiod and treatment on paired uterine horn masses. Not surprisingly, animals treated with E_2 had heavier uterine horn masses than did those that received empty capsules ($F_{1,35}=325.655$ $p<0.001$).

There was no effect of photoperiod on serum E_2 levels ($p>0.05$) (Fig. 3B). There was however, an overall effect of treatment ($F_{2,\ 52}=234.813$, $p<0.001$). Pair-wise comparisons found that OVx+E_2 animals in both photoperiods had higher levels of E_2 than did either OVx or Sham animals ($p<0.001$).

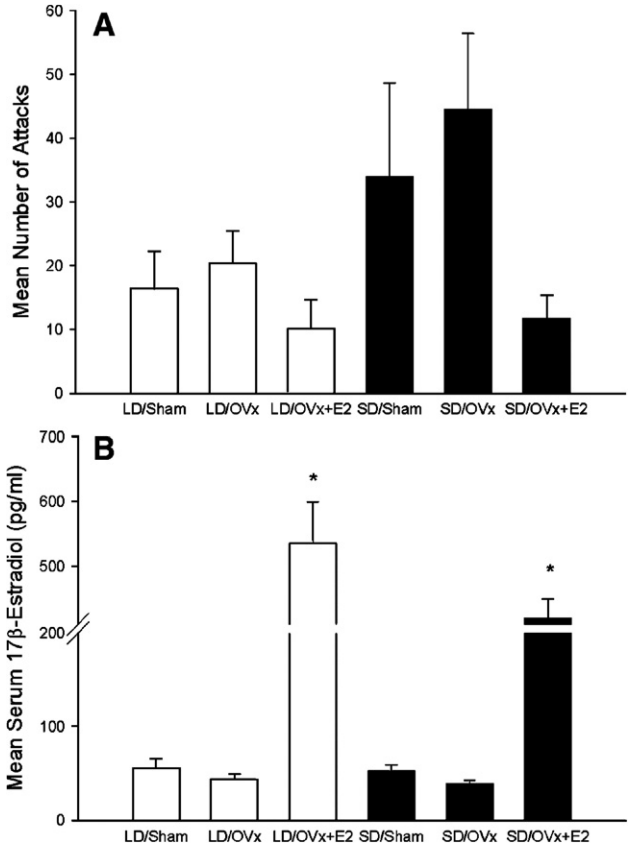

Fig. 3. Mean (+S.E.M.) (A) number of attacks for long-day sham (LD/Sham), long-day ovariectomized (LD/OVx), long-day ovariectomized plus 17β-estradiol (LD/OVx+E2), short-day sham (SD/Sham), short-day ovariectomized (SD/OVx) and short-day ovariectomized plus 17β-estradiol (SD/OVx+E2). (B) Serum 17β-estradiol. An asterisk (*) denotes statistically significant differences at $p<0.05$ for comparisons within photoperiod.

Sham and OVx animals did not differ in their mean serum E_2 levels ($p > 0.05$) in either photoperiod (Fig. 3B).

Discussion

The principal goal of this study was to characterize the aggressive behavior of long- and short-day housed female Siberian hamsters. In the present study, female hamsters, unlike some other rodent species, displayed appreciable amounts of aggression toward same-sex conspecific intruders. Furthermore, in all experiments, female hamsters housed in short days displayed increased aggression compared with long-day housed animals, consistent with previous reports in males of this species (Jasnow et al., 2000, 2002), as well as both males and females of another hamster species, Syrian hamsters (Badura and Nunez, 1989; Fleming et al., 1988).

In general, the aggressive behavior of female rodents has not been explored outside of the context of offspring defense. To our knowledge, a single account of the aggressive behavior of female Siberian hamsters has been published (Wynne-Edwards and Lisk, 1987), and this report suggests that female Siberian hamsters display significant amounts of aggression while in breeding condition. For example, although males showed significantly greater numbers of chases and bites than did females, female attacked at the same frequency as did males (Wynne-Edwards and Lisk, 1987). Our data similarly found that females exhibited appreciable amounts of aggression during the breeding season. Additionally, we hypothesized that females, like the males of this species, would show increases in aggressive behavior when housed under a short-day photoperiod. This hypothesis was generally supported. These findings are consistent with data from Syrian hamster females (Garrett and Campbell, 1980; Badura and Nunez, 1989; Fleming et al., 1988).

The aggression displayed in male Siberian hamsters is thought to be uncoupled from or perhaps inversely related to gonadal steroid concentrations (Jasnow et al., 2000). Therefore, we hypothesized that female aggression would show a similar independence from gonadal steroids. In all experiments our hypothesis was supported. In Experiment 1, there was no difference in the aggressive behavior of animals tested during Diestrus 1 (when serum concentrations of the gonadal steroids are relatively low) and animals tested in late Proestrus (when serum E_2 was twice the level of Proestrus animals). Additionally, OVx had no significant effect on aggression in long-day housed animals, although OVx individuals were marginally more aggressive than control animals.

The administration of either P or E_2 to short-day housed individuals had no significant effect on aggressive behavior; however, E_2-treated individuals were relatively less aggressive than short-day control individuals. In all cases it seems aggressive behavior may not be dependent on circulating levels of gonadal steroids. The non-significant trend toward decreased aggression in E_2 treated females is suggestive of an inverse relationship between this hormone and aggressive behavior. Although this possibility can not be ruled out, females housed in a short-day photoperiod had levels of E_2 that were not significantly lower than females housed in long days suggesting that changes in circulating levels of E_2 are not likely mediating changes in aggressive behavior. This finding was unexpected, especially in light of the smaller uterine size (i.e., reproductive mass) in short-day animals, however, a similar finding has been previously reported in this species when hamsters are gestated in short days (van den Hurk et al., 2002). Our intended goal of administering exogenous E_2 was to elevate presumably low circulating levels of the hormone in short-day hamsters to levels comparable to long-day animals; the E_2-implanted animals, however, appear to have been exposed to pharmacological levels of the E_2, due to their already high E_2 concentrations. To control for this, in Experiment 3 we ovariectomized both LD and SD animals, however, once again our E_2-replaced individuals were found to have supra-physiological levels of E_2. Interestingly, in both experiments in which ovariectomies were performed, removal of the gonads had no significant effect on circulating E_2 levels. This could be due to a "floor effect" in that the assays were not sensitive enough to detect small decreases in circulating E_2. Alternatively, these results suggest the possibility for other non-gonadal (e.g., neurosteroid) sources of E_2 production in female Siberian hamsters. Furthermore, in this study single doses of exogenous E_2 and P were administered separately; thus, the effects of differing doses of these hormones, both alone and in combination, on aggression cannot be determined from the present data. Experimental manipulations of both of these ovarian hormones in the same animals using a range of hormonal regimens in future studies will be necessary to more fully evaluate the effects of these hormones in female aggression.

There is little consensus as to the role of ovarian hormones in the mediation of non-maternal aggression either between or within rodent species. For example, OVx seems to have no effect on the aggressive behavior toward adult conspecifics in female rats (DeBold and Miczek, 1981, 1984), mice (Barkley and Goldman, 1978), and Syrian hamsters (Fleming et al., 1988). Other studies suggest that ovariectomy reduces (bank voles: Kapusta, 1998, Syrian hamsters: Payne and Swanson, 1971) or increases (Mongolian gerbils: Razzoli et al., 2003) aggression. Estrogen treatment has been shown to have no effect on the aggressive behavior of adult female mice (Barkley and Goldman, 1978) and Syrian hamsters (Floody and Pfaff, 1977), but suppresses aggressive behavior in bank voles (Lisk and Nachtigall, 1988; Razzoli et al., 2003). P, in contrast to E_2, is generally considered to have an inhibitory effect on aggressive behavior (Fraile et al., 1986), although some exceptions have been reported (Meisel and Sterner, 1989; Payne and Swanson, 1971).

The lack of effect of both removal of the gonads and administration of exogenous gonadal steroids on aggressive behavior in the present study is intriguing, but does not eliminate the possibility that gonadal steroids may still mediate increases in aggressive behavior in female Siberian hamsters. Physiological changes downstream of circulating hormones, including changes in steroid binding globulins or receptors within specific brain regions, can play an important role in the regulation of physiological and behavioral

responses (Breuner et al., 2003; Bittman and Blaustein, 1990; Ronchi et al., 1998). Consistent with this idea, photoperiodic changes in both estrogen and progestin receptor immunoreactivity have been reported in female Syrian hamsters (Mangles et al., 1998), although its relationship to aggression has not been explored.

Alternatively, previous investigations of the hormonal mediation of aggression in species that exhibit non-breeding aggression, or species in which aggression persists after gonadectomy, have considered adrenocortical steroids as potential alternative mediators of aggression (e.g., Demas et al., 2004; Hau et al., 2004; Holst and Buergel-Goodwin, 1975; Soma et al., 1999) (reviewed in Demas et al., 2007). Evidence for a role of adrenal hormones as mediators of territorial aggression in rodents, has been reported. For example, in a study of house mice, increases in aggressive behavior mediated by short-day like melatonin infusions were eliminated by the bilateral removal of the adrenal glands (Paterson and Vickers, 1981). Similarly, removal of the entire adrenal gland reduced aggressive behaviors in Siberian hamsters held in short days. Demedullation alone, which disrupts the production of catecholamines but not corticoids, however, had no effect on the melatonin-induced aggression displayed in these animals (Demas et al., 2004). These results support the hypothesis that adrenocorticoids play a role in mediating territorial aggression. However, it is not currently known which class of adrenocortical steroids (e.g., glucocorticoids or adrenal androgens) is mediating the behavior, but there is evidence suggesting the possible involvement of both types of hormones (Haug et al., 1989; Soma and Wingfield, 2001; Wommack and Delville, 2003; Van Duyse et al., 2004).

The present experiments represent an initial attempt at examining seasonal changes in aggression and the underlying hormonal mechanisms in female Siberian hamsters. Our results suggest that territorial aggression is significantly elevated in short-day hamsters compared with animals housed in long days but that this increase in aggression is not likely mediated by circulating gonadal steroids. Additional studies will be aimed at determining the precise endocrine mechanisms mediating short-day aggression in females of this species. Although this study investigated seasonal changes in aggression at the proximate level, little is known as to why Siberian hamsters display increased aggression in short days from an ultimate perspective. Information of the life history of this species is limited, although it has been postulated that increased aggression in the winter months is a necessary adaptation allowing for a rigorous defense of limited resources during the harsh winters these animal must endure in their natural habitats (Caldwell et al., 1984; Jasnow et al., 2000). Although this idea is intriguing, further studies are necessary to test it. Collectively, the present results provide the first evidence of seasonal changes in aggression in female Siberian hamsters.

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