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Adrenal hormones mediate melatonin-induced increases in aggression in male Siberian hamsters (*Phodopus sungorus*)

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Abstract

Among the suite of seasonal adaptations displayed by nontropical rodents, some species demonstrate increased territorial aggression in short compared with long day lengths despite basal levels of testosterone. The precise physiological mechanisms mediating seasonal changes in aggression, however, remain largely unknown. The goal of the present study was to examine the role of melatonin, as well as adrenal hormones, in the regulation of seasonal aggression in male Siberian hamsters (*Phodopus sungorus*). In Experiment 1, male Siberian hamsters received either daily (s.c.) injections of melatonin (15 μg/day) or saline 2 h before lights out for 10 consecutive days. In Experiment 2, hamsters received adrenal demedullations (ADMEDx), whereas in Experiment 3 animals received adrenalectomies (ADx); control animals in both experiments received sham surgeries. Animals in both experiments subsequently received daily injections of melatonin or vehicle as in Experiment 1. Animals in all experiments were tested using a resident–intruder model of aggression. In Experiment 1, exogenous melatonin treatment increased aggression compared with control hamsters. In Experiment 2, ADMEDx had no effect on melatonin-induced aggression. In Experiment 3, the melatonin-induced increase in aggression was significantly attenuated by ADx. Collectively, the results of the present study demonstrate that short day-like patterns of melatonin increase aggression in male Siberian hamsters and suggest that increased aggression is due, in part, to changes in adrenocortical steroids.

Keywords: Seasonal; Photoperiod; Pineal; Cortisol; Stress; Aromatase; Testosterone

Introduction

Individuals of many mammalian species experience potentially large fluctuations in environmental conditions across the seasons of the year (reviewed in Bronson and Heideman, 1994; Nelson et al., 1990). Consequently, numerous morphological, physiological, and behavioral responses have evolved that allow individuals to cope with changes within the environment. Although a variety of biotic (e.g., ambient temperature, food availability) and abiotic (e.g., social interactions) environmental variables fluctuate on a seasonal basis, changes in the ambient photoperiod (day length) provide the most reliable, "noise-free" cue with which to coordinate physiological and behavioral responses (Bronson and Heideman, 1994; Prendergast et al., 2003; Wingfield, 1983). Thus, individuals have evolved to utilize photoperiodic information to coordinate specific seasonal adaptations with the appropriate time of year. For example, most temperate-zone rodents maintained in short "winter-like" days (i.e., <12 h of light per day) within the laboratory undergo physiological and behavioral changes associated with winter, including gonadal regression, changes in body mass, pelage, thermoregulation, immune function and general activity (reviewed in Bartness et al., 1993; Demas, 2004; Goldman, 2001).

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The adaptive responses to changes in ambient photoperiod are mediated by a multisynaptic neural circuit originating with the perception of environmental light via retinal ganglion cells and terminating with the transduction of environmental day length information into a neuroendocrine signal within the pineal gland (reviewed in Goldman, 2001). Specifically, the pineal indole amine melatonin is secreted in abundance during darkness, whereas daylight inhibits pineal melatonin secretion (Bartness et al., 1993; Goldman, 2001). Thus, changes in ambient day length result in changes in the pattern of secretion of melatonin. In this manner, it is the precise pattern of melatonin secretion, and not the amount of hormone per se, that provides the biochemical "code" for day length (Goldman, 2001).

The majority of previous laboratory studies examining photoperiodic effects in rodents have focused primarily on physiological adaptations (e.g., reproduction, energy balance, immune function; reviewed in Bartness and Wade, 1985; Bronson and Heideman, 1994; Nelson and Demas, 1996). In addition to physiological responses, however, pronounced seasonal changes in behavior exist. Several studies have demonstrated photoperiodic changes in aggression in both male and female rodents (Badura and Nunez, 1989; Fleming et al., 1988; Garrett and Campbell, 1980; Jasnow et al., 2000). For example, male Syrian hamsters (*Mesocricetus auratus*) maintained in short days for 8 weeks undergo gonadal regression and display increases in aggression compared with long-day hamsters, despite basal serum concentrations of testosterone (T) (Garrett and Campbell, 1980). Interestingly, prolonged maintenance (i.e., >15 weeks) in short days triggers gonadal recrudescence and the short-day increases in aggressive behavior largely disappear, returning to long-day levels of aggression by 21 weeks (Garrett and Campbell, 1980). Recently, a similar pattern of aggression was reported in male Siberian hamsters (*Phodopus sungorus*). Specifically, male Siberian hamsters housed in short days (LD 8:16) for 10 weeks were more aggressive than long-day (LD 16:8) housed hamsters; after 20 weeks of short days, however, gonadal recrudescence occurred and aggression dropped to long-day levels (Jasnow et al., 2000). Similar patterns of aggression have been observed in female Syrian hamsters housed in short days (Fleming et al., 1988). In addition, exogenous T administration appears to reduce short-day-induced increases in aggression in Siberian hamsters (Jasnow et al., 2000). Interestingly however, when short-day-housed reproductively responsive and nonresponsive Siberian hamsters are compared, both groups of hamsters display elevated aggression compared with long-day animals, despite the lack of gonadal regression among individuals within the nonresponsive morph (J. Wen, A.K. Hotchkiss, G.E. Demas, R.J. Nelson, unpublished data). Collectively, these results suggest that photoperiodic changes in aggression are independent, or possibly inversely related, to circulating concentrations of gonadal steroids.

Despite the results discussed above, the precise neuroendocrine mechanisms underlying short-day increases in aggression in rodents remain largely unknown. One possible explanation for these results is that the increases in the duration of melatonin secretion occurring in short days subsequently lead to increased aggression. In support of this hypothesis, male house mice (*Mus musculus*) treated daily with exogenous melatonin for five consecutive days display significant increases in territorial aggression compared with control mice treated with saline (Paterson and Vickers, 1981). In addition, pinealectomy suppresses territorial aggression in mice (Paterson and Vickers, 1981), consistent with the results reported for female Syrian hamsters. These results are particularly intriguing given that house mice have traditionally been assumed to be photoperiodically nonresponsive (Nelson, 1990). Also, it is important to note that the dose of exogenous melatonin used in this study was supraphysiological, rendering these results more difficult to interpret. However, both pinealectomy and treatment with exogenous melatonin within species-typical physiological ranges also affect aggression in photoperiodic species. For example, pinealectomy eliminates the short-day increase in aggression in female Syrian hamsters, whereas exogenous melatonin treatment augments aggression in long-day-housed animals (Fleming et al., 1988). Short-term treatment with exogenous melatonin also increases aggression in male Syrian hamsters without altering serum T concentrations (Jasnow et al., 2002). Taken together, these results suggest an important role for melatonin in mediating photoperiodic changes in aggression in male and female hamsters.

The effects of melatonin on aggression in rodents may be due to direct actions of this hormone on neural substrates mediating aggression (e.g., hypothalamus, limbic system). Alternatively, melatonin-induced aggression may be indirectly due to changes in hypothalamo–pituitary–adrenal (HPA) activity, as adrenal hormones have been implicated in aggressive behavior (Haller and Kruk, 2003). In support of the latter hypothesis, changes in both the size and function of the adrenal gland are associated with changes in aggression. In addition, male house mice treated with melatonin display increased territorial aggression but decreased adrenal masses compared with saline-treated animals (Paterson and Vickers, 1981). The increases in aggression displayed by melatonin-treated animals, however, can be blocked by adrenalectomy (Paterson and Vickers, 1981). Experimental reductions of both adrenomedullary catecholamines, as well as glucocorticoids, are associated with decreased aggression in rodents (Crawley and Contrera, 1976; Haller and Kruk, 2003; Paterson and Vickers, 1981) and reductions of glucocorticoids via pharmacological blockade of adrenocorticotropic hormone (ACTH) release can attenuate melatonin-induced increases in aggression in mice (Paterson and Vickers, 1981). Collectively, these results suggest that the effects of exogenous melatonin on

aggression are mediated by its effects on the adrenal glands.

Given the pronounced effects of photoperiod and melatonin on aggression in Siberian hamsters, independent of T, the present study was undertaken to determine the physiological mechanisms regulating these pronounced seasonal changes in aggressive behavior. Because previous work on rodent aggression has revealed an important role for adrenal hormones, the present study investigated whether short-day melatonin signals lead to increased aggression via changes in adrenal hormones. In Experiment 1, we tested the hypothesis that short-day-like patterns of melatonin increase aggression and that increased aggression is independent of changes in gonadal steroid hormones. In Experiments 2 and 3, we tested the role of adrenomedullary and adrenocortical hormones, respectively, in melatonin-induced aggression.

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. Two weeks before the start of the experiments, hamsters were housed individually in polypropylene cages (40 × 20 × 20 cm) in colony rooms with a 24 h LD 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 nonaggressive intruders during behavioral testing and were group-housed (five animals per cage) in long days (LD 16:8) to keep aggression to a minimum (Brain, 1972). These animals were approximately the same weight as long-day experimental animals and were the same age as experimental animals. Nonaggressive intruders were used only once during behavioral testing. All animals were treated in accordance with the Indiana University Institutional Animal Care and Use Committee (IACUC).

Experiment 1: effects of exogenous melatonin on aggression

Twenty-five hamsters were used in Experiment 1. All animals were maintained in long days (LD 16:8) for the duration of the experiment. A subset of the animals ($n = 13$) was randomly selected and received daily s.c. injections of melatonin (15 μg/day in a volume of 0.1 ml; Sigma Chemical, Saint Louis, MO) dissolved in an ethanol/saline solution (1 part ethanol:10 parts saline) (Stetson and Tay, 1985) for 10 days, whereas the remaining animals ($n = 12$) received injections of the vehicle alone. All injections were administered 2 h before lights out. The melatonin injection protocol that was used in Experiment 2 was chosen for several reasons. First, the precise timing of melatonin injections (i.e., 2 h before lights off) has been shown to extend the normal long-day pattern of endogenous melatonin secretion; the resulting extended pattern of melatonin is interpreted by hamsters as a short day (Stetson and Tay, 1985). Thus, the pattern of melatonin generated in experimental animals, rather than being artificial or supraphysiological, accurately reflects typical short-day patterns. Second, melatonin treatment was administered on a relatively short-term basis (i.e., 10 days); this time period was chosen because it is not sufficiently prolonged to trigger gonadal regression, and unlike maintenance in short days, leaves gonadal steroid concentrations unaffected (Jasnow et al., 2002). This protocol allows the effects of exogenous melatonin to be tested directly, without subsequent changes in gonadal steroid hormones. Following 10 days of injections, resident–intruder aggression was assessed as described previously (Jasnow et al., 2000). Briefly, an intruder hamster was introduced into the home cage of an experimental hamster and the behavioral encounters were observed for 5 min. The cages of resident animals were left unchanged for 10 days before testing to allow establishment of a territory (Jasnow et al., 2000). Aggressive behavior was scored live and the number of attacks, the latency to initial attack, and the total duration of aggression were scored. Following behavioral testing, animals were euthanized with overdoses of sodium pentobarbital and reproductive condition was determined by weighing the testes at necropsy.

Experiment 2: the role of adrenal medullary catecholamines in melatonin-induced aggression

Forty hamsters were used in Experiment 2. The goal of this experiment was to test the hypothesis that melatonin-induced increases in aggression are due to increases in adrenal medullary catecholamines. To test this hypothesis, half of the hamsters received bilateral adrenal demedullations (ADMEDx) whereas the remaining animals received sham surgeries. ADMEDx was performed while the animals were anesthetized with sodium pentobarbital (50 mg/kg) according to a modification (Demas and Bartness, 2001) of the method of Tonge and Oately (1973). Briefly, bilateral incisions were made on the dorsum over the kidneys and the adrenal glands were visualized using a dissecting microscope. A small incision was made on the adrenal cortex and the adrenal medulla was extirpated using minimal pressure. Every effort was made to leave the adrenal cortex intact. We have previously demonstrated that this technique reliably and consistently results in adrenal epinephrine content <0.05%, as determined by high-pressure liquid chromatography (Demas and Bartness, 2001). Animals were allowed to recover from the surgeries for 2 weeks. Half of the animals from each surgical group were then administered daily s.c. injections of melatonin for 10 days; the remaining animals received injections of saline. Resident–intruder aggression was assessed and reproductive condition was

determined by weighing the testes at necropsy as described in Experiment 1.

Experiment 3: the role of adrenal cortical hormones in melatonin-induced aggression

Forty-six hamsters were used in Experiment 3. The goal of this experiment was to test the hypothesis that melatonin-induced increases in aggression are due to increases in adrenocortical hormones (e.g., cortisol). To test this hypothesis, half of the hamsters received surgical bilateral adrenalectomies (ADx) via the method of Drazen et al. (2003), whereas the remaining animals received sham surgeries. Briefly, a small incision was made in the skin rostral to the kidneys. The perirenal fat and prominent adrenal vein were located and the adrenal was isolated from the surrounding fat capsule and removed. The incision on the skin was sutured closed. Because the loss of aldosterone in ADx hamsters prevents proper regulation of salt balance in these animals, all ADx animals had access to 1% saline in a 5% sucrose solution in addition to their regular drinking water for the duration of the study. Although hamsters lack a sodium appetite, the sucrose adequately masks the saline solution, making it potable to the hamsters (e.g., Pieper and Lobocki, 2000; Salber and Zucker, 1974). Six ADx hamsters died as a result of the surgery. One week following surgeries, half of the animals in each group received s.c. injections of exogenous melatonin for 10 days as described in Experiment 1.

Blood collection and hormone assays

For all three experiments, animals were brought into the surgery room individually 24 h before behavioral testing, lightly anesthetized with either methoxyflurane vapors (Metofane, Mundelein, IL) or anhydrous diethyl ether (Sigma), and blood samples (~500 μl) were drawn from the retro-orbital sinus between 1000 h and 1200 h EST; animals were returned to their respective housing conditions. Blood samples were allowed to clot for 1 h, the clots were removed, and the samples centrifuged (at 4°C) for 60 min at 2500 rpm. Serum aliquots were aspirated and stored in sealable polypropylene microcentrifuge tubes at −80°C until assayed. Blood serum testosterone (Experiment 1) or cortisol concentrations (Experiments 2 and 3) were determined in separate enzyme immunoassays (EIA). Cortisol was measured because it is the predominant glucocorticoid in Siberian hamsters, with circulating concentrations ~100× that of corticosterone (Reburn and Wynne-Edwards, 1999). Both hormones were measured using commercially prepared EIA kits (Assay Designs, Ann Arbor, MI). These kits have been previously validated for use with Siberian hamsters (Demas et al., in press). The antisera used in these kits are highly specific. For the T assay, cross-reactivity is 7.20% for androstendione, 0.72% for DHEA, and 0.4 for estradiol. For the cortisol assay, cross-reactivity is 27.68%

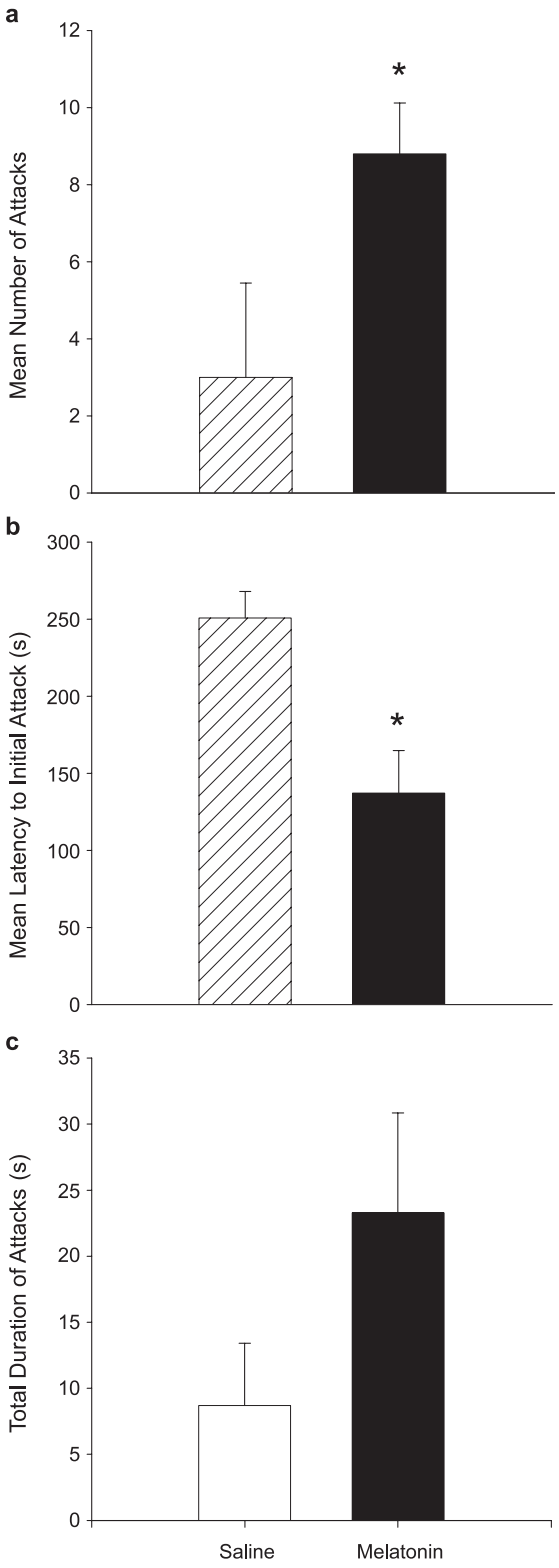


Fig. 1. Mean (±SEM) number of attacks (a), latency to initial attack (s) (b), and total duration of attacks (s) (c) in adult male Siberian hamsters treated with either s.c. injections of melatonin (15 μg/day) (n = 13) for 10 consecutive days or in control hamsters treated with saline. Significant differences between pairwise means are indicated by an asterisk (*) if $P < 0.05$.

for corticosterone. For both kits, cross-reactivity with all other steroid hormones is <0.01%. The sensitivities of the assays are 3.82 and 56.72 pg/ml for testosterone and cortisol, respectively. Intra-assay variability was <10% for all samples in both assays.

Statistical analyses

The data from all dependent measures from Experiment 1 were analyzed using separate independent, two-tailed Student's t tests (SPSS, Chicago, IL). In Experiments 2 and 3, data were analyzed using separate two-way (adrenal manipulation × melatonin treatment) analyses of variance (ANOVAs) (SPSS). Post hoc comparisons between pairwise means were determined using Tukey HSD tests when the overall ANOVA was significant. In all cases, differences between group means were considered statistically significant if $P < 0.05$.

Results

Experiment 1: hamsters treated with exogenous melatonin displayed increased aggression compared with control animals

In Experiment 1, melatonin-treated hamsters displayed an increased number of attacks compared with hamsters treated with saline ($t_{23} = 2.11$; $P < 0.05$) (Fig. 1a). Melatonin-treated hamsters also displayed a shorter latency to initial attack ($t_{23} = 3.48$; $P < 0.05$) (Fig. 1b), but did not differ significantly in the total duration of attacks ($P > 0.05$) (Fig. 1c). Melatonin-treated hamsters did not differ from control animals in either body mass (41.56 ± 1.25 g in melatonin-treated vs. 40.58 ± 1.13 g in saline-treated hamsters) or paired testes mass (0.876 ± 0.033 g in melatonin-treated vs. 0.970 ± 0.055 g in saline-treated hamsters) ($P > 0.05$ in both cases). There were also no significant differences in serum T concentrations (4.8 ± 0.41 ng/ml in melatonin-treated vs. 4.25 ± 0.38 ng/ml in saline-treated hamsters) ($P > 0.05$).

Experiment 2: ADMEDx did not affect melatonin-induced increases in aggression in hamsters

In Experiment 2, melatonin-treated hamsters displayed a significantly higher number of attacks ($F_{1,35} = 10.34$; $P < 0.05$) (Fig. 2a) and total duration of attacks ($F_{1,35} = 13.17$;

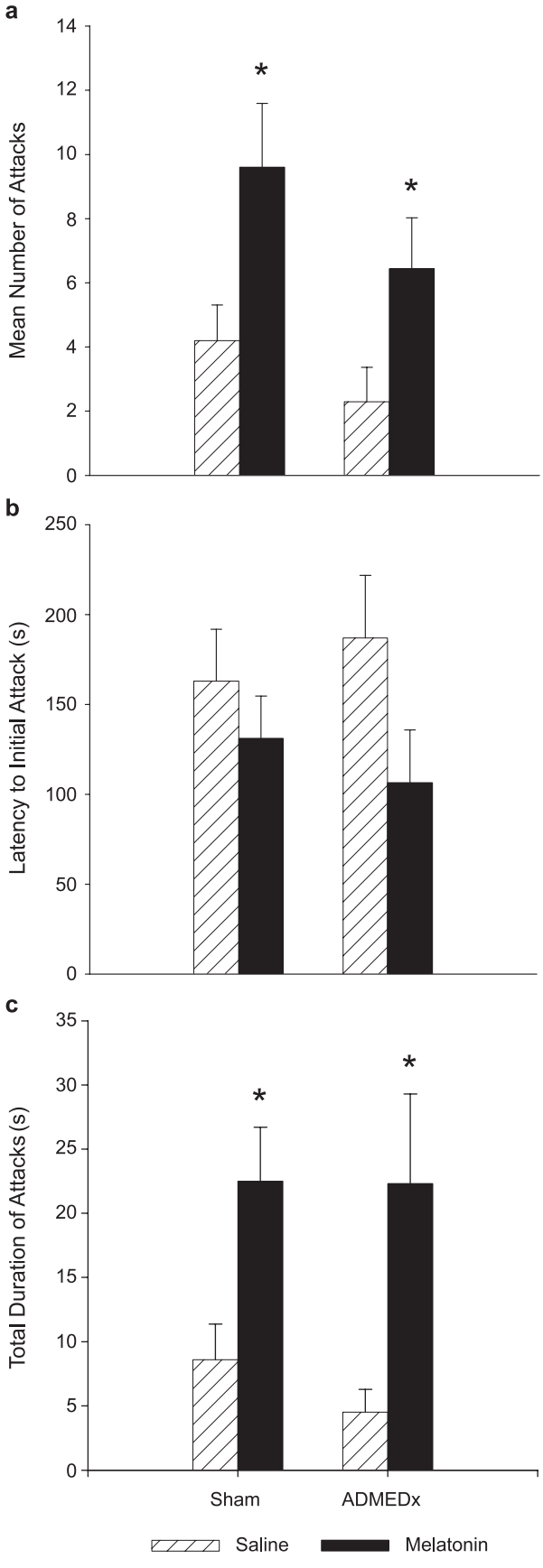


Fig. 2. Mean (±SEM) number of attacks (a), latency to initial attack (s) (b), and total duration of attacks (s) (c) in hamsters that received bilateral adrenal demedullations (ADMEDx) or sham operations (Sham) and subsequently treated with either melatonin or control (Saline) injections as described in Experiment 1. Significant differences between pairwise means are indicated by an asterisk (*) if $P < 0.05$.

$P < 0.05$) (Fig. 2c). Latency to initial attack was not significantly different between melatonin-treated and vehicle-treated animals ($P > 0.05$) (Fig. 2b). There were no significant differences between melatonin- and vehicle-treated hamsters in either body mass or paired testes mass (Table 1) ($P > 0.05$ in both cases). ADMEDx had no effect on serum cortisol concentrations ($P < 0.05$) (Fig. 3). Hamsters treated with exogenous melatonin had slightly higher levels of cortisol than saline-treated hamsters although this difference did not reach statistical significance ($P = 0.064$).

Experiment 3: ADx attenuated melatonin-induced increases in aggression in hamsters

In Experiment 3, there was a significant interaction between melatonin treatment and ADx in terms of the number of attacks ($F_{1,36} = 5.36$; $P < 0.05$) (Fig. 4a), the latency to initial attack ($F_{1,36} = 4.66$; $P < 0.05$) (Fig. 4b), and the duration of attacks ($F_{1,36} = 5.16$; $P < 0.05$) (Fig. 4c). Specifically, melatonin-treated hamsters displayed an increase in the number and duration of attacks and a decrease in the latency to initial attack; ADx, in contrast, attenuated the melatonin-induced increase in aggression (Fig. 4). There were no significant differences between melatonin- and vehicle-treated hamsters in body mass, paired testes mass, or serum cortisol concentrations ($P > 0.05$ in all cases) (Table 1). However, as in Experiment 2, melatonin-injected hamsters displayed a slight, nonsignificant increase in serum cortisol compared with saline-injected control animals ($P = 0.16$).

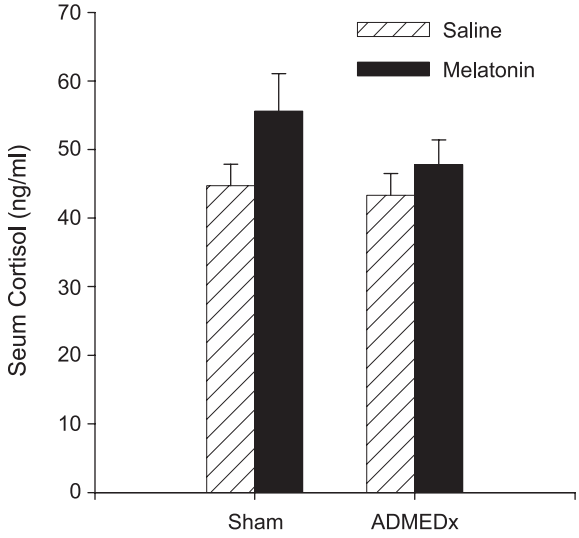


Fig. 3. Mean (±SEM) serum cortisol concentrations in hamsters that received bilateral adrenal demedullations (ADMEDx) or sham operations (Sham) and subsequently treated with either melatonin or control (Saline) injections as described in Experiment 1. Significant differences between pairwise means are indicated by an asterisk (*) if $P < 0.05$.

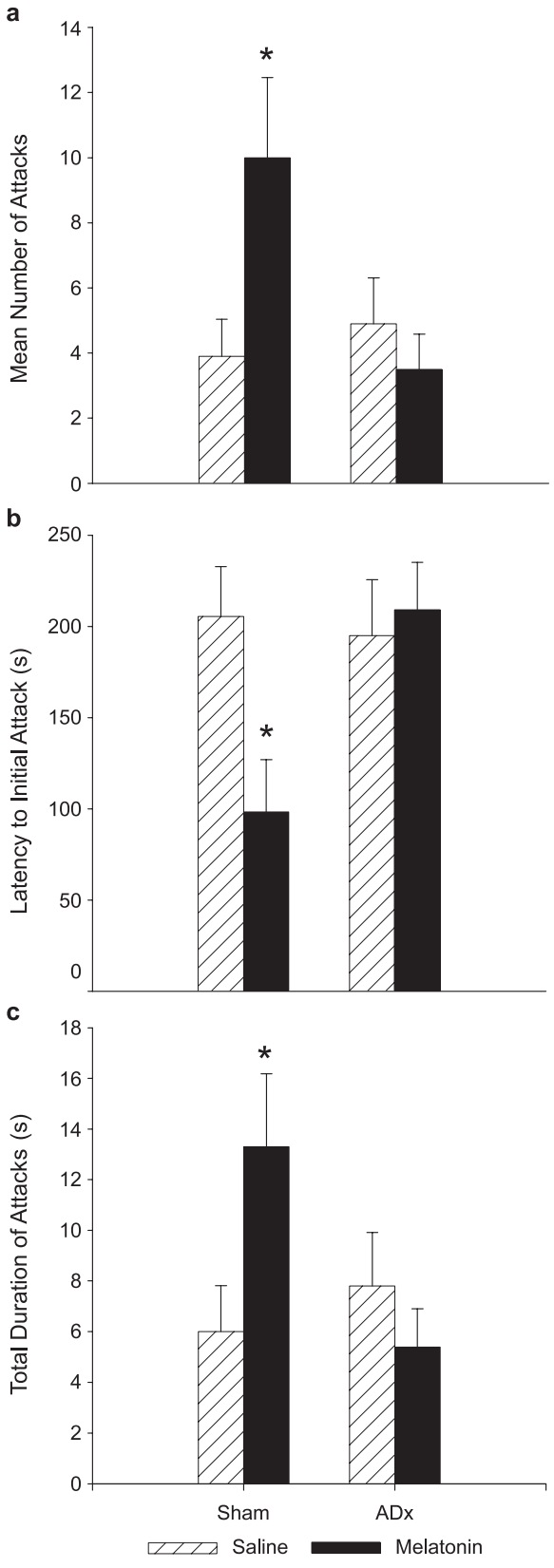
Discussion

The results of the present study support the hypothesis that seasonal changes in aggression in Siberian hamsters are due, in part, to alterations in adrenocortical hormones. These results also demonstrate that short-day patterns of melatonin increase resident–intruder aggression in male Siberian hamsters and that increased aggression could be attenuated by adrenalectomy. Daily injections of melatonin increased aggression in hamsters compared with saline-treated animals and blockade of adrenal hormones via bilateral ADx attenuated melatonin-induced increases in aggression. Specific disruption of adrenal medullary catecholamines via ADMEDx, however, did not affect melatonin-induced aggression, suggesting that melatonin- (and likely short-day) induced increases in aggression are mediated by adrenocortical rather than adrenomedullary hormones. Because ADx removes both adrenomedullary and adrenocortical hormones, however, we cannot rule out a role for catecholamines in photoperiodic changes in aggression. In other words, fluctuations in circulating catecholamines may interact with adrenocortical hormones to affect changes in aggression in Siberian hamsters. Future studies in which adrenocortical hormones are selectively attenuated using specific pharmacological agents (e.g., mitotane, metyrapone) will address this issue. Importantly, increased aggression reported in the present study was not due to changes in body mass or gonadal regression, as both these parameters were unaffected by exogenous melatonin treatment. Melatonin-induced aggression was also not likely affected by changes in testosterone, as the melatonin injection protocol used in the present studies does not alter serum T concentrations (Jasnow et al., 2002 and present

Table 1
Mean (±SEM) body and paired testes masses in hamsters receiving bilateral adrenal demedullations (ADMEDx) (Experiment 2), bilateral adrenalectomies (ADx) (Experiment 3), or sham surgeries (Sham) followed by injections of melatonin (Mel) or saline control (Sal)

Experiment 2	Sham/Sal	Sham/Mel	ADMEDx/Sal	ADMEDx/Mel
Body mass (g)	42.59 ± 1.14	40.57 ± 1.66	41.21 ± 1.32	38.41 ± 0.78
Testes mass (g)	0.842 ± 0.022	0.896 ± 0.096	0.797 ± 0.030	0.779 ± 0.039
Experiment 3	**Sham/Sal**	**Sham/Mel**	**ADx/Sal**	**ADx/Mel**
Body mass (g)	40.37 ± 1.54	39.32 ± 1.75	41.18 ± 1.47	41.13 ± 1.22
Testes mass (g)	0.694 ± 0.056	0.823 ± 0.036	0.819 ± 0.067	0.789 ± 0.038

study). Collectively, these results support the hypothesis that short-day increases in aggression may be mediated, in part, by the effects of melatonin on the HPA axis.



These results are consistent with previously published studies demonstrating photoperiodic changes in aggression in male Syrian and Siberian hamsters (Garrett and Campbell, 1980; Jasnow et al., 2000), as well as previous reports of increased aggression after exogenous melatonin treatment in Syrian hamsters (Badura and Nunez, 1989; Fleming et al., 1988; Jasnow et al., 2002) and house mice (Paterson and Vickers, 1981). The present results are also in accordance with several field studies in mammals published to date demonstrating high aggression during the nonbreeding season when T levels are basal. For example, male rat-like hamsters (*Cricetus triton*) display elevated aggression during the winter nonbreeding season, despite low levels of T (Zhang et al., 2001). Furthermore, seasonal changes in aggression have been shown to be independent of seasonal changes in T in wood rats (*Neotoma fuscipes*) (Caldwell et al., 1984). In primates, aggression and fecal T concentrations are significantly positively correlated during the breeding season, but uncorrelated with one another during the nonbreeding season (Cavigelli and Pereira, 2000).

Androgens also differentially regulate territorial aggression during the breeding (i.e., summer) and nonbreeding (i.e., winter) seasons in several nontropical avian species (Soma et al., 1999, Soma and Wingfield, 2001; Canoine and Gwinner, 2002). For example, blockade of androgen receptors with flutamide inhibits territorial aggression during the breeding season, but does not affect nonbreeding aggression in European robins (*Erithacus rubecula*) (Schwabl and Kriner, 1991). Furthermore, male song sparrows (*Melospiza melodia morphana*) display high levels of aggression during winter despite basal levels of circulating T (Wingfield and Hahn, 1994). Although castration has no effect on aggression during the nonbreeding season, treatment with the aromatase inhibitors fadrozole (FAD) or ADT markedly reduces nonbreeding aggression (Soma et al., 1999, 2000a). Furthermore, treatment with exogenous estradiol (E_2) can reverse the effects of fadrozole and return nonbreeding aggression to normal levels, suggesting a role for estrogens in aggressive behavior during this time of year (Soma et al., 2000b). Although the aromatase enzyme is responsible for the conversion of T to E_2, T is virtually undetectable during the nonbreeding season in these species; thus T is not likely the predominant source of aromatizable androgens.

It has been previously suggested that the adrenocortical androgen dehydroepiandrosterone (DHEA), rather than T, may play an important role in aggression during the nonbreeding season (Soma and Wingfield, 2001). This

Fig. 4. Mean (±SEM) number of attacks (a), latency to initial attack (s) (b), and total duration of attacks (s) (c) in hamsters that received bilateral adrenalectomies (ADx) or sham operations (Sham) and subsequently treated with either melatonin or control (Saline) injections as described in Experiment 1. Significant differences between pairwise means are indicated by an asterisk (*) if $P < 0.05$.

hypothesis is intriguing given that circulating concentrations of DHEA, unlike T, are high year-round in both birds (Soma and Wingfield, 2001) and rodents (Kriegsfeld and Nelson, 1998) and because DHEA, like T, can be aromatized to E_2. In support of this idea, circulating concentrations of DHEA have been positively correlated with aggressive vocalizations during simulated territorial intrusions (Hau et al., 2004). Furthermore, this idea is consistent with the rodent data suggesting a role for adrenal steroids in mediating short-day (i.e., nonbreeding season) aggression. Although the results of the present study suggest an important role for adrenocortical hormones in mediating melatonin-induced aggression, they do not distinguish among several steroid hormones of adrenocortical origin. As the avian literature suggests, DHEA, in addition to glucocorticoids, may mediate territorial aggression in rodents. For example, it was previously shown that bilateral ADx blocks the nocturnal rhythm in aggression in male Syrian hamsters, even in animals receiving cortisol replacement (Landau, 1975). Although this result was suggested to be due to disruption of the endogenous circadian rhythm in glucocorticoid secretion in ADx hamsters, the absence of DHEA in these animals may have contributed to changes in aggression. To our knowledge, however, there are no published reports of serum DHEA concentrations for Siberian hamsters. In addition, the role of aromatization of steroid hormones has not been examined within the context of seasonal changes in aggression in rodents. Ongoing work in our laboratory, however, is aimed at delineating the precise role of DHEA, and its potential aromatization to estrogens, in mediating season changes in aggression in Siberian hamsters.

Another possible mechanism by which adrenocortical hormones may regulate short-day or melatonin-induced aggression in hamsters is via changes in the neurohormone arginine vasopressin (AVP). Considerable evidence exists suggesting that AVP is negatively regulated by the HPA axis. For example, AVP gene expression is inhibited by glucocorticoids and the removal of negative feedback via adrenalectomy results in up-regulation of hypothalamic AVP gene expression (Davis et al., 1986; Kovacs et al., 1986; Sawchenko, 1987). Furthermore, AVP has been implicated in mediating aggression in several rodent species (Bester-Meredith et al., 1999; Goodson, 1998; Stribley and Carter, 1999; Winslow et al., 1993). Injections of AVP into the anterior hypothalamus stimulate aggression, whereas AVP antagonists injected into the same brain region inhibit aggression in Syrian hamsters (Ferris and Potegal, 1989; Ferris et al., 1997; Potegal and Ferris, 1989). The effects of photoperiod on AVP in the central nervous system, however, are less clear. Some studies have reported increased AVP immunoreactivity in short-day housed animals (Bittman et al., 1996; Duncan et al., 1995; Juszczak et al., 1997), while others have reported no differences or decreases in AVP immunoreactivity in hamsters exposed to short days (Albers et al., 1991; Duncan, 1998). Siberian and Syrian hamsters, however, have some noteworthy species differences in the distribution of AVP neurons (Shi and Bartness, 2000) and these differences may explain some of the differences described above. More recently, it has been demonstrated that maintenance in short days decreases AVP receptor binding in Syrian hamsters, although changes in receptor binding do not affect flank marking behavior (an AVP-dependent behavior) (Caldwell and Albers, 2003). Clearly, future studies are needed to examine the role of changes in AVP (V1a) receptors in Siberian hamsters and their relevance to behavioral changes seen in this species in response to photoperiod or melatonin treatment.

At an ultimate level of analysis, increased aggression during the short days of winter likely confers an evolutionary advantage to animals at a time when food availability is low and competition for limited resources is relatively high. Dominant animals may be more successful in obtaining or protecting limited resources compared with subordinate animals, and thus may have higher reproductive success relative to subordinate animals. Regardless of the adaptive significance of photoperiodic changes in aggression, the present results suggest that short-day increases in aggressive behavior are due, at least in part, to increases in the duration of melatonin secretion in short compared with long days. In addition, the short-day increases in aggression, unlike the more commonly studied reproductive aggression, appears to be independent of circulating gonadal steroid hormones (i.e., testosterone). Adrenal hormones, specifically of adrenocortical origin, appear to mediate melatonin-induced and likely short-day resident–intruder aggression. These data support the idea that breeding and nonbreeding season aggression is differentially regulated by the neuroendocrine system. Furthermore, the differential regulation of breeding and nonbreeding aggression in seasonally breeding rodents underscores the importance of studies that utilize ecologically relevant models to investigate the neuroendocrine mechanisms underlying territorial aggression.

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