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Aggressive behavior in female golden hamsters: development and the effect of repeated social stress

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Abstract

In male golden hamsters, agonistic behavior matures during puberty, changing from play fighting to adult-like aggression. In addition, this transition is accelerated by repeated social subjugation early in puberty. However, little is known about the development of agonistic behavior in females. In the present study, we compared the development of agonistic behavior in male and female golden hamsters. Furthermore, we also tested the effects of repeated social subjugation on the development of agonistic behavior during puberty. Hamsters were tested for agonistic behavior in the presence of a smaller intruder at different intervals during puberty. Several observations were made. First, the frequency of attacks remained stable in females, while varying in males. Second, the transition from play fighting to adult-like aggression occurred at earlier time periods in females than in males. Finally, a clear transitional period marked by attacks focused on the flanks was observable in males around mid-puberty. However, this transitional period was not apparent in females. In addition, juvenile females were exposed to aggressive adult males or females. In both cases, repeated exposure to stress had no statistically significant effect on the development of agonistic behavior. After 2 weeks of subjugation, exposure to aggressive adults had no effect on serum cortisol levels, indicating that juvenile females habituate to repeated social stress. These data show significant sex differences in the development of agonistic behavior and adaptation to repeated stress in juvenile golden hamsters.

Keywords: Agonistic behavior; Aggression; Puberty; Cortisol; Progesterone; Sex differences; Play fighting; Adolescence; Chronic stress

Introduction

In golden hamsters (*Mesocricetus auratus*), puberty is marked by drastic changes in social behavior, as agonistic behavior matures from play fighting to adult aggression (Goldman and Swanson, 1975). These behaviors, which can be studied as offensive responses toward a smaller intruder, have unique characteristics (Delville et al., 2003). As soon as they are capable of coordinated movements, golden hamsters engage in play fighting activity (Goldman and Swanson, 1975; Schoenfeld and Leonard, 1985). In hamsters, play fighting is characterized by a high frequency of attacks and bites (Goldman and Swanson, 1975; Pellis and Pellis, 1988a; Wommack et al., 2003). Indeed, juvenile hamsters attack each other constantly, often reversing roles. As the animals mature, attack frequency decreases gradually and reversals disappear. During fights between adults, residents typically attack and promptly bite the intruder, then retire for a period of time until the next attack. In addition, the hamsters target different parts on the body of intruders during play fighting and adult aggression (Pellis and Pellis, 1988a,b; Wommack et al., 2003). In early puberty, as the animals play-fight, their attacks are mainly focused on the cheeks and face of the intruders. Over time, the focus of the attacks moves gradually to the flanks followed later by the lower belly and rump. This transition in the focus of attacks occurs around mid-puberty as testicles start growing and serum testosterone levels start rising (Vomachka and Greenwald, 1979; Wommack et al., 2003).

However, these observations were reported in male hamsters. Little is known about play fighting or the transition from play fighting to adult aggression in female hamsters. Some studies have reported sex differences in play fighting activity, as males were more active than females (Guerra et al., 1992); however, these data focused on a single time point during early puberty. As play fighting

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activity varies greatly during early puberty in males (Goldman and Swanson, 1975; Wommack et al., 2003), comparisons between sexes require a longitudinal perspective.

Sex differences in aggressive behavior have also been reported in adult hamsters (Siegel, 1985). Several studies have noted that females are dominant toward males (Goldman and Swanson, 1975; Marques and Valenstein, 1977; Payne and Swanson, 1970; Tiefer, 1970). These findings have not always been consistent and may depend on the reproductive status or the age of the females (Siegel, 1985). In addition, it is not clear whether females are more aggressive during same sex interactions (Brain, 1972; Payne and Swanson, 1970; Tiefer, 1970). Moreover, the exact nature of the aggressive interactions previously reported in females is also unclear, as different types of aggression (offensive vs. defensive aggression) can be observed depending on the testing conditions. Tests in the presence of a smaller and younger intruder will favor offensive aggression (Miczek, 1979). Tests between animals matched for weight and age in a neutral arena will involve both offensive and defensive aggression by the protagonists (Blanchard and Blanchard, 1988).

In addition, the timing of the transition from play fighting to adult aggression can be altered by environmental stimuli. In juvenile male hamsters, repeated exposure to social subjugation during early puberty accelerates the onset of adult aggression in juvenile male hamsters and enhances aggression toward smaller intruders (Delville et al., 1998; Wommack et al., 2003). However, it is possible that females have different responses to social subjugation. Indeed, adult female hamsters are less vulnerable to social defeat (Huhman et al., 2003). Alternatively, these effects of stress may also differ across development. In males, repeated exposure to social subjugation after mid-puberty has different consequences than before mid-puberty (Delville et al., 2003).

The present studies had two separate goals. The first goal was to compare the development of offensive responding between male and female hamsters during puberty. The second aim was to determine the effect of repeated social subjugation on the development of offensive responding during puberty in female hamsters.

Methods

Animals and treatment

Golden hamsters were bred in a colony maintained in the laboratory and founded with animals purchased from Harlan Sprague–Dawley (Indianapolis, IN). Shortly after birth, litters were culled to six pups containing both males and females. All animals were weaned on postnatal day 25 (P-25) and individually housed in Plexiglas cages (8 × 13 × 5 in.). Golden hamsters are solitary and territorial (Dieterlen, 1959; Festing, 1986; Johnston, 1985; Murphy, 1985). Food and water were provided ad libitum. All animals used in experiments were housed under a reversed daylight cycle (14L/10D; lights on at 21:00). All experimental procedures were performed according to NIH guidelines and were approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin, an AALAC-approved facility.

Experimental design

Development of agonistic behavior

To assess the typical patterns of aggressive behavior as it develops over time, female and male hamsters ($n = 16$ and $n = 19$) were exposed to smaller (10–20%) and younger (5–10 days) conspecific same-sex intruders placed inside their home cage. A subset of the males mentioned in this study were described in a previous manuscript (Wommack et al., 2003). The offensive responses of the animals in the presence of the intruder were recorded and videotaped for later review. Each test lasted 10 min and was repeated approximately once a week from P-27 to P-62. To ensure that no dominant–subordinate relationships were formed, no individual was exposed to the same intruder more than once. All behavioral tests were performed at the middle of the dark phase under dim red light illumination. Body weights were taken once a week.

Furthermore, all females (resident and intruder) were exposed to an adult male for 1–2 min daily to ascertain the stage in their estrous cycle. Preliminary studies showed no effect of this brief exposure. Individuals were only tested for offensive responses on the first or second day of diestrus. Intruders were only used on the first or second day of diestrus as well.

Social stress

Repeated exposure to social subjugation was patterned on a previously described protocol modified for use with females (Wommack and Delville, 2002; Wommack et al., 2003). Animals were separated into three groups on P-27 (Control, $n = 16$; Female-Subjugated, $n = 15$; Male-Subjugated, $n = 15$). All animals (including controls) were exposed to an adult male for 1–2 min daily to minimize confounds from a possible Vandenbergh Effect in male-subjugated females (Lombardi and Vandenbergh, 1977; Vandenbergh, 1967). The possibility of a Vandenbergh Effect in male-subjugated females was further tested as follows. The first day of lordosis response was recorded and compared between treatment groups. In addition, serum levels of progesterone were assayed in females on P-41. All females that were sexually receptive in the presence of the male were placed back into their home cage for that day. When the females were not sexually receptive, they were exposed to one of the following treatments daily from P-27 to P-41. Animals subjugated by females (Female-Subjugated) were placed in the home cage of an aggressive adult female for 30 min. Animals subjugated by males (Male-Subjugated) were placed in the home cage of an aggressive

adult male for 30 min. Control animals were placed in an empty clean cage for the same period of time. Subjugations were observed to confirm that all animals were chased and bitten during the 30-min period. The frequency of attacks sustained during subjugation was recorded for each animal. The adult females used to subjugate subjects were also monitored for estrous cyclicity and were never used on estrous days.

Subjugated and control animals were tested for offensive responses in the presence of a smaller (10–20%) and younger (5–10 days) conspecific same-sex intruder once a week on P-27, P-34, P-41, P-55 and P-62 with a range of ±2 days from each test day. These tests were performed before any manipulations scheduled for that day. Control and subjugated animals were only tested for offensive responses on the first or second day of diestrus and intruders were only used on the first or second day of diestrus as well.

Description of behaviors

Several behaviors were observed and recorded during behavioral tests and video playbacks. These behaviors were analyzed separately as either *quantitative* or *qualitative* in nature. Quantitative observations consisted of frequency and target of attack, number of flank marks, contact time, number of pins and latency to attack. Contact time was defined as the duration of time the resident initiated and maintained contact with the intruder. A pin was counted when one animal used its torso and forepaws to push down another animal in a supine position. An attack was defined as an approach followed by an attempt to bite the intruder. Attacks were distinguished on the basis of the body part initially targeted on the intruders, rather than the location of the bites. Indeed, defensive behavior by the intruders often causes bites to be located away from the initial target of attacks. Qualitative observations related to the types of attacks performed by the subjects in the presence of an intruder. These types of attacks were separated on the basis of the initial body parts targeted during attacks. The initial targets of attacks were front (head and cheeks), side (including flank glands) and belly/rear (Pellis and Pellis, 1988a,b; Wommack et al., 2003). A fourth category of attack was also observed during testing. In this case, the resident would walk on top of a submissive intruder laying immobile on its back and start biting anywhere. These walk-in attacks were not included in the data analysis and accounted for less than 10% of all attacks in both sexes.

It was necessary to analyze these qualitative data to compare different stages of development. This was made possible by comparing the percentages of occurrences of each attack type. After reviewing all videos, percentages of attacks that were either frontal, side or belly/rear were calculated (number of attacks of a specific body part divided by total number of attacks during that test) for each individual and each test. For example, the percentage of frontal attacks that occurred during testing was calculated by dividing the total number of frontal attacks by the sum of frontal, side and belly/rear attacks. Walk-in attacks were not used to calculate this measure because these attacks were not targeted at specific areas. The averages of each type of attack were compared between genders or group over time.

Hormone assays

We tested the effects of chronic social subjugation on serum cortisol (to better characterize the stress of repeated social subjugation) and progesterone levels (to determine any effect of social subjugation on ovarian development). Females were divided into three groups (Control, Female-Subjugated, Male-Subjugated) as explained above. Animals from each group were further divided into Baseline and Response subgroups. Baseline animals were sacrificed on P-27 or P-41 straight from their home cages. Response animals were sacrificed immediately after exposure to their treatment (placement in empty cage or subjugation) on either P-27 or P-41. All animals were quickly decapitated and trunk blood was collected. The samples were spun at 5000 rpm and sera were collected and stored at −20 °C until assayed by EIA. Attention was taken to sacrifice animals on the day after estrus.

Serum cortisol levels were assayed with Cortisol Correlate-EIA™ kits (Assay Designs, Inc., Ann Arbor, MI). Each sample was assayed in duplicate from 20-μl aliquots. Intra-assay variability was 6.9%. Inter-assay variability was 9.3%. The antibody used has 26.7% cross-reactivity with corticosterone (which levels were shown to be under 500 pg/ml), 3.6% with progesterone, 4% with 11-deoxycortisol and less than 1% with other endogenous steroids.

Serum progesterone levels were assayed with Progesterone Correlate-EIA™ kits (Assay Designs). Each sample was assayed in duplicate from 20-μl aliquots. Intra- and inter-assay variability was 5.6% and 4.6%, respectively. The antibody used in the kit had 3.5% cross-reactivity with 17-OH-progesterone and less than 1% for other endogenous steroids. Corticosterone was also assayed and levels were found negligible.

Data analysis

Nonparametric data (number of flank marks, pins, attacks) were analyzed with nonparametric statistics separately with Kruskal–Wallis tests followed by Mann–Whitney tests for comparisons between groups on each test day. Nonparametric data were also analyzed through Friedman tests followed by Wilcoxon tests for comparisons between days within each group. Days of first lordosis were compared between groups with a Kruskal–Wallis test followed by Mann–Whitney tests. P values were two-tailed and considered statistically significant if $P < 0.05$. Serum levels of progesterone and cortisol were analyzed through non-

parametric tests (Kruskal–Wallis followed by Mann–Whitney tests) because of elevated and unequal variability between groups.

Parametric data from behavioral observations (durations, latencies, percentages, serum levels) were also analyzed separately for group effects (gender differences, subjugated vs. control) and developmental effects. Because of missing cells in the data sets, the behavioral data could not be analyzed through two-way ANOVAs. Gender differences were analyzed through *t* tests on each test day, and through one-way ANOVAs for comparisons over time for each gender independently. The effects of social stress were also analyzed through separate one-way ANOVAs followed by Fisher PLSD post hoc tests for each test day and one-way repeated ANOVAs for comparisons over time for each group independently. *P* values for *t* tests were two-tailed and considered statistically significant if $P < 0.05$. Body weight data were analyzed through two-way ANOVAs (independent variables: treatment groups and age).

Results

Development

Two types of data were compared between males and females across peri-pubertal development. Quantitative data included frequencies and durations for various types of aggressive responses such as attacks or pins. Qualitative data included percentages of attack types observed during the agonistic encounters, such as frontal, side and belly/rear attacks.

Different patterns of development were observed between males and females across puberty after analysis of quantitative data (Fig. 1). In males, changes (attack frequency, number of pins and duration of contact time) followed parallel courses over time. These changes were statistically significant [Attack Frequency: $\chi^2(5) = 28.5$, $P < 0.001$; Pins: $\chi^2(5) = 42.5$, $P < 0.001$; Contact Time: $F(5,0) = 9.9$, $P < 0.001$]. The development of these behaviors showed first an increase in frequency or duration between P-27 and P-34 (Attack Frequency: $Z = 3.3$, $P = 0.001$; Pins: $Z = 3.3$, $P < 0.001$), followed by a gradual decrease from P-41 to P-62 (Attack Frequency: P-34 to P-41: $Z = 2.2$, $P < 0.05$; Pins: P-34 to P-41: $Z = 3.2$, $P < 0.01$). In contrast, no such changes were observed in females. In females, the same data remained constant from P-27 to P-55 ($P > 0.05$).

Attack frequencies peaked on P-34 in males but not in females (Fig. 1). The difference between sexes was statistically significant on that day ($U = 65$, $P < 0.05$, Mann–Whitney). In addition, while attack frequencies declined afterwards in males, they remained somewhat elevated in females. By P-62, females were performing more attacks than males ($U = 11$, $P < 0.05$, Mann–Whitney). Pins differed statistically between sexes on P-49, 56 and 62 (P-49: $U = 33$, $P < 0.01$; P-55: $U = 42.5$, $P < 0.01$, P-62: $U = 5$, $P < 0.01$

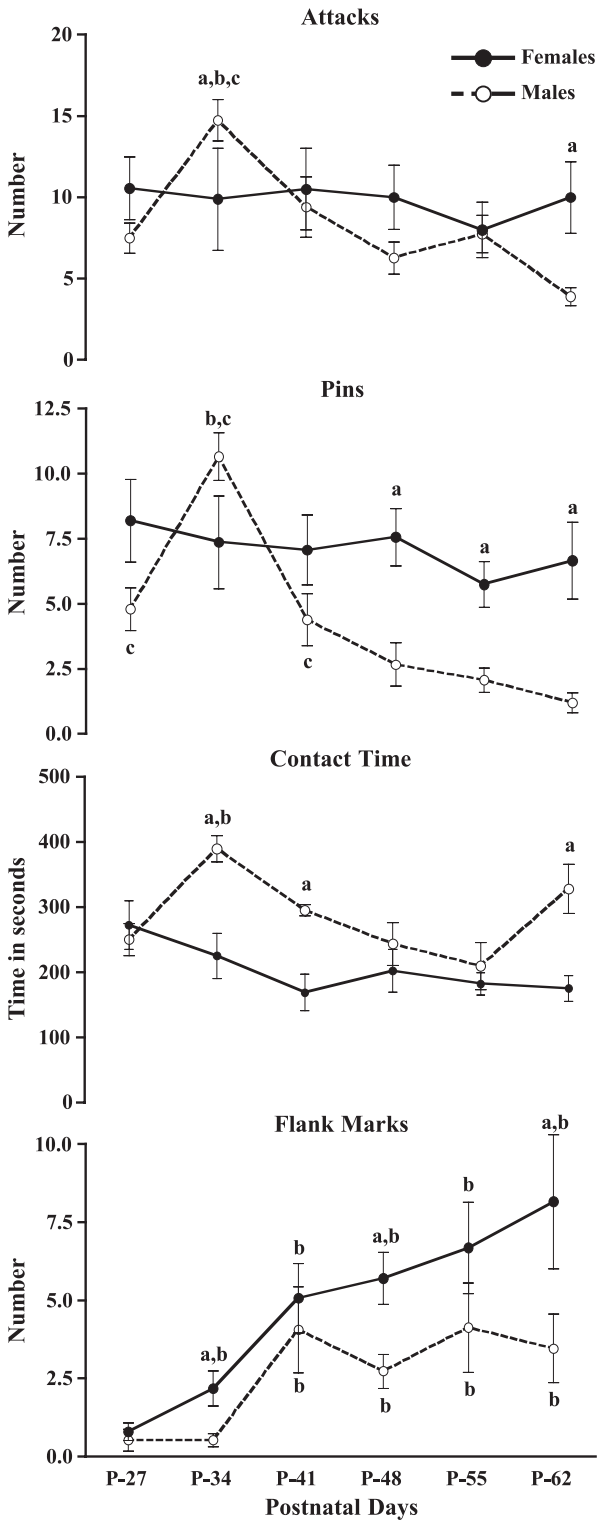

Fig. 1. Comparison of the duration of contact time and frequencies of attack, pin and flank marking in male and female golden hamsters ($n = 19$ and 16, respectively) tested for offensive responses during peri-pubertal development from postnatal day 27 (P-27) to P-62. Tests were performed for 10-min periods in the presence of a smaller and younger intruder. All data are expressed as mean ± SEM. a denotes statistical differences between sexes, b denotes statistical differences with P-27, and c denotes statistical difference with P-62, with $P < 0.05$, at least.

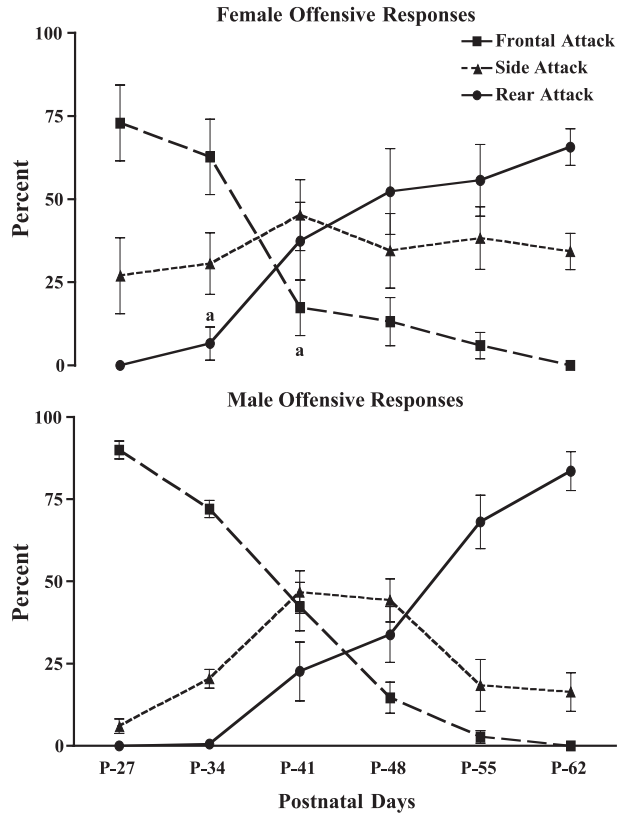

Fig. 2. Comparison of the proportion of attacks that were either directed at the face and cheeks (Frontal Attacks), side/flanks (Side Attacks), and lower belly and rear (Rear Attacks) in male and female golden hamsters (n = 19 and 16, respectively) tested for offensive responses during peri-pubertal development from postnatal day 27 (P-27) to P-62. Tests were performed in the presence of a smaller and younger intruder. All data are expressed as mean ± SEM. a denotes statistical differences between sexes, with $P < 0.05$, at least.

Mann–Whitney), as activity declined in males, while remaining elevated in females (Fig. 1). Contact times were higher in males than females on P-34 and P-41 [$t(29) = 4.1$, $P < 0.001$; $t(26) = 2.1$, $P < 0.05$; respectively]. Interestingly, although females performed more attacks than males on P-62, contact times were higher in males on that particular day [$t(19) = 2.5$, $P < 0.05$].

A totally different pattern of development was observed for flank marking behavior in both male and female hamsters across puberty (Fig. 1). In both sexes, flank marking activity during agonistic encounters increased from P-27 to P-62 [males: $\chi^2(5) = 22.5$, $P < 0.001$; females: $\chi^2(4) = 25.1$, $P < 0.001$]. In males, flank marking increased until P-41 (P-27 to P-41: $Z = 2.9$, $P < 0.01$) and remained stable through P-62. In females, flank marking increased significantly from P-27 to P-34 ($Z = 2.2$, $P < 0.05$) and kept increasing gradually until P-62. Both sexes started on P-27 with similar flank marking activity, but the behavior rose faster and to a higher level by P-62 in females (P-34: $U = 59$, $P < 0.05$; P-49: $U = 46.5$, $P < 0.05$; P-62: $U = 18$, $P < 0.05$; Mann–Whitney).

Analysis of qualitative data (percentages of attack types) also showed different patterns of development between males and females (Fig. 2). In males, puberty could be subdivided into three periods, each marked by a specific attack type, as previously described (Wommack et al., 2003). Frontal attacks were characteristic of early puberty from P-27 to P-34. Belly/rear attacks were characteristic of late puberty from P-55 to P-62. Side attacks were most predominant around mid-puberty between P-41 and P-49. In females, the developmental patterns were different. Early puberty was also characterized by frontal attacks between P-27 and P-34, and the end of puberty was also characterized by belly/rear attacks. However, the percentage of side attacks did not change across puberty, hovering around 25%, and never became characteristic of mid-puberty.

In addition, the relative frequency of frontal attacks decreased faster than in males. By P-41, females had a

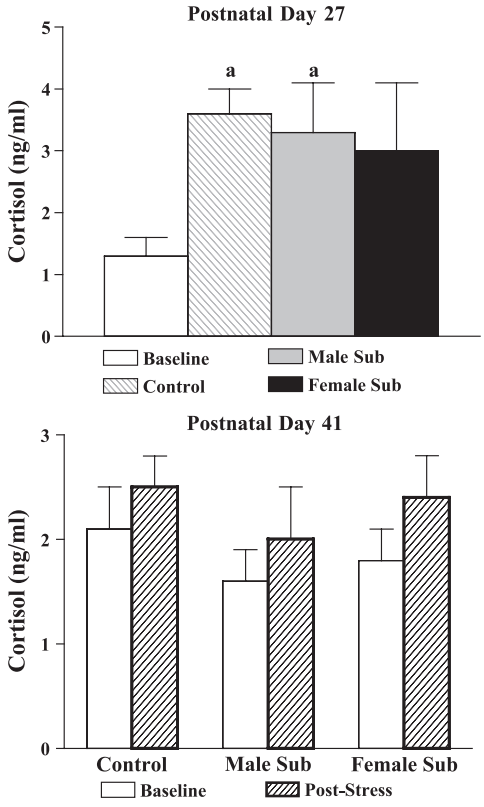

Fig. 3. Postnatal day 27: comparison of serum cortisol levels collected on postnatal day 27 (P-27, beginning of period of daily subjugation). Samples were collected either before (Baseline, n = 10) or just after a 20-min period in an empty clean cage (Control, n = 10) or in the home cage of an aggressive adult male (Male Sub, n = 9) or female (Female Sub, n = 9) hamster. Postnatal day 41: comparison of serum cortisol levels collected on postnatal day 41 (P-41, end of daily period of subjugation). Samples were collected either before (Baseline) or just after a 20-min period (Post-Stress) in an empty clean cage (Control) or in the home cage of an aggressive adult male (Male Sub) or female (Female Sub) hamster. Control Baseline: n = 5, Post-Stress: n = 7; Male Sub Baseline: n = 6, Post-Stress: n = 6; Female Sub Baseline: n = 6, Post-Stress: n = 6. a denotes a statistical difference with Baseline levels (P-27), with $P < 0.05$, at least.

lower percentage of frontal attacks than males [$t(25) = 3.1$, $P < 0.01$]. The opposite relationship was found with the belly/rear attacks. By P-34, females had a higher percentage of belly/rear attacks than males [$t(27) = 2.5$, $P < 0.05$]; though the percentage of side attacks did not differ from males.

Social subjugation

Juvenile female hamsters were socially subjugated by either adult males or females. Adult females turned out to be much more aggressive than adult males to the juvenile females. Adult females attacked and bit the juveniles about 15.8 times per day, while the daily number of attacks and bites by males averaged only 5.9. Besides these differences, the physical and sexual development of the juvenile females was equally unaffected by repeated social stress. There was no statistically significant difference in body weight between groups over peri-pubertal development. The first day of estrus averaged 33 days of age in all groups. These data were further confirmed by the analysis of serum progesterone levels collected on P-41. These levels averaged 1.5 ng/ml in all groups.

Regardless of the intensity of social stress experienced by the juvenile females, the development of agonistic behavior did not differ significantly between treatment groups. None of the behavioral measures differed significantly between groups on any day of testing.

In addition, serum cortisol levels were assayed on P-27 and P-41 on the first and last days of subjugation (Fig. 3). On P-27, baseline levels of cortisol averaged 1.3 ng/ml. These levels were twice as high after exposure to social subjugation by females or males or after placement in an empty clean cage [$H(3) = 11.4$, $P < 0.01$]. The increase over the baseline was statistically significant for male subjugated ($U = 19$, $P < 0.05$) and control individuals ($U = 6$, $P < 0.001$). By P-41, baseline levels ranged between 2.0 and 2.5 ng/ml between groups. Post-stress cortisol levels ranged between 1.5 and 2.1 ng/ml between groups. None of these data differed significantly between treatment groups or between baseline and post-stress.

Discussion

During the present studies, both sexes were observed performing offensive responses in the presence of intruders. Therefore, under similar conditions, male and female hamsters are equally capable of displaying offensive aggression. However, clear sex differences were also observed in the peri-pubertal development of agonistic behavior. These sex differences involved several aspects of the behavior. First, the development of attack and pin frequencies peaked early and was followed by a gradual decline until adulthood in males. In females, these frequencies remained constant and somewhat elevated throughout puberty. Fluctuations were also seen in the duration of contact time in males, while remaining stable in females. Second, the development of offensive responses in males can be clearly separated into different phases based on the prevalence of specific attack types. In males, a first phase is characterized by frontal attacks, which is followed by a phase characterized by a prevalence of side attacks, then a third phase with belly/rear attacks. In females, frontal attacks were also characteristic of early development and belly/rear attacks were only performed late in puberty. However, the frequency of side attacks remained stable throughout puberty. Finally, our previous data have shown that males do not habituate to repeated exposure to social stress (Wommack and Delville, 2003). This lack of habituation is associated with alterations in the development of agonistic behavior during puberty (Wommack et al., 2003). In contrast, females were perfectly capable of habituating to repeated social subjugation, regardless of its intensity. This last observation is supported by recent data collected in adult female hamsters (Huhman et al., 2003). Together, our data bring a new and more complete description of the development of offensive aggression in male and female hamsters, and point to critical sex differences.

Beside sex differences in the patterns of development of offensive responses, additional observations were also noted in the data. Our observations confirm that males are more active than females around P-34 (Guerra et al., 1992). However, as attack and pin frequencies decreased in males, they remained stable in females. By early adulthood (P-62), females were more likely to attack and pin their intruders than males. These observations confirm the importance of broad developmental studies as compared to studies focused on specific days. Our data also confirm that, as adults, females are more aggressive than males. Interestingly, females maintained a high frequency of pins in early adulthood. This behavior has been associated with play fighting in hamsters (Guerra et al., 1992, 1999). As such, it could be argued that female aggression includes elements of play fighting during adulthood in hamsters. It is also interesting to note that the duration of contact times was longer in males than in females in early adulthood, although males were less likely to attack than females. This observation suggests that females were more focused on aggression or more efficient at attacking their intruders. Alternatively, it is also possible that males spend more time in close contact with their intruders during olfactory investigations. Nevertheless, this observation is consistent with our conclusion that female hamsters are more aggressive than males.

During these studies, flank marking was also recorded during the agonistic encounters. Although hamsters start flank marking at the same time that they start play fighting (Ferris et al., 1995; Goldman and Swanson, 1975), there was a clear and gradual increase in flank marking activity throughout puberty. Flank marking can be observed under different contexts (Johnston, 1975, 1985). The behavior can be observed by placing a hamster in the recently vacated home cage

of a conspecific. The behavior also can be observed in absence of a stimulus after intra-hypothalamic injections of vasopressin (Ferris et al., 1984). In the present studies, flank marking was observed during social encounters. In hamsters, the behavior is typically used to communicate social status, and is mostly performed by dominant individuals during encounters (Ferris et al., 1987; Johnston, 1975, 1985). Over repeated pairings between the same individuals, hamsters substitute flank marking for overt aggression (Ferris et al., 1987). Females are known to flank mark, particularly during aggressive encounters (Johnston, 1977). It is possible that females were more likely to flank mark as they were clearly dominant to their intruders, and indicate dominance faster than males. However, it is also important to note that flank glands of hamsters are strongly testosterone-dependent (Johnston, 1981). It is unclear what type of scent was propagated by the females during these studies.

One important observation reported in our studies involves developmental changes in the relative frequencies of attack types between males and females. In females, the frequency of side attacks remained relatively stable during puberty as compared to males. In females, 25% of attacks by juveniles on P-27 were targeting the sides, as compared to less than 10% in males. In addition, the frequency of frontal attacks declined faster in females than in males. By P-41, no more than 20% of attacks by females were focused on the face, while in males 40–50% of attacks were still focused on the face. These two additional observations suggest that the development of agonistic behavior is faster in females than in males, at least during its first phase (play fighting).

The present studies also included a description of effects of repeated social subjugation during the play fighting phase in females. Previous studies in male hamsters have described an acceleration of the development of agonistic behavior followed by enhanced aggression toward smaller and younger intruders (Delville et al., 1998; Wommack et al., 2003). No such observation was made in the present studies. In addition, our study includes two different groups subjected to different intensities of social subjugation. Adult females were much more likely than males to attack peripubescent intruders. During our previous studies with males, juvenile hamsters were exposed to 5–10 bites per day over 20-min periods (Wommack et al., 2003). In this study, juvenile females were exposed to 15–20 attacks per day by adult females over 20-min periods. Two conclusions can be drawn from these observations. First, these observations support our previous suggestion that females are more aggressive than males. Second, females are capable of adapting to repeated social subjugation, even under more severe conditions.

These conclusions are further supported by serum levels of cortisol. At first, exposure to a clean cage as well as exposure to social subjugation by males or females caused a 2- to 3-fold increase in serum levels of cortisol. However, 2 weeks later, these stimuli no longer caused any increase in serum cortisol levels. In males, social subjugation as well as isolation into a clean cage caused a 2- to 3-fold elevation in serum cortisol levels on the first day of stress (Wommack and Delville, 2003). Two weeks later, exposure to social subjugation still caused a 2- to 3-fold elevation of serum cortisol levels, while placement in a clean cage no longer affected these levels (Wommack et al., 2003). These observations may explain the lack of effect of repeated social stress on the development of agonistic behavior in female hamsters. Females were capable of habituating to repeated stress while males were not. It could be argued that upon weaning, juvenile female hamsters will encounter both adult males and females depending on population density. The present study shows that females are capable of adapting to repeated social subjugation by adults. This capacity may be advantageous for females. They would be able to remain within the relative safety of areas that are already populated. In addition, they would also be able to take over territories vacated by deceased adults.

It is also interesting to note that serum levels of cortisol in females ranged between 1 and 2 ng/ml before stress and between 3 and 4 ng/ml after stress on P-27. In males, serum levels of cortisol ranged between 3 and 5 ng/ml before stress and between 10 and 15 ng/ml after stress on P-27. These data suggest another sex difference. The hypothalamo-pituitary-adrenal axis is less active in females than in males.

In summary, the present data describe the development of offensive aggression in female hamsters, as compared to males. Several aspects of development differed between sexes. These differences may point to sex differences in the neural mechanisms controlling the behavior (Joppa et al., 1997). In addition, our data also show that females are more resilient to repeated exposure to social stress than males, and suggest sex differences in the neural mechanisms underlying adaptation to stress. These findings are consistent with previous data showing that females are less susceptible to stress in learned helplessness paradigms (Steenbergen et al., 1989, 1990). Furthermore, this resistance to stress may depend on the stage of the estrous cycle (Jenkins et al., 2001).

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