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Repeated social stress and the development of agonistic behavior: individual differences in coping responses in male golden hamsters

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Abstract

In male golden hamsters, repeated social subjugation during puberty accelerates the development of adult aggressive behavior and enhances its intensity in the presence of smaller individuals. The current study is focused on the characterization of the hormonal and behavioral responses to social subjugation during puberty. Subjugation consisted of daily exposure to an aggressive adult for 20-min periods from postnatal day 28 (P-28) to P-42, while controls were placed into an empty clean cage. Plasma cortisol levels were measured prior to or immediately after treatment on P-28 and P-42. On P-28, exposure to an aggressive adult or a clean and empty cage caused an increase in plasma cortisol levels. However, only social subjugation resulted in elevated cortisol levels on P-42, showing that juvenile hamsters habituate to an unfamiliar environment but not to social subjugation. In addition, we found a relationship between the frequency of submissive responses during social subjugation and the development of aggressive behavior. The transition from play fighting to adult aggression was most accelerated in the least submissive animals. These data show that behavioral response to social subjugation determines the development of aggressive behavior in golden hamsters. Our data also suggest that submissive behavior is a form of coping that attenuates the behavioral consequences of social subjugation in male golden hamsters.

Keywords: Aggression; Cortisol; Play fighting; Puberty; Submission; Development; Chronic stress

1. Introduction

Repeated social subjugation during puberty has unique consequences in male golden hamsters [1]. Subjugated individuals are more aggressive towards smaller targets in early adulthood [2,3]. In addition, social subjugation accelerates the peri-pubertal development of agonistic behavior, as subjugated animals initiate adult-like responses at earlier time periods [3]. These effects contrast with the consequences of repeated social subjugation during adulthood in male hamsters [4]. At that time, social subjugation completely inhibits aggressive behavior, producing a state described as conditioned defeat [5,6]. Similar observations have been reported in socially subjugated adults in other mammals [7]. In adult rats and guinea pigs, social stress decreases aggression, enhances HPA activity, and in extreme cases, causes death [8–12].

In previous studies on adolescent subjugation in hamsters, experimental animals were exposed to an aggressive adult for 20 min daily for 2 weeks while controls were placed in a novel cage during the same time [2,3,13]. However, stress responses were not characterized in these studies. In adult hamsters, single exposure to a social stressor or isolation causes an increase in plasma cortisol levels [14,15]. Long-term enhancement of HPA activity has also been observed in subordinate adults [16] as a consequence of chronic exposure to a social stressor. It is unclear whether the stress responses of hamsters habituate differentially depending on the intensity or type of stressor. It is likely that hamsters are capable of adapting to repeated isolation but not repeated social subjugation. This difference would explain the behavioral observations on the development of agonistic behavior in socially subjugated individuals [1].

Stress responses are not limited to endocrine changes but also include behavioral components. Defeated hamsters display submissive postures [5]. During previous studies on adolescent subjugation, behavioral responses to the

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social stressor were not characterized [2]. In addition, individuals differ in their behavioral responses to stressors, and these differences are associated with unique behavioral outcomes. In rats, different behavioral responses have been characterized during stressful situations [17]. For example, individuals that were likely to respond actively to a shock prod were also likely to be more aggressive [18]. It is possible that in golden hamsters, consequences of social subjugation may be determined by an individual's response to the daily stressor.

The present study is focused on characterizing both behavioral and endocrine responses to social stressors during puberty. In addition, we correlated these responses with the effects of social subjugation on the development of agonistic behavior in male golden hamsters.

2. Materials and methods

2.1. Animals and treatment

The animals (male golden hamsters) were bred in the laboratory from a colony that originated from Harlan Sprague–Dawley (Indianapolis, IN). Five days after birth, each litter was culled to six pups including males and females. On postnatal day 25 (P-25), all animals were weaned and singly housed in plexiglass cages (20 × 33 × 13 cm). Within 2 days following weaning, each animal was briefly (a few seconds) observed in the presence of an adult intruder. Individuals that immediately fled from the adult were considered to be inherently fearful animals (approximately 1 in 12) and were removed from the experiment. All animals received food and water ad libitum and were housed under a reversed light/dark cycle (14 L:10 D, lights on at 9:00 am).

2.2. Experimental design

Repeated exposure to social subjugation was performed according to a previously described protocol [2,3,13]. On P-28, animals were separated into two groups (subjugated, $n = 19$, and control, $n = 16$). Animals in the experimental group were placed in the home cage of an aggressive adult for 20 min daily. Controls were placed in clean empty cages during this time. It is important to note that a single exposure to a clean and empty cage causes a stress response in adult hamsters [14]. Subjugation began on P-28, a time corresponding to the onset of puberty [19], and ended on P-42. By that day, testes had grown to approximately 50% of their weight. Therefore, this period roughly corresponds to the first half of puberty [1]. During the 2 weeks of subjugation, experimental animals were cycled through a group of aggressive adults ($n = 8$) each day. Adult males were screened for aggression in the presence of a smaller and younger intruder. Adults that were not aggressive during this test were not used in the experiment. During daily subjugation, both the number of submissive postures performed by each individual as well as the number of bites/attacks they received from the aggressive adult were recorded. Both groups were tested for offensive aggression in the presence of smaller, younger intruders on P-28, P-35, P-45, and P-70, and agonistic behaviors were observed. Daily subjugation and aggression tests were performed during the second half of the dark phase. All aggression tests were recorded with a Sony digital camera and later reviewed using Macintosh iMovie software. Tests for offensive aggression were conducted approximately 1 h prior to daily subjugation. All animals were weighed twice a week.

2.3. Behavioral observations

2.3.1. Offensive responses

Offensive responses were recorded as previously described during aggression tests on P-35, P-45, and P-70 [3]. For each individual, the total number of attacks, and the percentages of attacks that were play-fighting (PF Att), side, adult (Ad Att), pins, and contact time was observed. Specifically, attacks were defined as an approach to the intruder followed by an attempt to bite. The region of the intruder's body targeted for attack was used to distinguish between PF Atts, side attacks, and Ad Atts. PF Atts were directed towards the face and cheeks of the intruder, while Ad Atts were directed toward the belly and rear. The percent of attacks that were either PF Att or Ad Att were calculated for each individual. Side attacks are directed towards the dorsolateral region of the intruder's trunk, including the flank glands. Pins were scored when the resident attacked the intruder and forced him to remain supine while additional bites were attempted. Contact time was recorded as the total amount of time during the encounter that the resident initiated and maintained contact with the intruder.

2.3.2. Submissive responses

Hamsters respond to social subjugation by performing a number of stereotypical defensive behaviors, including upright postures and submissive behaviors, such as tail-up and on-back postures [5,20]. The most impressive expression of submission is the on-back posture during which individuals remain supine and motionless for up to minutes at a time. Although on-back postures have been observed in adults as an expression of conditioned defeat [5], this behavior is most commonly observed in juvenile hamsters [20]. On-backs were scored only when the intruder lay supine and motionless independently of the resident's actions and were not scored when intruder remained supine while using his forelimbs to divert the attacks of the resident.

2.4. Cortisol assays

We measured the cortisol response to social subjugation after the first defeat (P-28) and after 2 weeks (P-42) of social subjugation. Individuals were sacrificed by rapid decapitation prior to or immediately following a 20-min period in the home cage of an aggressive adult or a clean and empty cage ($n=7-9$ per group). After decapitation, trunk blood samples were collected and centrifuged at 5000 rpm for 5 min. Sera were saved at -20 °C. We assayed cortisol levels to determine the hormonal response of juveniles to a single exposure and repeated exposures to a social or nonsocial stressor. All assays were performed with Cortisol Correlate-EIA kits (Assay Designs, Ann Arbor, MI). Each sample was assayed in duplicate from 10-μl aliquots. Intra-assay variability was 9.0%. Inter-assay variability was 13.8%.

2.5. Data analysis

On P-28 and P-42 separately, plasma cortisol levels were compared between groups with ANOVAs followed by Fisher's PLSD post hoc test. Pearson's correlations were performed between behavior displayed during subjugation (on-backs and bites/attacks received) and plasma cortisol levels in subjugated individuals on P-42, separately for baseline and post-defeat levels. Additionally, we looked for correlations between behavior during daily subjugation and aggressive behavior on each day of testing (P-35, P-45, P-70). Pearson's correlations were again used for parametric data (e.g., %PF Att) while Spearman's rank correlations were performed for nonparametric data (e.g., number of attacks). Bonferroni corrections were applied to adjust the P values for multiple uses of data. If the correlation was significant, then individuals were separated into two subgroups: above and below the group average. Behavioral responses and aggressive behaviors were then compared between subgroups by Student's t tests or ANOVAs followed by post hoc tests (when including controls).

3. Results

3.1. Cortisol response

On P-28, exposure to either an aggressive adult or a clean and empty cage resulted in a threefold increase in plasma cortisol concentrations from baseline [$F(2,22)=4.6$, $P<.05$, ANOVA] (Fig. 1). The elevations were statistically significant in isolated animals ($P<.01$, Fisher's PLSD). Following 2 weeks of daily subjugation, significant differences were again observed between groups [$F(3,27)=3.7$, $P<.05$]. Exposure to an aggressive adult resulted in a threefold increase in plasma cortisol ($P<.05$). However, individuals exposed to a clean and empty cage on this day showed no increase in cortisol from baseline concentrations (Fig. 1). Importantly, on P-42, baseline cortisol concentrations did not differ between groups. In this experiment, no significant correlations were found between cortisol concentrations and daily on-backs.

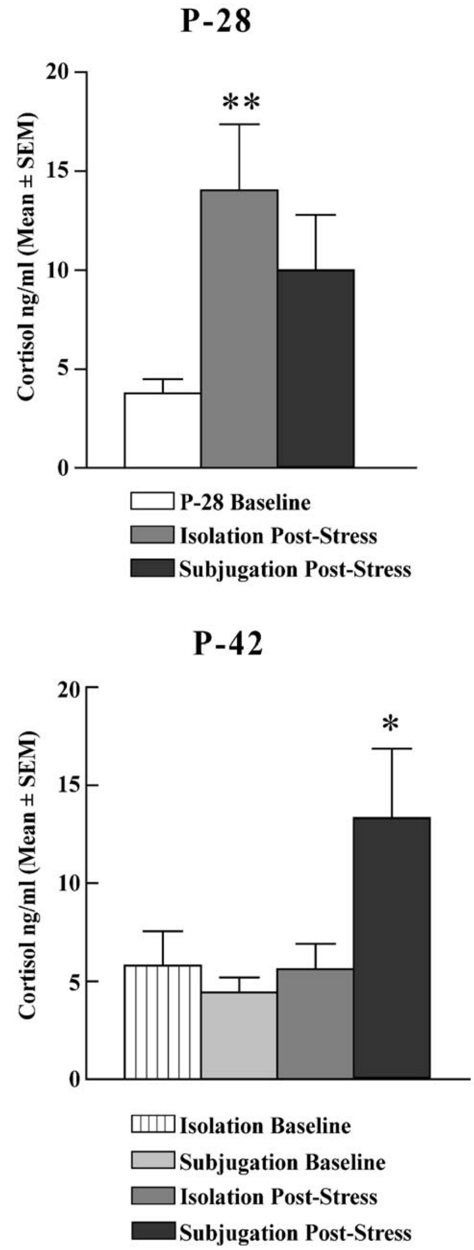

Fig. 1. Plasma levels of cortisol were assayed in samples collected just before (baseline) or just after (post-stress) a 20-min exposure to a clean empty cage (isolation) or an aggressive adult (subjugation). Post-stress cortisol levels were compared with the appropriate baseline cortisol levels after the first exposure to isolation or subjugation (P-28) or following 2 weeks of daily exposures (P-42) ($n=7-9$ for each group), * $P<.05$; ** $P<.01$, Fisher's PLSD as compared to baseline.

3.2. Individual differences in submissive behavior

Correlations were attempted between averages of on-backs performed and bites/attacks received during daily subjugation from P-28 to P-35 and offensive responses tested on P-35. These correlations were not statistically significant.

Correlations were also attempted between averages of on-backs performed and bites/attacks received during daily subjugation from P-28 to P-42 and offensive responses on P-45 and P-70. Most behavioral data from the tests performed on P-45 and P-70 did not correlate significantly with the daily averages of on-backs and bites/attacks recorded during subjugation. Nevertheless, there were two exceptions from behavior recorded on P-45. On that day, the average number of on-backs performed by the hamsters was positively correlated with PF Att (r=.753, $P<.001$, Bonferroni) and negatively correlated with Ad Att ($r=-.704$, $P<.001$, Bonferroni). The distribution of the daily averages of on-backs appeared to be bipolar, with two separate subpopulations. As such, individuals with daily on-back averages lower than the group mean (2.6 ± 1.18) were placed into a low on-back (LOB) ($n=9$) subgroup, while individuals with averages higher than the group mean were placed into a high on-back (HOB) subgroup ($n=10$). Overall, HOB animals were at least twice as likely to perform on-backs during daily subjugation as compared to LOB individuals (Fig. 2). This difference was statistically significant [$t(17)=-7.11$, $P<.001$, Student's t test].

The percentages of PF Att and Ad Att differed strongly between LOB individuals and controls during tests on P-45 (Fig. 3). HOB individuals were intermediary between LOB and controls. Comparisons between LOB, HOB, and control groups showed statistically significant differences in both percentages of PF Att and Ad Att [respectively, $F(2,32)=15.0$, $P<.001$; $F(2,32)=14.9$, $P<.001$]. LOB individuals performed a lower percentage of PF Att than both HOB and control animals ($P<.01$). LOB individuals also displayed a higher percentage of Ad Att than HOB and control hamsters ($P<.01$). Finally, HOB individuals also performed a lower percentage of PF Att than control animals ($P<.05$).

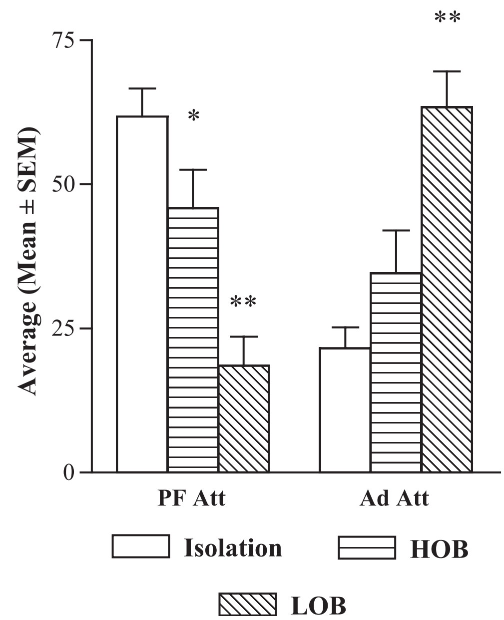

Fig. 3. Percentages of attacks that were either PF Att or Ad Att during tests performed in the presence of a smaller and younger intruder on P-45. Comparisons were made between animals that were repeatedly placed into an empty clean cage (isolation, $n=16$) and animals exposed to aggressive adults (LOB, $n=9$, and HOB, $n=10$) separated according to daily averages of on-backs performed during subjugation from P-28 to P-42. * $P<.05$; ** $P<.01$, Fisher's PLSD, as compared to isolation.

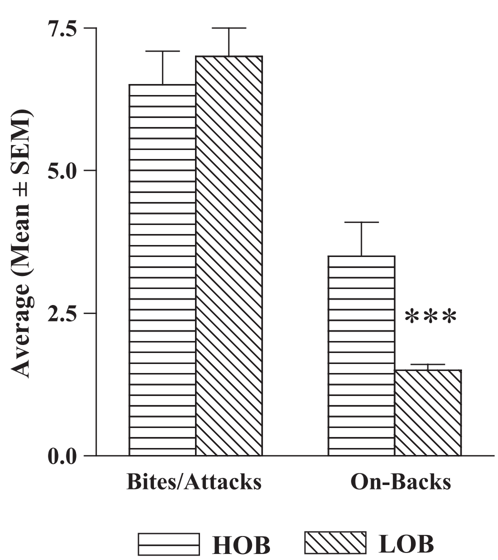

Fig. 2. Daily averages of bites/attacks received by juveniles and on-backs performed during social subjugation from P-28 to P-42. Subjugated animals were distributed into two subgroups according to the number of on-backs they performed during daily subjugation, HOB ($n=10$) and LOB ($n=9$). *** $P<.001$, Student's t test, two-tailed.

The degree to which an individual was submissive did not appear to affect the amount of physical subjugation received from the aggressive adult during subjugation. The daily averages of bites/attacks received during subjugation were similar between HOB and LOB subgroups (Fig. 2).

4. Discussion

The initial exposure to either a social or nonsocial challenge was a stressful experience for juvenile hamsters. After the first exposure (P-28) to either a novel cage or an aggressive adult, juveniles had elevated plasma cortisol levels. The cortisol elevations observed in juveniles following a single exposure to a novel environment or social defeat are consistent with previous findings in adult hamsters [14,15]. After 2 weeks, though, exposure to a clean and empty cage no longer resulted in increased plasma cortisol levels. Only subjugated individuals maintained the threefold increase from baseline as observed after the first exposure. Thus, the ability of juvenile hamsters to habituate to repeated exposure to an unfamiliar environment but not repeated social subjugation is the primary difference between these two stressors. Additionally, no differences in baseline cortisol levels were observed between groups following 2 weeks of subjugation. Although these data are consistent with previous reports of lesser vulnerability to chronic subjugation in juveniles [2,3], they also contrast with the cortisol elevations observed in chronically subjugated adult hamsters [16]. The cause of this developmental difference is unclear at this time.

Exposure to aggressive adults did not solely result in increased plasma levels of cortisol. Juvenile hamsters displayed extremely submissive postures in the presence of an aggressive adult. They remained on their back for extended periods of time, exposing their belly to their aggressors. This on-back submissive behavior was the most impressive display performed by the juveniles during subjugation. Interestingly, a highly significant correlation was found between the daily averages of on-back responses and the development of agonistic behavior, particularly on P-45. The most submissive animals were most likely to behave like the controls in the presence of an intruder, as they were most likely to use PF Atts. In contrast, the individuals that were least likely to perform on-backs during subjugation were most likely to perform adult attacks when paired with a smaller intruder, thus making them much different than control animals. Our previous data showed that social subjugation accelerates the development of agonistic behavior [3]. The present data show that the submissive responses to the aggressive adults determine the outcome of the subjugation. In this case, the least submissive animals were most likely to have an accelerated development of their agonistic behavior.

It is important to note that no significant correlation was observed with the aggressive behavior tested on P-70. This finding indicates that the role of submissive responses during subjugation is specific to the development of agonistic behavior, from play fighting to adult-like aggression. In addition, it is also interesting to note that no correlation was found with the average numbers of bites/attacks received from the adults during subjugation. This other finding emphasizes the importance of behavioral responsiveness to subjugation (i.e., submissive behavior) as a factor in determining the development of agonistic behavior. Coping has been defined as a conscious or unconscious effort to diminish stressor intensity and/or endure the experience as painlessly as possible [21]. As such, it could be argued that the on-back submissive posture is a behavioral coping mechanism, as individuals that perform more submissive postures are less affected by social subjugation. The basis for individual differences in submissive behavior will be closely examined in future experiments.

During these studies, we found a behavioral difference to stress responsiveness to be predictive of differences in later behavior. Behavioral differences in stress responsiveness have been found in a number of studies [10,17]. However, these differences have been observed following the primary stressor. For example, in the Blanchard studies, subordinate rats housed in a visible burrow system were characterized as stress responsive or nonresponsive based on observations outside of the social environment [12]. On the other hand, one study reported that differences in subordinate behavior directly correlated with outcomes of future social encounters [22]. Rats were housed in a triad. In this configuration, a dominant and two subgroups of subordinates were observed. One subgroup of subordinates engaged in a great deal of playful contact with the dominant while the other subgroup was avoidant. Interestingly, once the dominant rat was removed from the triad, the individual that was less playful became dominant of the other, more playful, subordinates. As such, this study showed that individual differences in behavioral responsiveness to social stressors influence the outcomes of future aggressive encounters.

Previous results showed that repeated exposure to social subjugation accelerates the development of agonistic behavior and enhances aggressive responses toward smaller individuals [2,3]. It has been argued that the significance of these results can be found within the effects of high population density on aggressive behavior [1]. Indeed, increased population density has been associated with enhanced aggression in mice [23–26]. Under high density of population, juvenile hamsters would then initiate territorial behavior faster and become more aggressive as adults [1]. Our new data show individual differences in responsiveness to social subjugation. This observation suggests that two subpopulations of juvenile hamsters exist. The least submissive individuals will show the greatest acceleration in the development of their agonistic behavior and would be most likely to attempt establishing or colonizing a new territory at the periphery of the population of adult males. In contrast, the most submissive individuals would be most likely to remain in the proximity of the adults while keeping their capacity to become territorial at later times. Each strategy could be beneficial depending on environmental pressures.

In conclusion, social subjugation during puberty causes significant hormonal and behavioral effects. Social subjugation is a stressor to which individuals are incapable of hormonally adapting. Importantly, the effects of repeated social stress were limited to immediate HPA activations, as subjugation did not affect baseline cortisol levels in juvenile hamsters. Individual response strategies to subjugation are predictive of how greatly an animal will be affected by this stressor, as highly submissive juveniles are considerably less affected by social subjugation than nonsubmissive individuals. These differences suggest that submissive behavior is some form of coping and may attenuate physiological responses to social stress other than the immediate cortisol response. Future studies will address this issue and will also focus on distinguishing neurobiological factors associated with individual differences in responsiveness to social subjugation.

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