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Development of social play in hamsters: sex differences and their possible functions

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Abstract

In several rodent species social play appears to be necessary for proper deployment of species-specific patterns of aggressive and reproductive behavior. Specifically, in male Syrian hamsters (*Mesocricetus auratus*), play has been linked to the development of adult aggression. We quantified several types of social play behavior in same-sex peer groups of Syrian hamsters three times per week for three consecutive weeks after weaning, which included postnatal days 22–42 (PD22 to PD42). Male hamsters increased playful contact during PD36–PD42, whereas females showed peak playful contact during PD29–PD35. These findings suggest that the motivation for social play increases during mid-adolescence in males, but dissipates in females. To investigate the effects of social play deprivation, one hamster per litter remained pair-housed with its mother for three weeks after weaning its littermates. In adulthood, both play-deprived and play-exposed animals received acute social defeat stress followed by social interaction testing. Play deprivation led to increased defeat-induced social avoidance in both males and females. In males, play deprivation increased fighting back during social defeat stress, whereas in females it reduced aggressive behavior during conditioned defeat testing. We suggest that social play deprivation disrupts neural circuits regulating aggression in a sex-specific manner, perhaps related to sex differences in territorial defense, but has similar effects on neural circuits regulating stress responsivity. Overall, these findings suggest that juvenile social play functions to promote coping with stress and appropriate social behavior in adulthood.

Keywords

social play; play deprivation; aggression; stress; social defeat; Syrian hamster

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1. Introduction

Play among juveniles is an important feature of the behavioral development of many different species, including humans, non-human primates, other mammals and birds (Burghardt, 2005). There are multiple proposed functions of such juvenile social play, although they are not mutually exclusive, such as facilitating muscle growth, strengthening social relationships, establishing dominance, and practicing adult activities including aggression, sex, and foraging. However, few have received empirical support. Play involves many different types of behaviors including motor and coordination effects, social interactions and similarities to adult fighting and aggression (Graham and Burghardt, 2010; Pellis, Pellis, and Bell, 2010). Some social play behaviors include submissive behaviors, such as laying on one's back or side, and others include more aggressive or dominance behaviors such as chasing, biting, or pinning. Given the diversity in social play, it is unlikely that a common function exists among animals differing widely in phylogenetic position, life history, ontogeny, and sociality (Burghardt and Pellis, 2018). Here we present evidence documenting that play deprivation in juvenile hamsters has different effects on males and females, in spite of having similar effects on defeat-induced avoidance of conspecifics and demonstrates that play is embedded deeply in ecological and social system organization.

One empirically supported hypothesis on the function of social play in rodents is that it leads to structural and functional changes in brain regions that enable the display of appropriate and adaptive behavior in adulthood (Pellis, Pellis, and Bell, 2010; Vanderschuren et al., 2016). The prefrontal cortex regulates those aspects of social play that allow animals to respond appropriately in specific contexts. Rats with lesions of the orbitofrontal cortex fail to modify play behavior in response to the identity of their social partner (Pellis et al., 2006). Lesions of the medial prefrontal cortex (mPFC) reduce the complexity of defensive tactics during play fighting in rats, which shortens the duration and complexity of play bouts (Bell et al., 2009).

Additionally, temporary pharmacological inactivation of frontal cortical regions in rats reduces social play (Van Kerkhof et al., 2013). Play deprivation during the juvenile period leads to alterations in the dendritic morphology of cortical neurons. Play deprived animals have longer apical dendrites in the mPFC compared to play-exposed animals, suggesting that play deprivation disrupts the pruning process (Bell et al., 2010).

Play deprivation can also lead to inappropriate social behavior in adulthood. In rats, social isolation early in life reduces affiliative behavior with conspecifics in adulthood (Hol et al., 1999) leading to inappropriate forms of aggressive behavior in adulthood. Isolated rats show longer latencies to submit to a more aggressive animal in a social defeat encounter, and fail to show normal avoidant behavior following social defeat stress (van den Berg et al., 1999; von Frijtag et al., 2002). Opportunities for social play with juvenile peers alleviates many of the consequences of social isolation (Einon et al., 1978; Panksepp et al., 1984). Consistent with animal research, children that are deprived of social play exhibit more violent behavior later in life (Frost and Jacobs, 1995), suggesting that opportunities for social play reduce impulsive and inappropriate behavior in adulthood.

In laboratory rodents, social play consists of frequent bouts of play fighting. Play fighting differs from adult aggression in that juveniles bite the face and neck of their opponents, whereas adults preferentially attack the flanks (Pellis and Pellis, 1987; Pellis and Pellis, 1988a). Juveniles are also more likely to counter-attack their partner as compared to adults, leading to role reversals in play fighting (Pellis and Pellis, 1990). Furthermore, juvenile rodents use defensive tactics that prolong play bouts and allow for increased rates of attack (Cervantes et al., 2007). These characteristics suggest that while play fighting might prepare animals for adult aggression, the development of aggressive behavior is not its sole purpose; social play also is involved in the ontogeny of sensorimotor development and an array of social, cognitive, and emotional behavior (Vanderschuren and Trezza, 2013).

In the late juvenile period, studies of the role of play fighting in the formation of dominance relationships have mixed results. While in marmots social dominance in adulthood is correlated with outcomes of juvenile social play (Blumstein et al., 2013), in meerkats the frequency of juvenile social play does not influence social cohesion, aggression or dispersion (Sharpe 2005a; Sharpe and Cherry 2003; Sharpe, 2005b). In rats, dominant males exhibit adult-like patterns of play, whereas subordinates vary their play depending on the identity of their social partner, suggesting that subordinates use social play to build relationships with dominants (Pellis and Pellis, 1991). In Syrian hamsters (*Mesocricetus auratus*), however, dominant hamsters show a higher rate of play fighting than subordinate hamsters (Pellis and Pellis, 1993). In addition, juvenile hamsters show elevated neural activity following play fighting in brain regions activated following offensive aggression in adults, such as the anterior hypothalamus, ventrolateral hypothalamus, bed nucleus of the stria terminalis, medial amygdala, and mPFC (Cheng et al., 2008). These findings support the view that offensive components of play fighting promote the development of neural circuits that control adult aggression in hamsters.

In most mammals, males engage in more rough and tumble play than females (Burghardt, 2005) and this is also true in human children (DiPetro, 1981). Guerra et al., (1992) studied Syrian hamster play behavior in both same and opposite sex pairs and found that male-female pairs showed less play fighting than same-sex pairs. However, they studied social play for less than a week; how rates of play in male-female pairs change throughout adolescence is thus unknown. Since female hamsters exhibit high rates of territorial aggression in adulthood (Payne and Swanson, 1970), as compared to males, it is important to revisit with more detail the issue of sex differences in this species and its possible role in the neural mechanisms underlying play.

We tested for sex differences in social play in Syrian hamsters by recording bouts of play fighting during the first three weeks post-weaning, which represent postnatal days 22–42 (PD22–42) and are a time of peak social play (Cervantes et al., 2007). To test potential functions of social play deprivation without social isolation, we play deprived male and female hamsters by pair-housing them with their mother for three weeks after weaning their littermates, as mothers rarely, if ever, play with their offspring (Guerra et al., 1999). Animals housed with same sex littermates had ample play opportunities. In adulthood (PD70), we exposed hamsters to social defeat stress to determine whether social play altered how animals respond to stress in adulthood. In Syrian hamsters, exposure to acute social defeat

stress leads to a complete loss of territorial aggression and elevated submissive and defensive behavior when tested in a novel social encounter the next day and is termed the conditioned defeat response (Huhman et al., 2003). Previously, we reported that the play deprivation in male hamsters leads to an elevated conditioned defeat response, longer apical dendrites in mPFC pyramidal neurons, and alters aggressive behavior in males, as play-deprived animals were more likely to counter-attack the trained aggressor during social defeat exposure compared to play-exposed animals (Burleson et al., 2016). Overall, the aim of the present study was to identify any sex differences in social play, the effects of play deprivation on responses to social defeat stress in female hamsters, and compare these results with previously reported findings in male hamsters.

2. Results

2.1 Social Play

In males, time spent in social play increases during PD36–42 whereas, in females, social play peaks in PD29–35 (Figure 1). We found a significant interaction between sex and week in for the amount of time spent in playful contact during PD22–P42 ($F_{(2,49.3)}=6.27$, $P=.004$). When examining individual behaviors regardless of sex, there is a significant effect of duration of play for component 3 that contained pins and head attacks ($F_{(2,72.01)}=3.27$, $p=0.044$). Overall, pins and head attacks developed slowly, peaked during PD29–35, and then decreased in relative frequency (Figure 2). There were no effect of sex on principle component 3 or on the other principal components that contained the other play behaviors (Table 2).

2.2 Conditioned Defeat Response

We quantified the aggressive behavior subjects received during social defeat to determine whether play deprivation altered the social defeat experience. Play-exposed (females: 451.3 ± 12.7 sec; males: 224.4 ± 9.6 sec) and play-deprived (females: 429.7 ± 16.8 sec; males: 200.5 ± 15.4 sec) animals did not significantly differ in the amount of aggression received during social defeat stress ($F_{(1,39)}=2.59$, $P=0.12$). Because play-deprived and play exposed animals did not significantly differ in the amount of aggression received, differences in their responses to social defeat are unlikely due to differences in the social defeat experience itself. Interestingly, females received more aggression during social defeat than males ($F_{(1,39)}=278.77$, $P=.0001$), consistent with previous research showing that female hamsters are more aggressive than males (Rosenhauer et al., 2017). To assess whether play deprivation altered how animals respond to aggression, we recorded whether animals fought back against the resident aggressors during the first social defeat episode. In females, 4 of 9 play-deprived animals fought back against the resident aggressor, whereas 4 of 13 play-exposed animals fought back ($\chi^2=0.14$, $P=.63$). In contrast, 4 of 10 play-deprived males fought back against the resident aggressor and 0 of 11 play-exposed males fought back ($\chi^2=5.44$, $P=.02$).

Both male and female play-deprived animals showed an elevated conditioned defeat response compared to play-exposed animals (Figure 3). Specifically, there was a significant defeat x social play interaction indicating that play-deprived animals showed greater defeat-

induced increase in submissive and defensive behavior compared to play-exposed animals ($F_{(1,68)} = 11.48$, $P = 0.001$, Figure 3a). The effect of play deprivation on submissive and defensive behavior was statistically significant in both females ($F_{(1,33)} = 5.99$, $P = 0.020$) and males ($F_{(1,35)} = 6.16$, $P = 0.018$). With regard to aggressive behavior, we found a significant sex x social play interaction ($F_{(1,68)} = 9.62$, $P = 0.003$, Figure 3b). Play-deprived females showed less aggression compared to play-exposed females ($F_{(1,33)} = 8.76$, $P = 0.005$), while social play experience did not significantly alter aggression in males ($F_{(1,35)} = 2.29$, $P = 0.14$). We also found a significant sex x social play interaction in the duration of affiliative behavior ($F_{(1,68)} = 12.52$, $P = 0.001$, Figure 3c). Play-deprived females showed greater affiliative behavior than play-exposed females in non-defeated conditions ($F_{(1,33)} = 5.35$, $P = 0.027$), while social play experience did not alter affiliative behavior in males ($F_{(1,35)} = 0.59$, $P = 0.45$). Finally, we found a significant sex x defeat interaction in the duration of nonsocial behavior ($F_{(1,68)} = 8.05$, $P = 0.006$, Figure 3d). Social defeat increased nonsocial behavior in both play-exposed and play-deprived females ($F_{(1,33)} = 12.99$, $P = 0.001$), whereas social defeat did not lead to changes in nonsocial behavior in males ($F_{(1,35)} = .09$, $P = 0.77$). Overall, these data indicate that play deprivation had a similar effect on submissive and defensive behavior in males and females, however the effects on other types of behavior were sex-specific.

2.3 Aggression and Play

To examine the relationship between juvenile social play and adult aggressive behavior we correlated the amount of aggression that non-defeated animals showed during their social interaction test with the amount of social play they showed as juveniles. In both male and female animals, we found that the duration of aggression was negatively correlated with both the amount of time spent in playful contact ($r = -.585$, $p = .014$, $n = 17$, Figure 4a) and the number of ventrum attacks ($r = -.531$, $p = .028$, $n = 17$, Figure 4b). The other behaviors that were observed had no significant correlations with the amount of aggression (Table 3). Overall, hamsters that spent more time playing and attacked the ventrum more often showed less territorial aggression (Figure 4). Interestingly, these correlations appear due to sex differences in patterns of social play and adult aggression. Juvenile males showed more social play than juvenile females, whereas adult females showed more aggression than adult males. With regard to the animals exposed to social defeat, there were no significant correlations between juvenile social play and the amount of submissive and defensive behavior shown at conditioned defeat testing.

3. Discussion

This study identified sex differences in the pattern of adolescent social play and the consequences of play deprivation in Syrian hamsters. Female hamsters show a peak in playful contact during PD29–35, whereas male hamsters show elevated rates of playful contact during PD36–42. However, with regard to play behavior that is unique to juveniles (principle component 3), both male and female hamsters show a peak in the rate of pins and head attacks during PD29–35. Consistent with condition defeat responses in males (Burleson et al., 2016), female hamsters deprived of opportunities for social play during adolescence showed an elevated conditioned defeat response following acute social defeat

stress in adulthood. In addition, play-deprived male hamsters responded to social defeat stress by fighting back against the aggressor (Burleson et al., 2016), while play-deprived females showed reduced aggressive behavior during conditioned defeat testing.

Social play in Syrian hamsters can be organized into three components; 1) attacks and full supine postures, 2) behaviors prolonging participation such as partial supine, and 3) behaviors characteristic of juvenile forms of playful aggression such as head attacks and pins. Although males and females did not differ in the overall rate of attacks and supine postures, juvenile forms of playful aggression peaked during PD29–35 in both males and females. These findings are consistent with reports in male rats and hamsters that attacks, particularly those directed at the head and neck, decrease with age (Pellis and Pellis, 1987; Taravosh-Lahn and Delville, 2004). However, a juvenile peak in the rate of attacks and pins is less clear in female hamsters. When tested with smaller, same-sex intruders throughout development, female hamsters do not show a clear peak in attacks and pins (Taravosh-Lahn and Delville, 2004). However, when we observed same-sex, peer groups of female hamsters in their home cages, we found that the rate of head attacks and pins peaks during PD29–35 in both females and males. Males, however, spent more time in playful physical contact during PD36–42 compared to females, consistent with previous research showing that males contact each other more during the juvenile period (Guerra et al., 1992; Taravosh-Lahn and Delville, 2004). Overall, these findings indicate that the transition from play fighting to adult-like forms of aggression occurs earlier in female hamsters than in males.

We found that both playful physical contact and ventrum attacks were negatively correlated with amount of aggression directed with novel intruders in adulthood. Although these correlations suggest that social play during adolescence is negatively associated with aggressiveness in adulthood, the correlations primarily result from sex differences in rates of aggression and social play. When comparing mean rates of social play for the entire three-week study period, females tended to show lower levels of playful contact and ventrum attack than males. Adult females were also much more likely than males to show aggression toward a novel, same-sex intruder in adulthood. Together, these results are consistent with previous research showing that male hamsters display more social play than females as young juveniles, whereas females display more territorial aggression than males as adults (Huhman et al., 2003; Taravosh-Lahn and Delville, 2004).

Although it is not known whether sex differences in social play contribute to sex differences in adult aggression, it is noteworthy that similar brain regions and neurochemicals regulating both social play and aggression. In male hamsters, play fighting and adult aggression lead to elevated neuronal activity within a similar neural network, including in vasopressin cells in the anterior hypothalamus (Cheng et al., 2008). Vasopressin acts in the anterior hypothalamus to promote both social play and adult aggression in hamsters (Cheng and Delville, 2009; Ferris et al., 1997). These findings suggest the possibility that play fighting might specifically regulating the development of neural circuits that control aggression in adult hamsters. Syrian hamsters are solitary, territorial rodents and it is perhaps not surprising that social play supports the development of territorial aggression. In species that living in complex social groups, social play during adolescence may modify social competency and thereby alter a range of adult social behavior, including affiliative, sexual,

and agonistic behavior. Interestingly, in rats, the vasopressin system regulates the development of social play in a sex-specific manner (Paul et al., 2014; Veenema et al., 2013). Overall, sex differences in social play might lead to differential development of the neural circuits and neurochemicals that regulate adult social behavior.

Early studies investigating the development of social behavior indicated that prolonged social isolation leads to increased aggressiveness (Luciano and Lore, 1975). Subsequent studies confined social isolation to periods of peak social play and found that socially isolated rats no longer show enhanced fearfulness as well as offensive aggression, although they exhibit hyper-defensive behavior (Einon and Potegal, 1991). Early social deprivation leads to an increased frequency and intensity of shock-induced aggression, although isolated juveniles given opportunities for play fighting did not show such changes in defensive aggression (Potegal and Einon, 1989). Play deprivation in rats disrupts appropriate responses to aggression because play deprived juvenile males do not readily display submissive postures during an aggressive encounter with a larger opponent in adulthood (van den Berg et al., 1999). Similarly, play-deprived rats do not modify ongoing social and nonsocial behavior when confronted with a larger aggressive animal in adulthood, which leads to their sustaining more severe aggression and elevated wounding (Einon and Potegal, 1991; Von Frijtag et al., 2002). These studies are consistent with our previous finding that play deprived male hamsters are more likely to fight back against larger, resident animals in a social defeat encounter and suggest that play deprivation disrupts appropriate responses to aggression (Burleson et al., 2016). Surprisingly, play deprivation did not increase the likelihood of female hamsters fighting back against resident aggressors during social defeat encounters. In fact, in non-defeated females, play deprivation increased affiliative behavior and decreased aggression in a social interaction with a smaller intruder. These findings suggest a sex difference in the effects of play deprivation, in which play deprivation leads to a hyper-defensive state and inappropriate responses to aggression in males but not females. Because male and female rodents have subtle differences in the neural circuits controlling aggression (Hashikawa et al., 2018), it is possible that these circuits are differentially affected by play deprivation.

In this study, social play deprivation in adolescence was associated with an elevated conditioned defeat response in adult female hamsters. We have previously showed that in male hamsters, play deprivation increases the conditioned defeat response and disrupts the pruning of apical dendrites in the mPFC (Burleson et al., 2016). Structural changes in the mPFC can have long-lasting effects on social and emotional behavior because in adulthood mPFC activity is associated with regulation of impulsive aggression (De Bruin et al., 1983), negative feedback on the neuroendocrine stress response (Radley et al., 2009), resistance to the development of learned helplessness (Maier and Watkins, 2010; Christianson et al., 2014), and extinction of conditioned fear (Do-Monte et al., 2015; Giustino and Maren, 2015). In male hamsters, dominant animals show a reduced conditioned defeat response and elevated c-Fos expression in mPFC neurons that send efferent projections to the basolateral amygdala (BLA), suggesting that neural activity in BLA-projecting mPFC neurons promotes resistance to the conditioned defeat response (Dulka et al., 2018). A key next step would be to determine whether impaired pruning of apical dendrites during adolescence disrupts defeat-induced neural activity of BLA-projecting mPFC neurons. Although neuroanatomical

data are not available for female hamsters in this study, our current findings suggest that social play functions to promote stress coping in both male and female hamsters. Overall, these findings support the view that social play promotes structural plasticity in the mPFC and thereby affects emotional self-regulation in adulthood (Leussis and Andersen, 2008; Bell et al., 2010; Baarendse et al., 2013).

The sex differences in social play identified in this study are consistent with the view that male hamsters show higher rates of social play during early adolescence and that females transition from play fighting to adult forms of aggression more quickly than do males. We also found a sex difference in the effect of play deprivation on certain aspects of agonistic behavior. Although play deprivation alters how males respond to aggression from larger opponents, we found that play deprivation did not increase defensive aggression in females. On the other hand, we found that play-deprived females show an elevated conditioned defeat response, suggesting that play deprivation disrupts the ability to cope with social defeat stress in both females and males. Future studies should address the possibility that the neural circuits regulating aggressive behavior are differentially influenced by play deprivation in males and females, but that the neural circuits that control coping with social stress are not. This study, along with an increasing number on a wide range of species and play types, are documenting the complexity of understanding the consequences and adaptive functions of play in ontogeny and the critical role of studying the neural bases of such play diversity.

4. Methods

4.1 Subjects

Syrian hamsters (*Mesocricetus auratus*) obtained from our breeding colony (derived from animals purchased from Charles River Laboratories, Wilmington, MA) were housed in polycarbonate cages (12 cm × 27 cm × 16 cm) containing corncob bedding, cotton nesting materials, plastic shelters, paper cups, and a wire mesh top. Food and water were available *ad libitum* and cages were in a temperature-controlled colony room (20 °C) on a 14:10 hour light/dark cycle with lights off at 1:00 pm. Behavioral observations occurred in a testing room during the first three hours of the dark phase of their light cycle. Handling animals periodically habituated them to any stress from human contact or during transport from the colony to the testing room. All procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee and are in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

4.2 Play Deprivation and Play Exposure during Adolescence

We targeted PD22-PD42 for play deprivation because social play peaks during this juvenile period in Syrian hamsters (Cervantes et al., 2007; Pellis and Pellis, 1988b). Control male and female hamsters were weaned at PD21 into same-sex peer groups of three animals per cage (n=21 males, n=24 females). One pup per litter remained pair-housed with their mother until PD42 (n=18 males, n=15 females). We housed juveniles with their mothers after the typical weaning age to play deprive them without the potential confounding effects of complete social isolation, a method that was totally effective as we never observed social play in

mother-pup dyads of either sex. Play deprived animals were rehoused with their littermates on PD43.

To quantify social play in the non-deprived juveniles, a subset of animals from 6 litters (n=18 males, n=18 females) were video recorded in their home cage under red light conditions for 15 minutes three times per week. Observers later coded social play from video files using an established ethogram derived from previous research on rats and hamsters (Pellis and Pellis, 1987; Pellis and Pellis, 1993; Table 1). To assess reliability, two coders trained on a novel set of videos until they achieved reliability on each behavior described in the ethogram and the average inter-observer spearman correlation was .795 $\pm$ 0.221.

4.3 Social Defeat Stress and Agonistic Behavior in Adulthood

In early adulthood (PD63), animals were housed individually so that they could establish a territory before social defeat exposure. Because the conditioned defeat response is characterized by the loss of territorial aggression, it is necessary for subjects to establish territories prior to social defeat stress. On PD70, animals were exposed to social defeat stress (n = 21 males, n = 22 females) or non-defeat control procedures (n = 18 males, n = 15 females). Data on male hamsters reported previously are presented here to assess sex differences (Burleson et al., 2016). Social defeat stress involved exposure to a same-sex animal that was older and larger (>4 months old and >190 g). Resident aggressors were prescreened to ensure that they reliably attacked and defeated the subjects. The social defeat procedure consisted of three, 5-min aggressive encounters in the resident aggressor's home cage, with a 5-min inter-trial interval during which the subject was returned to its home cage. To correct for potential variation in latency to submit, the first defeat episode did not begin until after the subject submitted to the first attack of the resident aggressor. The second and third defeat episodes were less variable because the subject submitted immediately. No defeat control animals were placed in the empty home cages of three separate resident aggressors for 5-min each. Empty cage exposure controls for the olfactory cues associated with social defeat episodes, but does not increase plasma glucocorticoid levels (Dulka et al., 2018). Social defeat encounters were video recorded and we quantified the total duration of aggression and the total number of attacks subjects received. We also recorded whether subjects fought back against the resident aggressor during the first social defeat encounter. We carefully monitored aggressive encounters for wounding and animals receiving minor scratches were treated with antiseptic solution. Two females wounded during social defeat stress were excluded from analysis.

We tested animals for a conditioned defeat response 24 hours after social defeat stress. Conditioned defeat testing consisted for a 5 min social interaction test during which a non-aggressive intruder was placed in the subject's home cage. Non-aggressive intruders were younger, play-exposed animals that displayed affiliative and nonsocial behavior during testing. All testing sessions were video recorded and the behavior of each subject quantified using Noldus Observer by a researcher blind to treatment conditions. We quantified the total duration of submissive and defensive behavior (flee, avoid, upright and side defensive posture, tail-up, and stretch attend), aggressive behavior (attack, chase, and upright and side

offensive posture), affiliative behavior (sniff and approach), and nonsocial behavior (locomotion, grooming, nest building, and feeding) (Albers et al., 2002). Researchers trained to quantify agonistic behavior on a separate set of videos and achieved greater than 90% agreement on the duration of submissive, defensive, and aggressive behavior.

4.4 Statistical Analysis

Behaviors from the video were averaged across observation days into their corresponding week, with a total of three weeks. In order to correct the time that the hamsters were not playing, we divided the individual behaviors by the time that the hamsters played to create a ratio of behaviors per second of active play. The individual behaviors subjected to a principal component analysis in order to account for correlated behaviors. The resulting three components were rotated with a varimax rotation to simplify the loadings of the components. The three components cumulatively explained over 70 percent of the variance (Table 1). Mixed model ANOVAs with cage (the peer group of three) as a random factor were used to analyze the relationship between the three components and total playtime with the sex and week in order to examine whether there was a difference in the development of play behaviors over time and between sex. Two-way and three-way ANOVAs were used for analysis of amount of aggression received during social defeat and behavior displayed during conditioned defeat testing, respectively. For defeated animals, we calculated Pearson correlation coefficients for the amount of submissive and defensive behavior shown at testing and measures of juvenile social play. For non-defeated animals, similar correlations were calculated using the amount of aggression they showed at social interaction testing.

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Highlights

Male hamsters display juvenile play fighting for a longer period than females

Play deprivation alters the expression of adult aggression in a sex-specific manner

Play deprivation increases defeat-induced social avoidance in both males and females

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Figure 1: Social play was recorded in same-sex peer groups for three weeks post-weaning. The mean ($\pm$ SE) time spent in playful contact is shown for males ($n=18$) and females ($n=18$) during PD 22–42. Males spent more time in playful social contact during PD36–42 than females (* $p < .05$).

Figure 2:

The average scores for principle components 1 (attacks), 2 (participates), and 3 (juvenile play) are shown during PD22–42. For principle component 3, greater scores indicate more pins and head attacks. Although there was no sex difference, the use of pins and head attacks peaked during PD29–35 (* $p < .05$).

Figure 3:

Play deprivation increased the conditioned defeat response in both males and females. Mean durations ($\pm$ SE) of A) submissive and defensive, B) aggressive, C) affiliative, and D) nonsocial behavior are shown during a 5-min social interaction test with a non-aggressive intruder. Animals exposed to social defeat stress prior to testing include play-exposed females ($n = 13$), play-deprived females ($n = 9$), play-exposed males ($n = 11$), and play-deprived males ($n = 10$). Non-defeated animals include play-exposed females ($n = 9$), play-deprived females ($n = 6$), play-exposed males ($n = 10$), and play-deprived males ($n = 8$). # indicates a significant social defeat $\times$ play deprivation interaction ($p < .05$). * indicates a significant main effect of play deprivation ($p < .05$). ^ indicates a significant main effect of social defeat ($p < .05$).

Figure 4: Scatterplot of the duration of aggression shown by non-defeated animals during social interaction testing compared to their average rate of A) playful contact and B) ventrum attacks during PD22–42. These measures of social play were negatively correlated with duration of aggression. Open circles indicate males (n = 18) and closed circles indicate females (n = 18), although circles may represent more than one animal.

Table 1:

Ethogram of social play

Behavior	Description
Playful contact	Affiliative contact with front and/or rear paws
Attack	Head lunge toward partner. Can target either head, neck, ventrum, or flank
Pin	Subject stands over partner, with paws pushing partner onto its back
Evasions	A swift movement away from a social partner that follows playful contact and ends the play bout.
Supines	Participation in playful contact that includes A) partial supine –when the animal is on its side during playful contact, B) full supine – the animal is on its back during playful contacts

Table 2:

List of behaviors belonging in each principle component. The cumulative variance explained is 70.32%. PC 1: 34.46%, PC 2: 15.29%, and PC 3:15.29%.

PC 1 (Attacks)	Scores	PC 2 (Participates)	Scores	PC3 (Juvenile Play)	Scores
Attacks	.952	Participates	.890	Pins	.733
Ventrum Attacks	.892	Partial Supine	.833	Head Attacks	.740
Neck Attacks	.664				
Full Supines	.663				

Table 3:

Social play behaviors not significantly correlated with duration of aggression during the social interaction test.

Behaviors	R-Value	P-Value
Total Attacks	-.394	.118
Total Participates	-0.326	.202
Pin	-.109	.676