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Adult-Juvenile Play Fighting in Rats: Insight into the Experiences that Facilitate the Development of Socio-Cognitive Skills

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Rats reared with playful peers during the juvenile period have a modified prefrontal cortex and improved executive functions, whereas ones reared with less playful partners, such as an adult, do not. It has been hypothesized that peer-peer play fighting creates unique experiences that tax executive functions and so influence the refinement of the prefrontal cortex. The present study compares the rough-and-tumble play of juveniles interacting with another peer with that of juveniles interacting with an adult. The juveniles interacting with adults engage in as much play as those that interact with juveniles. However, they experience fewer attacks from their adult partners and experience fewer bouts of close-quarter wrestling. Moreover, the juveniles in these juvenile-adult pairs experience fewer opportunities to perform role reversals in which the attacker becomes the defender. These findings are consistent with the hypothesis that the turn taking typical of the play fighting with peers is critical for the development of executive functions.

Rearing rats in social isolation over the juvenile period (i.e., from weaning to the onset of sexual maturity) leads to young adults with a wide variety of social, cognitive and emotional deficits (e.g., Arakawa, 2002, 2003; Byrd & Briner, 1999; da Silva, Ferreira, Carobrez, & Morato, 1996; Einon & Morgan, 1977; Einon, Morgan, & Kibbler, 1978; Pellis, Field, & Whishaw, 1999; van den Berg et al., 1999). In turn, these effects are associated with wide ranging changes in the development of various neural systems (e.g., Hall, 1998; Fone & Porkess, 2008). One change is in the function of the medial prefrontal cortex (mPFC; Baarendse, Counotte, O'Donnell, & Vanderschuren, 2013). This finding is important because it provides a link to one of the potentially important experiences of which individuals are deprived by isolation rearing - social play with peers. This is for two reasons.

First, isolation rearing does not produce a failure to develop species-typical behavior but rather, may disrupt the effectiveness of how species-typical behavior patterns are executed and modified in contextually relevant ways (Martin & Caro, 1985). Thus, one implication is that at least some of the deficits reflect reduced higher-level control over the regulation of species-typical behavior. Given the important regulatory control afforded by the executive functions of the prefrontal cortex (e.g., short-term memory, attention, emotional regulation, impulse control, decision making; Dalley, Cardinal, & Robbins, 2004; Goldberg, 2001) and the way that social play can provide experiences that are relevant to varying the context within which behavior patterns are executed (Petrů, Špinka, Lhota, & Sípek, 2008), it is plausible that the functional changes in areas of the prefrontal cortex arising from isolation rearing (Baarendse et al., 2013), are, at least in part, due to the individual's lack of experience of social play with peers (Pellis, Pellis, & Himmler, 2014; Vanderschuren & Trezza, 2014).

Second, even though being reared in isolation deprives the developing rat of more than just play with peers (Bekoff, 1976), studies using less draconian rearing regimes also implicate the importance of play with peers as a key experience in altering these higher order functions. Juveniles reared with exposure to non-playful

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or less playful partners also exhibit deficits in cognitive and social behavior (Burleson, Pedersen, Seddighi, et al., 2016; Einon et al., 1978; Schneider, Hannusch, Schmahl, et al., 2014; Schneider, Pätz, Spanagel, & Schneider, 2016; Schneider, Bindila, Schmahl, et al., 2016) and altered development of the medial prefrontal cortex (mPFC; Bell, Pellis, & Kolb, 2010; Burleson et al., 2016; Himmler, Pellis, & Kolb, 2013). These changes are consistent with some of the effects seen in animals that were fully isolated as juveniles (Pellis et al., 2014; Vanderschuren & Trezza, 2014). These considerations strongly implicate peer-peer play as one of the important experiences in the juvenile period, influencing the development of the mPFC and the executive functions that are supported by this area of the brain (Pellis & Pellis, 2009). The issue that arises from this conclusion is that of identifying the experiences that are generated by play that have these developmental influences.

Play fighting, the main form of social play exhibited by rats, involves attack and defense of the nape of the neck, which is gently rubbed with the snout if contacted (Pellis & Pellis, 1987; Siviy & Panksepp, 1987). One rat approaching and lunging at its partner's nape initiates bouts of play fighting (Himmler, Pellis, & Pellis, 2013). A rat drugged with a psychoactive drug that still allows it to move about and explore its enclosure attracts such attacks from normal peers even though it does not launch attacks and does not respond with defensive actions when attacked (Deak & Panksepp, 2006; Field & Pellis, 1994; Pellis & McKenna, 1995; Thor & Holloway, 1983). This is important because exposing otherwise isolated juveniles daily to a drugged partner does not prevent the development of cognitive deficiencies as does exposing such rats daily to a peer that is not drugged (Einon et al., 1978). That is, the opportunity to launch playful attacks is not sufficient to provide the necessary experiences to facilitate the development of these higher order functions.

In a series of elegant studies by Schneider and colleagues (Schneider et al., 2014; Schneider, Bindila, Schmahl, et al., 2016; Schneider, Pätz, Spanagel,, et al., 2016), rats from a highly playful strain, Wistar rats (Himmler, Modlińska, Stryjek et al., 2014), were housed with rats from a low playing strain, Fischer 344 (Siviy, Baliko, & Bowers, 1997; Siviy, Love, DeCicco, Giordano, & Seifert, 2003), over the juvenile period. As adults, the Wistar rats reared with Fischer 344 rats had a changed pain threshold and had deficiencies in social cognition. Analysis of the play engaged in at the juvenile period showed that the Wistar rats launched many attacks, but received few (corroborating the pattern seen in the drug studies), and few of the attacks by the Wistar rats led to the Fischer 344 rats defending themselves. Most critically, few of the defended attacks led to the recipient adopting tactics that involved close-quarter wrestling and reciprocation of roles (Schneider, Bindila, Schmahl, et al., 2016; Schneider, Pätz, Spanagel,, et al., 2016). These findings suggest that it is the experiences derived from the reciprocal exchanges that arise from playful wrestling that provide the training suitable for facilitating the development of the executive functions of the prefrontal cortex.

Indeed, in the juvenile period when the frequency of play fighting peaks in occurrence (Thor & Holloway, 1984), rats are most likely to inject maneuvers that compromise their ability to maintain control of their postural stability and so their ability to effectively deflect the countermoves of their partner (Foroud & Pellis, 2002, 2003; Pellis, Pellis, & Foroud, 2005). Such self-handicapping likely explains the finding that about 30% of play fights results in role reversals, where the original attacker reverts to adopting the defensive role (Himmler, Himmler, Pellis, & Pellis, 2016). The loss of control and the experience of unpredictability that such self-handicapping produces has been hypothesized to be the way that play trains juveniles to become more resilient in a world in which unexpected events are likely to occur (Špinka, Newberry, & Bekoff, 2001). Furthermore, it has been hypothesized that such resiliency is in part derived from improved executive functions that derive from play-induced changes to the development of the prefrontal cortex (Pellis, Pellis, & Bell, 2010; Pellis et al., 2014; Vanderschuren & Trezza, 2014).

Mother rats rarely launch playful attacks on their offspring and rarely respond with a playful defense when attacked by their offspring. Rather, in litters containing both a mother and offspring, the young preferentially play with their peers (Cramer, Thiels, & Alberts, 1990; Pellis & Pellis, 1997; Thiels, Alberts, & Cramer, 1990). Using this evidence for impoverished adult-juvenile play, juvenile females were reared with either a peer or with an adult female over the juvenile period, with those reared with an adult showing less pruning of the dendritic arbor of neurons in the mPFC (Bell et al., 2010; Himmler, Pellis & Kolb, 2013). The conclusion drawn was that, with the adult, the play experienced by the juveniles was insufficient to provide the hypothesized training needed for modifying the development of the mPFC (Pellis, Pellis, & Bell, 2010; Pellis, Pellis, & Himmler, 2014). It is possible that when reared alone with an adult, rather than with a whole litter, the juvenile compensated by playing more with the adult. However, if the findings from Schneider and colleagues (Schneider, Bindila, Schmahl, et al., 2016; Schneider, Pätz, Spanagel,, et al., 2016) are of general validity, we posit that it is not the overall amount of play that is important, but rather that that play experienced with an adult partner is deficient in wrestling and reciprocation. The present paper tests this prediction.

Juvenile female rats were housed either with a peer or an adult female and their play was tested using a standardized test procedure (Himmler, Pellis, & Pellis, 2013). The play present in peer dyads was compared with that of the dyads composed of a juvenile and an adult. Based on the findings from Schneider and colleagues (Schneider, Bindila, Schmahl, et al., 2016; Schneider, Pätz, Spanagel, et al., 2016), we predicted that the juveniles interacting with the adult should receive fewer playful attacks and likely initiate more attacks of their own to compensate. The likelihood of defending against an attack should be either unchanged or elevated, and the types of defense by the adult should be ones that reduce the likelihood of prolonged wrestling. Finally, the juveniles playing with adults should experience less reciprocation.

Method

Subjects

A total of 24 Long Evans hooded female rats were used. The rats were obtained from Charles River Laboratories (St. Constant, Quebec). Eighteen were around 24 days of age and six were around 74 days of age. It should be noted that females were used in the rearing manipulation studies (Bell et al., 2010; Himmler, Pellis & Kolb, 2013) and that is why females were the subjects of the present study. Females were used in the original studies because our experience has been that housing a juvenile with an adult male leads to a greater risk of cannibalism by the adult (Arelis, 2006), and since they were to be housed together until after the onset of sexual maturity, when young males are likely to be sexually motivated by the presence of a cycling female (Pellis & Pellis, 1990), this could change the nature of the experiences involved. They were housed in the animal facility at the Canadian Centre for Behavioral Neuroscience. All subjects were housed in dyads with six dyads being composed of juveniles and the other six of a juvenile and an adult. The rats were maintained at a constant 21-23°C on a 12:12-hour light-dark cycle, with lights off at 1930, and were kept in 46cm X 25cm X 20cm polyethylene tubs, with processed corncob bedding. Food and water were provided ad libitum. All animals were handled and cared for in accordance with the Canadian Council for Animal Care regulations.

Procedure

Play was tested when the juveniles were 34 and 36 days, which is within the peak period for play behavior in rats (Pellis & Pellis, 1990; Thor & Holloway, 1984), during the first three hours in their light phase. For three days before play testing, all animals were habituated (in their dyads) to the testing enclosure for 30 minutes a day. Short periods of social isolation preceding testing is known to increase the frequency of play fighting (Panksepp & Beatty, 1980; Niesink & van Ree, 1989); the present study used 24 hours of social isolation (Pellis, Field, Smith, & Pellis, 1997). Rats were tested twice, with a 24-hour rest period between the two test days to avoid having animals socially isolated longer than 24 hours. Test trials lasted for 10 minutes each, providing enough time to capture most aspects of playful interactions. Both habituation and testing occurred in complete darkness as it has been shown that social behaviors such as play increase when in the dark compared to light conditions (Himmler, Pellis & Pellis, 2013). Play trials occurred in a 50cm X 50cm X 50cm Plexiglas box encased in a soundproofed chamber (61cm X 61cm X 84cm). The inside of the Plexiglas box

was filled with 1-2cm of CareFresh® bedding. A DVD103 Sony Handycam was used to film all play trials at a 45° angle using the night-shot option to film in the dark. The play trials involved testing pair mates. In this way, the juveniles from the juvenile-juvenile pairs played together and the juveniles and adults from the juvenile-adult pairs played together.

Behavioral Analysis

Attack and defense. A playful attack was scored when the tip of the snout of one partner was either in contact with its partner's nape or when one partner made a targeted movement towards the nape of the other. The recipient of the attack could either respond to the attack using one of many defenses or simply ignore the attack. Playful defense of the nape was scored as involving one of two forms: (a) evasion, which involves the defender moving its nape away from its attacker and does so by running, jumping, or pivoting away, and (b) facing defense, in which the defender moves its nape away by turning to face its attacker in order to block access to its nape by opposing its teeth between its partner and its own nape. Although facing defense can itself involve different tactics (Himmler, Pellis & Pellis, 2013), our previous experience has shown that, as the body size difference of the participants becomes greater, it becomes more difficult to make such discriminations (Pellis, Himmler, Himmler, & Pellis, 2017). Therefore, for the present study, in which one group involved an adult that was over twice as large as its juvenile partner, defense was simply scored as to whether it involved evasion or facing defense. The initial 2-3 video frames from the onset of a defensive movement were used to determine the type of defensive tactic first attempted by the defending rat rather than recording the eventual outcome of an attack (Himmler, Pellis & Pellis., 2013).

Previous analyses have shown that persistent attack or changes in attack can lead to a defender switching to other defensive tactics (e.g., Pellis & Pellis, 1987, 1997; Pellis, Castañeda, McKenna, Tran-Nguyen, & Whishaw, 1993). Thus, while the first few frames can identify the initial, and so preferred, defensive tactic used by the defender, they may not be sufficient to determine the subsequent outcomes of combined attack and defense (Himmler, Himmler, Stryjek et al., 2016). Given the need to evaluate playful experiences that reflect reciprocal exchanges, two further measures were scored that assess the outcome of the effects of the combined effects of the maneuvers by both partners.

Reciprocation. A postural configuration that is considered to reflect a cooperative phase of play fighting in rats is the pin, in which one partner is lying on its back and the other is standing on top (Panksepp, 1981). The partners in this configuration often continue to compete for access to each other's napes (Pellis & Pellis, 1987), and so offer the opportunity for prolonged actively contested bodily contact. In Long Evans rats, the most common way for a partner to be pinned is for the recipient of a nape attack to roll over on to its back (Himmler, Himmler, Stryjek et al., 2016), but, as noted above, whether the defender actively rolls over onto its back or is pushed over is more difficult to assess when one partner is of a much larger size (Pellis et al., 2017). Therefore, to be consistent in assessing the prevalence of this postural configuration across the two groups, the occurrence of pins, as defined by Panksepp (1981), was simply scored when that posture was present rather than how it was achieved. As more cooperation leading to reciprocity was expected among peers than in pairs with an adult, it was predicted that discordant pairs should exhibit a lower frequency of pinning (see also Schneider, Bindila, Schmahl, et al., 2016; Schneider, Pätz, Spanagel, et al., 2016).

As noted above, during play fighting, the defender may launch counterattacks, which, if successful, can lead to a role reversal, as the original attacker adopts a defensive role (Pellis & Pellis, 1987, 1990). Also, as noted above, during the peak juvenile period, the animal in the dominant position is likely to adopt postures and perform movements that facilitate the success of counterattacks (Pellis et al., 2005). Therefore, another useful behavioral measure to assess the degree of reciprocity is to score the likelihood that in a particular playful bout there is a role reversal between the attacker and defender (Himmler, Himmler, Pellis et al., 2016). The play fights from the two types of pairs were scored to determine the likelihood that the partners would reverse roles. The criterion for selection was that the recipient of a nape attack had to respond with a defensive action that maintained bodily contact. Given that adults launched few playful attacks in the adult-juvenile pairs, the criteria for selecting play fights to sample were different for the two types of pairs. For the juvenile-juvenile pairs, the first ten play fights meeting the criterion were scored. Based on the different proportions of attacks by the juveniles and the adults in the juvenile-adult pairs (see Results), the sequence of sampled play fights was increased to 15. To match the relative proportion of attacks by juveniles and adults, two of the play bouts included in the sample involved ones that were initiated by adults attacking the juvenile. As above, the first two attacks by adults and the first 13 attacks by the juveniles that met the criterion were sampled as they appeared in the videotaped material.

Statistical Analysis

The actions by both members of a pair can continually modify each other's actions (Pellis & Pellis, 2015). This makes comparing the behavior of one relative to the other in the juvenile-adult pairs difficult in terms of identifying which may be deviating the encounter from its typical course compared to juvenile-juvenile interactions. For outcome measures such as pins and role reversals, this did not matter, as what was compared was the frequency of these measures occurring in pairs. Thus, what was compared was the

relative frequency of the measures in juvenile-juvenile pairs versus juvenile-adult pairs. However, for the measures of attack and defense, which assesses the behavior of an individual in pair, a different approach was used to ensure that statistical comparisons were between independent samples. The behavior of one of the partners from the juvenile-juvenile pairs was used to compare with the behavior of the adults in the juvenile-adult pairs, and the other juveniles from the juvenile-juvenile pairs were used to compare with the juveniles from the juvenile-juvenile pairs. Choosing which juvenile to use as the control for the adult and which for the juvenile was based on relative differences in their size.

The rats in the juvenile-adult pairs were significantly discordant in size (*Mean +/- SEM* given in grams: juvenile = 101.87 +/- 4.76; adults = 222.57 +/- 4.34), $t(5) = 40.18$, $p < 0.001$. There were no significant differences in weight between the juveniles in the juvenile pairs and the juveniles in the juvenile-adult pairs, $p > 0.05$. Critically, the relative difference between pair mates (weight of heaviest-weight of lightest/weight of lightest x 100) was significantly greater for the juvenile-adult pairs (*Mean +/- SEM* given as percent difference: 120.70 +/- 3.00 versus 8.03 +/- 2.27), $t(10) = 30.00$, $p < 0.001$. The physical asymmetry may be important for accounting for some of the differences in the execution of playful combat tactics. Therefore, to compare the play between the two groups as fairly as possible, the behavior of the heaviest juveniles from the juvenile-juvenile pairs was compared to that of the adults and the behavior of the lightest juveniles from the juvenile-juvenile pairs was compared to that of the juveniles from the juvenile-adult pairs.

All comparisons were pairwise with the scores from subjects in the juvenile-juvenile pairs contrasted with those from subjects in the juvenile-adult pairs tested using independent t-tests. Even though multiple t-tests were conducted, all the differences in the scores compared were predicted, so corrections to reduce the risk of Type 1 errors was not deemed to be necessary. Differences were considered significant for p values of ≤ 0.05. For graphical or tabular representation of the data, values are given for group means and standard error.

One observer scored the measures of attack and defense (LW) and another scored the pins and role reversals (SMP). Two methods were used to ensure inter-observer reliability. First, the scores for the juvenile pairs were compared to those from several previous studies from the same laboratory using the same experimental procedures and scoring techniques (e.g., Himmler, Stryjek, Modlinska et al., 2013; Kisko, Himmler, Himmler, Euston, & Pellis, 2015; Pellis & Pellis, 1990; Smith, Forgie, & Pellis, 1998). In all cases, the values scored in the present study fell within the range of previously published values. Second, because we have not scored adult-juvenile play before, for these pairs, the respective measures were re-scored by another observer (VCP) who was not privy to the hypothesis being tested, and so the predicted differences, at the time of scoring. The scores between observers were significantly correlated, $r > 0.80$.

Results

Attack and defense

Compared to the matched juveniles from the juvenile-juvenile pairs, the adults launched significantly fewer attacks, did not differ in the likelihood of defending themselves and used facing defense significantly less often (Table 1). Rather, the adult was more than twice as likely to evade the playful attacks by the juveniles, and most of the facing defense that did occur involved the adult partially rotating the forequarters and kicking the juvenile away with its hind foot. That is, there were few opportunities to engage in prolonged wrestling involving grappling with one another.

Table 1

Comparison of the Adult's Play Behavior with Matched Juveniles From the Juvenile Pairs

Play measures	Adults *M+/- SEM*	Matched juveniles *M+/- SEM*	*t*	*p*
Playful attacks/10min	6.17 (4.40)	40.33 (12.50)	6.32	< 0.001
Percent of attacks eliciting defense	80.67(10.98)	80.57(9.32)	0.01	> 0.05
Percent of defenses involving facing defense	34.77(16.26)	63.45(12.04)	3.47	< 0.01

Compared to matched juveniles from the juvenile-juvenile pairs, the juveniles in juvenile-adult pairs launched significantly more playful attacks and they were just as likely to use facing defense (Table 2). Although, the likelihood of defense was not statistically significant at the 0.05 level (Table 2), juveniles tended to respond to attacks from adults more often than juveniles attacked by other juveniles ($p = 0.06$). That is, the juveniles playing with adults increased their attack and defense thus increasing playful encounters, and along with maintaining a high rate of facing defense, increased the occurrence of prolonged wrestling.

Table 2

Comparison of the Behavior of the Juveniles Playing with Adults with Matched Juveniles from the Juvenile Pairs

Play measures	Juveniles playing with adults *M+/- SEM*	Matched juveniles playing with juveniles *M+/- SEM*	*t*	*p*
Playful attacks/10min	80.00(19.85)	51.33(18.80)	2.57	< 0.05
Percent of attacks eliciting defense	92.60(11.78)	71.62(22.66)	2.06	> 0.05
Percent of defenses involving facing defense	66.12(23.27)	64.45(12.04)	0.06	> 0.05

Reciprocity

The juvenile-juvenile pairs had significantly more pins per 10min (*Mean +/- SEM*: 20.17 +/- 3.28) than did the adult-juvenile pairs (4.50 +/- 1.89), $t(10) = 4.14$, $p < 0.01$. Similarly, role reversals, represented as the percentage of play bouts in which the attacker became the defender, were significantly more frequent in the juvenile-juvenile pairs (*Mean +/- SEM*: 30.00 +/- 2.58) than in the adult-juvenile pairs (13.33 +/- 3.33), $t(10) = 3.95$, $p < 0.01$. The numbers for both measurements likely underestimate the difference between the juveniles from the two groups. Regarding pins, it was invariably the juvenile that was pinned by the adult. Moreover, pinning between juveniles often involved both partners continuing to attempt to access each others' napes (see Pellis & Pellis, 1987), whereas, when an adult pinned a juvenile, the adult typically held the juvenile on its back briefly then moved away. In the few cases in which the juvenile was in the superior position, the adult never fully rotated onto its back, but rather remained on its side with the hind foot on that side maintaining

contact with the ground, and quickly maneuvered to withdraw from the juvenile. That is, the pin was not a platform for continued active wrestling involving bodily contact.

The qualitative experiences in the role reversals among juveniles compared to the cases involving adults also appeared to differ. In the latter, a role reversal occurred when the juvenile persisted in gaining access to the nape of the prone adult and after the adult repeatedly kicked the juvenile with its hind foot or pushed it with its hip. The adult would then rotate its forequarters toward the juvenile and push it over. In most cases, it was not possible to be certain about whether the adult was launching a counterattack at the juvenile's nape. Rather, the adult would push the juvenile over, hold it down and when the juvenile stopped struggling, it would let it go and move away. In contrast, role reversals among juveniles were clearly associated with counterattacks directed at the partner's nape and most, 63.16%, occurred from the pin configuration. Further highlighting the asymmetry in pattern of role reversals involving juveniles and adults is that, for the scored encounters when an adult attacked a juvenile, no cases led to a role reversal.

Discussion

Summing together the behavior of both pair mates, the occurrence of nape attacks did not differ between the juvenile-juvenile pairs and the juvenile-adult pairs (*Mean +/- SEM*: 92.83 +/- 9.81 versus 86.17 +/- 8.09), nor did the likelihood of such attacks leading to defensive actions by the attacked partner (75.58 +/- 3.63 versus 78.92 +/- 5.31). This would suggest that the total amount of play experienced by juveniles from the two groups did not differ quantitatively. However, there were significant qualitative differences that likely led to differences in the types of playful experiences that were present. As predicted from other studies that matched a playful partner with a less playful one (e.g., Reinhart, Metz, McIntyre, & Pellis, 2006; Schneider, Bindila, Schmahl, et al., 2016; Schneider, Pätz, Spanagel,, et al., 2016), the adult female partners only launched 13.3% of the playful attacks compared to the approximately 50% by their counterparts in the juvenile pairs and half as likely to use defensive tactics that led to wrestling (Table 1). The juveniles in these pairs increased their frequency of playful attacks, tended to increase their likelihood of defending themselves and maintained juvenile-typical levels of facing defense (Table 2). Without the compensatory increase in attack and defense by the juveniles playing with an adult, there would have been a 58.9% decrease in close-quarter wrestling compared to the juveniles in the juvenile-juvenile pairs. Even with the compensatory changes, however, there was a 39.3% reduction.

Most critically, the defensive actions by the adult females acted to block body contact with the juvenile, so even the close-quarter encounters that did occur rarely generated the kinds of wrestling, such as the body-to-body contact produced by the pin configuration (Himmler, Himmler, Stryjek et al., 2016; Panksepp, 1981), that occurs when juveniles play together. This was dramatically revealed by the large decrease in the likelihood of role reversals when juveniles played with adults. That is, the kinds of maneuvers that generate the opportunities and adjustments of their own and their partner's movements that leads to ensuring reciprocal exchanges in juvenile-juvenile pairs (Himmler, Himmler, Pellis, et al., 2016; Pellis et al., 2005), are greatly reduced when juveniles play with adults. Indeed, even when role reversals did occur, they arose because the adult used its superior size to overpower the juvenile, rather than the juvenile having the opportunity to modify its behavior to facilitate its partner's ability to retaliate successfully (Pellis et al., 2005). Thus, the findings from the present study are consistent with those from Schneider and colleagues (Schneider, Bindila, Schmahl, et al., 2016; Schneider, Pätz, Spanagel, et al., 2016).

It remains to be tested whether the impoverished play experienced by juveniles interacting with adult pair mates in a dyadic test paradigm represents the totality of their play experiences when reared with such a partner over the entire juvenile period. It is possible that, as the juvenile approaches puberty, and decreases the size difference with its partner, its behavior may more closely match that of the adult partner (Pellis et al., 2017). In this way, the same degree of impoverishment in play experience may not be uniform over time. Interestingly, in this regard, Schneider and colleagues (Schneider, Bindila, Schmahl, et al., 2016; Schneider, Pätz, Spanagel, et al., 2016) found that the Wistar rats interacting with the Fisher-344 partners experienced impoverished playful reciprocation whether tested as dyads or observed engaging in spontaneously occurring play in their home cages over the course of the rearing period. Detailed developmental studies are needed, but the present findings do support the potentially important role of reciprocal exchanges during play mediating the influence of juvenile play on the development of the mPFC and associated social skills (Pellis, Pellis, & Bell, 2010). In addition, while the present findings are consistent with this hypothesis, it has not yet been demonstrated that the reduced pruning of the neurons for the mPFC in rats reared with an adult partner (Bell et al., 2010; Himmler, Pellis, & Kolb, 2013), is correlated with impoverished social skills. Even so, it should be noted that hamsters that have been reared with an adult partner as juveniles show reduced pruning of the dendritic arbor of the neurons of the mPFC and also have impoverished social skills that are known to involve the mPFC (Burleson et al., 2016). Moreover, the Wistar rats reared with Fisher-344 partners have impoverished social skills (Schneider, Bindila, Schmahl, et al., 2016). Thus, while the causal link between being reared with an adult and subsequent social skills needs to be established in rats, the present findings in combination with the others noted above make this connection plausible and worth testing further. At the least, the present findings help further characterize the kinds of play experiences in the juvenile period that are derived from similarly playful peers that may be important to refining the neurobehavioral mechanisms that underpin social competency.

For example, the present study further confirms that the experiences derived from playfully attacking a partner are by themselves unlikely to sufficient to modify neurobehavioral systems. Juveniles interacting with adults increase the frequency of playful attacks by 36% (Table 2), so, if anything, they are gaining more experiences in attacking than juveniles playing with juveniles. What appears necessary is for the partner that is attacked to not only defend against the attacks, but also to do so in a manner that leads to wrestling, and for that wrestling to generate opportunities for role reversals. Indeed, the present study suggests that a reduction of as little as 40% in playful wrestling may have negative consequences on the development of the mPFC that have been previously found when a juvenile is reared with an adult (Bell et al., 2010; Himmler, Pellis, & Kolb, 2013).

A role reversal entails an attacker losing its dominant position and assuming a defensive role as the defender launches a successful counterattack (Himmler, Himmler, Pellis, et al., 2016). Such role reversals can involve the animal in the dominant position engaging in movements that self-handicap its ability to defend against the counterattack (Foroud & Pellis, 2003). For play fighting to remain playful, such reciprocation is needed (Dugatkin & Bekoff, 2003; Palagi, Cordoni, Demuru, & Bekoff, 2016), to allow both animals to have the chance to gain the upper hand for at least some of the time during encounters (Pellis, Pellis, & Reinhart, 2010). However, it is not only cooperation that is involved, as there are more counterattacks (Pellis & Pellis, 1990; Pellis, Pellis, & Dewsbury, 1989) than successful role reversals (Himmler, Himmler, Pellis et al., 2016), indicating that there is also competition to maintain the advantageous position (Pellis & Pellis, 2017). Therefore, it must be the blending of cooperation and competition that creates the opportunities to gain experiences during play fighting that are important for developing social skills. Such opportunities were severely curtailed in the mixed-age pairings analyzed in the present study.

A model for how play fighting produces the experiences that promote the development of the mPFC and associated executive functions is that, because of the need to maintain some degree of reciprocity, a playing rat must monitor its own behavior and that of its partner, thus engaging short term memory about wins and losses. If the play is too unbalanced, in that the same individual has won most of the preceding encounters, that individual needs to refrain from exercising its advantage when able to do so (i.e., use impulse control) and even self-handicap itself to increase the chances of its partner in reversing the encounter to its favor. Placing itself in a losing situation involves loss of control and this may require emotional regulation to moderate the intensity of the emotional response. Finally, all these factors need to be considered so that a decision on what to do next is made. All these factors involve executive function capacities that engage the mPFC (Euston, Gruber, & McNaughton, 2012). Due to the larger size of the adult, and its apparent failure to restrain its behavior to accommodate its larger size, a juvenile has little prospect of reversing roles when attacked by an adult. Moreover, the greater size of the adult allows it to counterattack more successfully, especially when it adopts a facing defense, so quickly reversing a juvenile's attack into a defense. That is, the unrestrained use of the adult's size advantage diminishes the experiences generated by following the rules of reciprocity.

While play fighting is rewarding (Vanderschuren, Acterberg & Trezza, 2016), gaining access to a partner's nape without there being some competition to gain that contact is not as rewarding as playing with a partner that actively defends its nape (Pellis & McKenna, 1995). Indeed, in some species, an attacker will forgo directly contacting the exposed play target of a partner and initiate a wrestle first, then maneuver to overcome the partner's defenses to gain access to that target (Pellis & Pellis, 2017). Moreover, it is well established that, for many species, animals prefer to play with age-matched partners (Biben, 1998; Fagen, 1981), presumably because such pairings involve animals of comparable skill (Thompson, 1998), thus maximizing the experience of attack and defense. Exceptions do occur, and in such cases, mismatched partners sustain playful fighting because the older, larger or more dominant animal markedly self-handicaps itself (LeResche, 1976). Thus, by playing with a partner of a comparable skill level, or with a superior partner that is willing and able to restrain its superior ability, juveniles can generate the kinds of balance between cooperation and competition and losing and regaining of control that generates the experiences that are important for training executive functions and the prefrontal cortex (Pellis & Pellis, 2017).

Conclusion

When living with peers and adults, juvenile rats preferentially engage peers in play fighting (Cramer et al., 1990; Pellis & Pellis, 1997; Thiels et al., 1990). Even older juveniles are seemingly unable to attenuate their actions to make playing with younger juveniles reciprocal (Pellis et al., 2017). Thus, as shown in the present study, even though juveniles can sustain high levels of play with adults, the frequency of playful wrestling is reduced and the adults do not play in a way to ensure that experiences of reciprocity are gained. This may be different for species in which young are typically raised without peers and so rely on play with a parent (e.g., Snyder, Zhang, Zhang, et al., 2003; Winn, 1989).

At the least, the present study shows that it is not the overt performance of behavior patterns related to attack and defense during play fighting that are critical, but rather, the subtle decision making that arises from negotiating a relatively reciprocal exchange in gaining and losing the advantage while competing for access to their partner's napes. At a broader level, the present study underscores the importance of directly assessing the behavior of animals undergoing manipulations of their rearing experiences (Taborsky, 2016).

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