



## **The Development of Juvenile-Typical Patterns of Play Fighting in Juvenile Rats Does Not Depend on Peer-Peer Play Experience in the Peri-Weaning Period**

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Play fighting in rats involves attack and defense of the nape. To protect the nape, rats use a variety of defensive tactics, with different strains having specific preferences. Targeting of the nape is established before weaning and defense matures over the course of the week preceding and the week proceeding weaning. Thus, it is possible that experience from engaging in immature forms of play is needed to consolidate the nape as the playful target and for the development of the juvenile-typical pattern of defense. Two experiments were conducted to evaluate this possibility. For the first experiment, male rats were reared over the week post-weaning in either pairs or alone, and their play tested with unfamiliar partners when juveniles (31-34 days). For the second experiment, during the week preceding weaning, male and female rats were placed into one of three conditions: (1) with the mother and no peers, (2) with same-sex siblings but no mother, or (3) with both the mother and same-sex siblings. The subjects were tested in same-sex, same-condition pairs when juveniles (31-34 days). Rats from all conditions, in both experiments, attacked the nape during play fighting and developed the same juvenile-typical patterns of playful defense. This suggests that the experience of peer-peer play in the peri-weaning period is not necessary for the development of the attack and defense components of juvenile-typical play.

Play fighting in rats typically involves the attack and defense of the nape, which if contacted is nuzzled with the snout (Pellis & Pellis, 1987; Siviý & Panksepp, 1987). To protect the nape, the defender either evades, by fleeing or swerving away, or turns to face to block the attacker. When turning to face the attacker, the defender can either rotate onto its back (supine defense) or use a variety of tactics that involve remaining standing on one or both of its hind paws (standing defense) to ward off its partner (Pellis & Pellis, 1987). Playful attack begins to emerge at around 15-17 days of age (Baenninger, 1967; Bolles & Woods, 1964; Thiels, Alberts, & Cramer, 1990) and the tactics of playful defense do not attain their juvenile-typical pattern until 28-30 days (Pellis & Pellis, 1997). Moreover, play reaches its peak frequency between 30-40 days of age (Meaney & Stewart, 1981; Panksepp, 1981; Pellis & Pellis, 1990; Thor & Holloway, 1984).

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Comparison of play behavior in juveniles from wild rats and four strains of domesticated rats showed that play fighting in all these rats involved the attack and defense of the nape, and all use the same repertoire of defense tactics to defend the nape (B. T. Himmler et al., 2013c; S. M. Himmler et al., 2014c). However, strains differ in their frequency of use of these different defensive tactics, with the largest difference being between Sprague-Dawley (SD) and Long-Evans (LE) rats. SD rats tend to use evasive tactics more frequently than facing defense, while LE rats use facing defense more frequently than evasive tactics. Moreover, when using facing defense, LE rats rotate to supine more often than SD rats. This strain-typical preference is maintained irrespective of whether attacked by same-strain or opposite-strain partners (S. M. Himmler, Lewis, & Pellis, 2014b).

The study on the effects of the strain of the attacker revealed an unexpected result: rats housed in mixed strain groups converged in their use of defensive tactics to ones that were intermediate between the two strains, and these altered preferences in defense remained the same irrespective of the strain of the attacker. That is, as little as seven days of exposure to partners from different strains in the week preceding weaning is sufficient to change strain-typical preferences in use of defensive tactics. Given that the development of play fighting from the week preceding to the week proceeding weaning is piecemeal (Bolles & Woods, 1964; Pellis & Pellis, 1997), the findings from the cross-housing experiment (S. M. Himmler et al., 2014b) suggest that the practice that is gained from playing in an immature form prior to the juvenile period may be necessary for the consolidation of the pattern of play that is typical of juveniles.

In order to test this hypothesis, two experiments were conducted that manipulated the experience of peer-peer play in the peri-weaning period. For the first experiment, rats were socially isolated for the same seven-day time period (24-30 days) that was effective in changing strain-typical preferences in defense due to housing with another strain (S. M. Himmler et al., 2014b). Given that LE rats showed a marked change in patterns of playful defense when reared in mixed strain groups than did the SD rats (S. M. Himmler et al., 2014b), for this experiment, LE rats were used. If peer-peer playful experiences are needed in order to develop strain-typical patterns of playful defense in the juvenile period, then social isolates should exhibit strain-atypical playful defense as juveniles.

If the play following isolation is atypical, this may not, however, be due to the lack of peer play interactions, as complete social isolation produces various abnormalities in the development of emotional regulation, as well as in cognitive and social skills (e.g., Baarendse, Counotte, O'Donnell, & Vanderschuren, 2013; Byrd & Briner, 1999; da Silva, Ferreira, Carobrez, & Morato, 1996; Hall, 1998; Lukkes, Mokin, Sholl, & Forster, 2009, Von Frijtag, Schot, van den Bos, & Spruijt, 2002), with some impairments evident when isolation is limited to the first week preceding weaning (Arakawa, 2002; 2003; 2007a, b). Therefore, if the rats in the post-weaning social isolation experiment were to show atypical patterns of play as juveniles, this could be due to an indirect effect of isolation on emotional, cognitive, and social development, and not necessarily due to the lack of practice of play fighting with peers.

In contrast, if the pattern of play is juvenile-typical following post-weaning social isolation, this may not in itself show that peer-peer play with littermates is not necessary. In the S. M. Himmler et al. (2014b) experiment, what was shown was that living and playing with a strain of rat that plays differently in the week preceding weaning can change the manner in which an individual plays. It may be the case that play with same-strain peers in the week preceding weaning, when play first begins to emerge (Bolles & Woods, 1964; Pellis & Pellis, 1997), provides the critical social experience for the development of juvenile-typical play, with the pattern of play only subject to change later if the post-weaning experiences are in conflict with those that occurred prior to weaning. That is, to capture the critical role of peer-peer interactions in the maturation of juvenile-typical play, depriving infants of such experiences over a wider swathe of the peri-weaning period may be needed.

Therefore, the second experiment was designed to control for these two confounding factors. First, the infant was housed with an adult female, which eliminates the effects of complete social isolation and provides it with a variety of social experiences (e.g., grooming, huddling), but little-to-no experience of play, and certainly no play with a peer (Einon, Morgan, & Kibbler, 1978). Second, infants were denied the opportunity for peer-peer play over the whole peri-weaning period (15-28 days) during which play fighting matures (Pellis & Pellis, 1997). Therefore, in Experiment 2, individual pups were reared with only their mothers as social companions and the play of these pups as juveniles was compared to the play of juveniles that had been reared with both a mother and siblings. However, given that weaning rats and cats early can affect the frequency of their play in the juvenile period (e.g., Bateson & Young, 1981; Brunelli, Shindledecker, & Hofer, 1989; Guyot, Bennett, & Cross, 1980; Janus, 1987; Ikemoto & Panksepp, 1992; Shimozuru et al., 2007), an additional control group was used. Pups were reared over the peri-weaning period with peers alone, in the absence of their mother.

With domestication, animals are reared for many generations in environments free of stressors, such as predation and food shortages and live in an atypical social organization; this may have reduced the critical importance of some early developmental experiences in shaping later juvenile behavior (Bateson & Martin, 2000). Thus, while for Experiment 1, the males from a domesticated strain were used for direct comparison to the results from S. M. Himmler et al. (2014b), for Experiment 2, wild rats born and raised in the laboratory (see Method) were used, diminishing the potentially confounding effects of domestication. Note also, that in the first experiment, only males were used, but in the second, both sexes were used. The reason that males were used in Experiment 1 was to parallel the study by S. M. Himmler et al. (2014b), but while some studies reveal little or no difference in the play fighting of males and females (e.g., B. T. Himmler et al., 2013c; S. M. Himmler et al., 2014c; Panksepp, 1981), others have revealed both quantitative and qualitative differences (e.g., Meaney & Stewart, 1981; Pellis, 2002). Therefore, in Experiment 2, both males and females were used to increase the likelihood of detecting peer-influences on the development of play fighting.

While the present study is primarily focused on the development of the tactics of defense, the potential effects of peer-peer play experience on the development of these tactics could arise indirectly due to experience-induced effects on the quality of playful attack. Even though from the very outset, pre-weaning rats focus their playful attacks

on their peers' napes and this does not appear to change with age under normal rearing conditions (Pellis & Pellis, 1997), it is possible that the tight focus on the nape is maintained by experience with attacking the nape. That is, in the absence of peer-peer play in the peri-weaning period, the targeting of the nape may degrade and this could indirectly affect the pattern of playful defense that emerges in the juvenile period. Therefore, in addition to scoring the tactics used for defense in the play fighting of juveniles, measures of the accuracy of playful attacks were also scored. Finally, while play-deprived rats may begin by playing in a typical manner, their lack of experience may erode their ability to modulate their actions in a way that enables play to remain playful (Pellis, Pellis, & Reinhart, 2010b). Therefore, measures that evaluate the ability for play facilitating actions to be deployed by the rats (e.g., see Kisko, Himmler, Himmler, Euston, & Pellis, 2015) were also scored.

## Method

### Subjects

A total of 151 rats were used in these studies. Of these, 24 Long-Evans (LE) male rats were used for Experiment 1. These rats were obtained from Charles River Laboratories (St. Constant, Quebec) at around 23 days of age and housed at the Canadian Centre for Behavioral Neuroscience. All animals were housed in their respective conditions at 24 days of age. The rats were maintained at a constant 21-23°C on a 12:12-hour light-dark cycle 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.

The remaining 127 rats were derived from a wild-type stock (WWCPS – Warsaw Wild Captive Pisula Stryjek) and were bred and housed at the vivarium at the Department of Psychology, Helena Chodkowska University of Management and Law, Warsaw, Poland (Stryjek & Pisula, 2008), and were handled in a way that minimizes human contact (Stryjek, 2008, Stryjek, 2010; Stryjek & Modlińska, 2013).

All WWCPS rats were housed in Tecniplast© Eurostandard Type IV cages (61cm×43.5cm×21.5cm) with dust-free softwood granules Tierwohl Super© as bedding. Food (Labofeed H, WP Morawski, Kcynia, Poland) and water were provided ad libitum. The day/night cycle was set at 12/12h, and the temperature was maintained at constant 21-23°C. All rats kept in the laboratory were housed, bred and cared for in accordance with the Regulation of the Polish Minister of Agriculture and Rural Development of 10 March 2006 on laboratory animal care, and the experimental procedures were approved by the 4th Local Ethics Commissions in Animal Experimentation, Warsaw, Poland.

### Apparatus

All play trials were in a 50cm×50cm×50cm Plexiglas box, with the floor having a 1-2cm layer of Softzorb® bedding for LE rats and Tierwohl Super© bedding for WWCPS rats. Based on previously established protocols, following each trial, the box was thoroughly cleaned with Virkon© and the bedding replaced in order to ensure that the experimental box was free of smells from the rats previously tested. Even though this may introduce some novelty to the testing enclosure that could affect playfulness (e.g., Vanderschuren, Niesink, Spruijt, & Van Ree, 1995), the pre-test habituation appears sufficient to ensure that the effects of strain and experimental treatment can be detected (B. T. Himmler et al., 2013c; S. M. Himmler et al., 2014c; Kisko et al., 2015). Play trials were recorded with a DVD103 Sony Handycam for the LE rats and a LC-308D camera for the WWCPS rats. Both cameras were equipped with the night-shot option and were placed so that video recordings were recorded from an oblique (45°) angle.

### Procedure

In all groups, play was tested between 31-35 days, which is within the peak period for playful interactions for rats (Thor & Holloway, 1984) and before the age at which, in males, dominance relationships begin to form (Takahashi & Lore, 1983; Pellis & Pellis, 1991). All rats were tested for their play

in a standard paradigm (Himmler, Pellis, & Pellis, 2013a). They were habituated to the test enclosure for 30 minutes per day, for three consecutive days, prior to testing. Following habituation, each subject was socially isolated for 24 hours prior to testing, as brief periods of social isolation increase playfulness, and then tested for 10 minutes. Both habituation and testing sessions were conducted in complete darkness, as play increases in frequency when in the dark as compared to normal light levels, low light or red light. Placement into, and removal from, the testing cage, was done with the experimenter wearing protective gloves.

## **Behavioral Analysis**

Playful interactions were first inspected at full speed, then in slow motion and frame-by-frame. Whereas Long-Evans rats can be easily identified from pair mates due to black and white pelage patterns, all WWCPs rats have a brown coat, and thus pair mates could not be readily tracked as individuals. Therefore, the play behaviors of both LE and WWCPs were scored and summed for pairs as we have done previously (B. T. Himmler et al., 2013c).

Playful interactions begin when one partner approaches and attacks their partner's nape. The recipient of the attack can then either respond to the attack or simply ignore it. If the recipient defends against the attack, the type of defense can be recorded (B. T. Himmler et al., 2013a). Therefore, the frequency of playful attacks per trial, the probability of defense (percentage of all nape attacks that were defended) and the probability of each type of defense tactic (percentage of each tactic used when defensive action was taken) were all recorded.

**Playful attack.** A playful attack was scored when one rat's nose was either in contact with its partner's nape, or when one rat made a targeted movement towards the nape of the other, but a defensive movement by the recipient precluded actual contact. If the recipient initiates a defensive action before the attacker reaches the nape, the point of contact on the defender's body was also scored, thus enabling the relative frequency of nape directed play fights versus non-nape directed play fights to be evaluated (B. T. Himmler et al., 2013c; S. M. Himmler et al., 2014c). The total frequency of attacks per pair per the 10 min trials was scored.

To assess the quality of the execution of playful attacks, three aspects of how rats move during an attack were measured: *aim*, *vigor* and *maintenance*. The first two were measured at the onset of the attack and the third was measured in the cases in which the defender lay on its back to protect its nape (i.e., pin, see below). All three aspects of the execution of attacks were scored on a three point scale (0, 1 or 2).

For *aim*, if the attacker failed to make contact with the nape (i.e., over or undershoot the target), that attack was given a score of "0," whereas if the attacker had clearly targeted and made contact with the nape, that attack was given a score of "2." Attacks that were intermediate between these two were given a score of "1." For *vigor*, if the attacker had walked over or simply moved its snout towards the nape of the other animal, a score of "0" was given for the attack. However, if the attacker had pounced or made swift movements towards the nape of the other animal, the attack was given a score of "2." Attacks that were intermediate between the two were given a score of "1." For *maintenance*, a "0" was given if the attacker either walked over to the supine defender or held the defender down with its forepaws, but in neither case made any movements of the snout toward the nape. A score of "2" was given if the attacker continued to target or maintained snout contact with the nape of the supine defender. Attacks that were intermediate between these two were given a score of "1."

For *aim* and *vigor*, a total of 10 playful attacks per pair were used and for *maintenance*, eight per pair were used. As these represented only a subset of the total attacks that occurred in the 10 min trials, to ensure that all pairs were sampled similarly, the minute in which the peak frequency of attacks was identified for each pair. The first eight or ten cases, depending on the measure, occurring during this peak period, were used. For *maintenance*, two male pairs and one female pair of WWCPs rats did not meet the minimum of eight supine configurations and so were not included in this analysis.

**Playful defense.** Attacks to the nape can be defended using two major types of tactics: The first tactic is evasion, in which the defender moves its nape away from its attacker and does so by running, leaping or swerving away and thus faces away from its partner. The second tactic is facing defense, in which the defender moves its nape away by turning to face its partner, so blocking access by opposing its teeth between its partner and its own nape. Facing defense can also take one of three forms: (i) complete

rotation, in which the defender rolls completely over onto its back, (ii) partial rotation, in which the defender rotates its forequarters, but maintains contact with the ground with one or both of its hind feet, and (iii) other, in which defensive actions involve rotations or other movements in other dimensions (e.g., rotating vertically in a horizontal plane). The type of defensive tactic used was determined by the movements occurring in the first two to three video frames, to ensure that what was recorded was the tactic first attempted by the defender (B. T. Himmler et al., 2013a). Based on the total frequency of attacks and defenses scored per pair, the probability that an attack led to a defensive maneuver and the probability of which defensive maneuvers were used were calculated. Given that previous studies have shown that the biggest strain differences are in the use of evasion and complete rotation (B. T. Himmler et al., 2013c; S. M. Himmler et al., 2014b, c), for simplicity, unless other tactics emerged as significantly different, only data on these two tactics will be presented graphically.

**Outcomes of play fights.** Playful interactions can last for a few seconds, and irrespective of the initial defensive tactic used, can lead to a number of different outcomes. For example, the playful interaction can end with one partner on its back, with the other standing on top, in what has been called a 'pin' configuration (Panksepp, 1981), or the partners may end up standing on their hind legs facing and holding one another (rearing) (Poole & Fish, 1975; Silverman, 1978). Which outcomes arise can provide insight into the motivational organization of the behavior. Some studies have shown that, an increase in rearing, especially if coupled with boxing (i.e., hitting one another with the forepaws), has been associated with increased aggression (e.g., Hurst, Barnard, Hare, Wheeldon, & West, 1996; Reinhart, Pellis, & McIntyre, 2004; Taylor, 1980). In contrast, increases in pinning have been interpreted as an increased motivation for playful contact (e.g., Panksepp, Siviy, & Normansell, 1984; Pellis & McKenna, 1995; Varlinskaya, Spear, & Spear, 1999). Therefore, to assess whether the motivational substrate was altered by the different rearing conditions, rearing, with and without boxing, as well as pinning were scored.

Rearing was scored when both partners were standing on their hind legs facing each other. Once in the rearing position, boxing was scored if one, or both rats, slapped the other on the face (Grant & Mackintosh, 1963). Irrespective of the duration of the rearing position, each bout was scored as a single event. Pinning was scored if the rats ended in a position with one partner on its back and the other standing on top (Panksepp, 1981). The frequency of rearing and pinning was scored as the absolute frequency per pair per trial.

During play fighting, rats may also launch counterattacks after successfully defending their nape from their partner (Pellis & Pellis, 1990). Successful counterattacks to the nape lead to role reversals, in which the original attacker is put on the defensive (Pellis, Pellis, & Foroud, 2005). For play to remain playful, interactions need to be reciprocal with the frequency of reversals providing a measure of the reciprocity (Pellis et al., 2010b). Therefore, changes in the frequency of role reversals can provide insight into altered social competence (Kisko et al., 2015). A sequence of attack and defense that led to the original attacker becoming the defender was recorded as a role reversal. For each pair, the percentage of attack-defense sequences that led to a role reversal was calculated and these were used to calculate group means.

## **Experiment 1**

A total of 24 rats were used. Twelve were singly housed at 24 days of age and were not exposed to a social partner until testing began between 31-33 days. The other 12 animals were housed in pairs at 24 days of age for the duration of the experiment. Given that, by necessity, the socially isolated animals were tested with unfamiliar partners, the individual subjects from the pair-housed condition were also tested with unfamiliar partners by using rats from different dyads. In this way, any group differences would be due to rearing effects, not the identity of the play partner.

## **Experiment 2**

Of the 127 wild rats used in this study, 21 were adult females used for breeding. Sixteen of the adult females provided the young for the mother-only and sibling-only groups, and also the rearing companions for the mother only group. The 16 adult females gave birth to a total of 73 pups (36 male and 37 female), with 16 of the pups (8 male and 8 female) being used for the mother-only group, 24 pups (12 male and 12 female) being used as the experimental animals for the sibling-only group, and the remaining 33 pups (16 male and 17 female) being used as the partners for the sibling-only groups. The other 5 adult females gave birth to 33 pups (16 male and 17 female) and these were used for the control group

containing both siblings and the mother. Of the 24 pups born to these 5 females, 12 males and 12 females were used to form the control groups, with the remaining 9 pups (4 male and 5 female) being used for other experimental purposes (see below for a more full description of the rearing conditions used for Experiment 2).

The WWCPs rats used in this study were of the F3 generation. With further generations of breeding within a laboratory context, there is the increased risk that the domestication process would begin to change various aspects of behavior (Barnett & Stoddart, 1969; Blanchard, Flannelly & Blanchard, 1986). At the same time, wild captured rats or their offspring were not included in the experiment, as there is no possibility of assessing, let alone controlling for, the conditions in which such animals had developed. Also, for the wild caught animals, the drastic change in environmental conditions may have had a profound effect on their levels of stress, and consequently, on their behavior during tests, as well as on their ability to raise offspring. Therefore, using the F3 generation allows us to control the conditions for the rearing environment experienced by the WWCPs rats, while reducing the potential early effects of domestication (B. T. Himmler et al., 2013c; Stryjek, Modlińska, Turlejski, & Pisula, 2013).

**Rearing conditions.** Single pregnant females were placed in separate, standard cages (Tecniplast© Eurostandard Type IV) with food and water provided ad libitum. After birth, the health of females and their litters was monitored. All rats were kept under identical conditions until day 15, when the pups were randomly divided into one of three groups (siblings-only, mother-only, and sibling-and-mother). All rats remained in these experimental conditions until day 27, when they were randomly paired with a sex and rearing condition matched partner. After pairing, all animals remained with the same partner for the remainder of the experiment (i.e., until they were 35 days of age).

*Siblings-only (SO).* A total of 57 (28 male and 29 female) pups from 10 litters were used in this condition. On day 15, pups were taken from their mothers and placed in incubators (Happy Chick II mini) (67cm×41cm×32cm), in groups of 6-8 siblings of the same sex. The incubator was equipped with a thermostatically controlled red light, heat lamp, which ensured a constant ambient temperature of 35°C. All pups were fed standard fodder ad libitum. To make the standard food accessible to the pups, it was mashed and soaked with substitute milk (Bebilon Comfort 1, Nutricia, Poland). The feed was replaced twice a day until the rats were able to consume unmashed standard fodder. In order to ensure the pups were receiving sufficient nutrition, two steps were taken. First, for the first two days of separation from the mother (day 15 and 16), the pups were also fed milk by a pipette two times daily. Second, the feed was weighed at each inspection to monitor the amount of food ingested by the pups and the pups were weighed daily to ensure that they were gaining weight. The rats remained in the incubator until 27 days old, at which time 12 male and 12 female rats were randomly selected as the experimental animals and placed in same-condition, same-sex pairs. These pairs (6 male and 6 female) served as the experimental pairs for this condition.

*Mother-only (MO).* The litters of 16 mothers were reduced to a single pup (8 male and 8 female). The single pups remained with the mother and did not receive any peer-peer interactions until day 27, at which time the rats were randomly placed in same-condition, same-sex pairs. These pairs (4 male and 4 female) served as the experimental pairs for this condition.

*Control-Mother and siblings (CO).* A total of 33 (16 male and 17 female) pups from 5 litters were used for this condition. Pups were reared with both mother and groups of 4-8 siblings until day 27, at which time 12 male and 12 female rats were randomly selected as the experimental animals and placed in same-condition, same-sex pairs. These pairs (6 male and 6 female) served as the experimental pairs for this condition.

## Statistical Analyses

The data for Experiment 1 were analyzed using two-tailed independent sample t-tests. For Experiment 2, the data were analyzed using a two-way analysis of variance (ANOVA), with sex and group (mother-only, sibling-only, mother and sibling) as independent variables. For pairwise comparisons, the least significant difference test was used for post hoc tests. For multiple comparisons, the Bonferroni correction was used when needed. Because the measures for the aim, vigor and maintenance were ordinal (i.e., scores of 0, 1 or 2), a non-parametric test, the Kruskal-Wallis non-parametric one-way analysis of variance, was used, and for pairwise comparisons, Mann-Whitney U tests were used. Differences were considered significant for  $p$  values  $\leq 0.05$ . For graphical representation of interval data, values are given

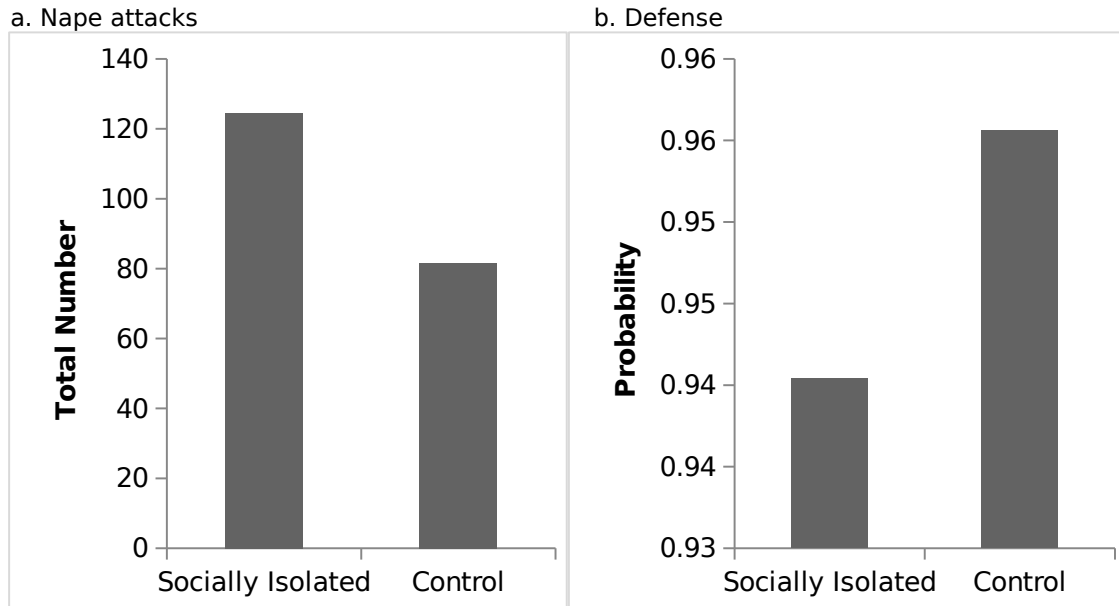
for group means and standard deviations, and ordinal data are given as group medians and ranges.

Inter-observer reliability for the same scorers was previously evaluated for the standardized measurements of playful attack and defense (B. T. Himmler et al., 2013c; S. M. Himmler et al., 2014c). However, because the measurements of aim, vigor and maintenance were new, these were evaluated for inter-observer reliability. For each of these measurements, 12 examples (two for each condition for each sex for the WWCPs rats), previously scored by one observer (B. T. H.), were re-scored by another observer (S. M. H.). Pearson's correlation revealed a high degree of inter-rater reliability (*aim*:  $r = 0.834$ ; *vigor*:  $r = 0.946$ ; *maintenance*:  $r = 0.908$ ). All correlations were significant ( $p < 0.05$ ).

## Results

### Experiment 1

**Playful attack.** There were no significant differences between groups for the proportion of playful interactions that began with the defense of the nape rather than contact on other areas of the body ( $p > 0.05$ ). All groups attacked the nape in over 90% of cases. The frequency of launching nape attacks was significantly different,  $t(10) = 5.736$ ,  $p = 0.0001$ , with socially isolated rats attacking more often (Figure 1a). With regard to the execution of attacks, there were no significant differences between groups for *aim* ( $p > 0.05$ ), *vigor* ( $p > 0.05$ ), or for *maintenance* ( $p > 0.05$ ) (Table 1).



**Figure 1.** (a) The total number of attacks per 10 minutes and (b) the probability of defending against a playful attack for LE rats.

**Playful defense.** There was no significant difference for the probability of defense ( $p > 0.05$ ) (Figure 1b). Both groups defended their napes in  $\geq 90\%$  of cases. Also, there were no significant group differences for the probability of using either of the defensive tactics: evasion ( $p > 0.05$ ) or complete rotation ( $p > 0.05$ ) (Table 1).

**Outcomes of play fighting.** The probability of defense involving rearing revealed a significant difference between groups with the control group rearing more than isolates,  $t(10) = -2.781$ ,  $p = 0.019$  (Table 1), but the probability that rearing led to boxing did not differ significantly between groups ( $p > 0.05$ ). There was no significant difference between groups for pinning ( $p > 0.05$ ) or in the probability of role reversals ( $p > 0.05$ ) (Table 1).

Table 1. *The three aspects of play measured for LE rats*

	Attack <sup>a</sup>			Defense <sup>b</sup>		Outcome <sup>b</sup>		
	Aim	Vigor	Maintenance	Evasion	CR	Rearing	Pinning	Role Reversals
<b>LE-Paired</b>	1.85 (1.6-2.0)	1.6 (1.4-1.8)	1.88 (1-2)	0.31 ± 0.02	0.43 ± 0.08	0.06 ± 0.01	0.69 ± 0.12	0.28 ± 0.08
<b>LE-Socially Isolated</b>	1.6 (1.3-2.0)	1.6 (1.3-1.8)	1.75 (1-2)	0.30 ± 0.05	0.42 ± 0.03	0.04 ± 0.01	0.62 ± 0.07	0.31 ± 0.06
<b>p</b>	ns	Ns	ns	ns	ns	< 0.05	Ns	ns

Note. <sup>a</sup>The scores for these measures are shown as medians and ranges (as shown in the parentheses), and the statistical comparisons were done using the Mann-Whitney U. <sup>b</sup>The scores for these measures are shown as mean ± SD, and the statistical comparisons were done using independent t-tests.

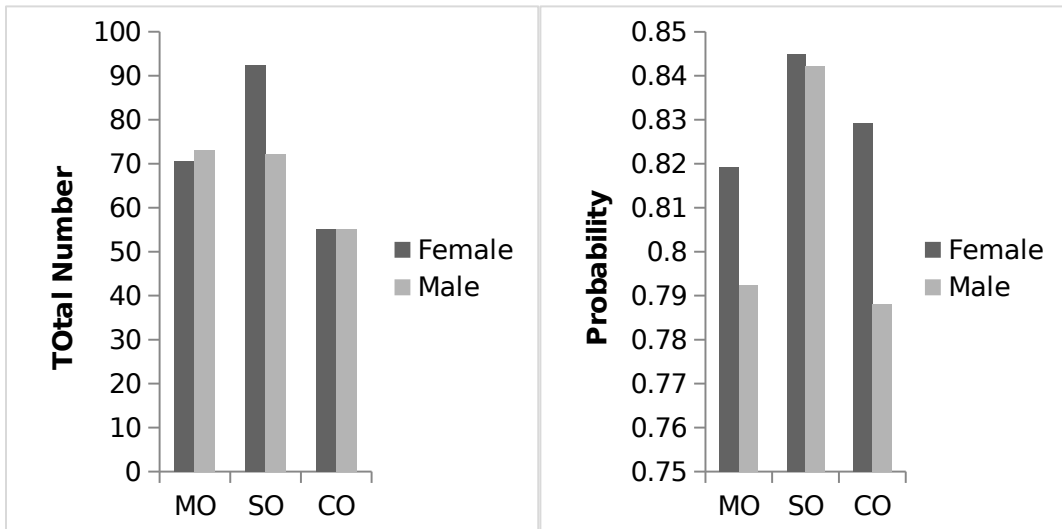
## Experiment 2

**Playful attack.** A 2 x 3 ANOVA for the proportion of playful interactions that began with the attack of the nape rather than contact on other areas of the body failed to reveal any significant difference for sex ( $p > 0.05$ ), group ( $p > 0.05$ ), or interaction between sex and group ( $p > 0.05$ ). All groups attacked the nape in over 90% of cases.

A 2 x 3 ANOVA for the total number of playful attacks did not show a significant sex difference ( $p > 0.05$ ), but did reveal a significant main effect for group,  $F(2, 26) = 4.632$ ,  $p = 0.019$ . Pair wise comparisons revealed that rats in the SO group launched more playful attacks than those in the CO group ( $p < 0.05$ ), but neither group differed from the MO group (Figure 2a). Even though there was no significant interaction for sex and group ( $p > 0.05$ ), inspection of Figure 2a indicates that most of the increase in the frequency of play by the SO group was likely due to the females.

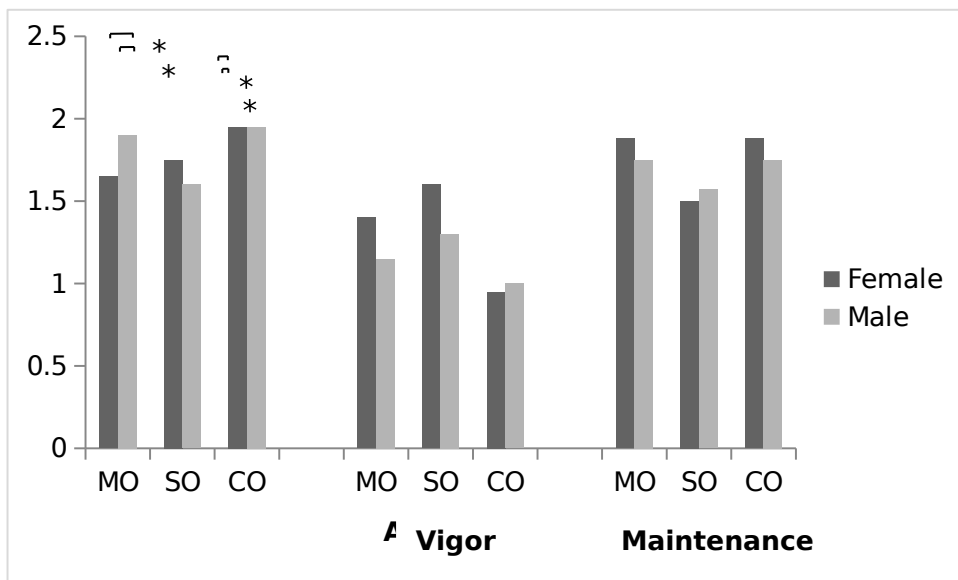
a. Nape attacks

b. Defense



**Figure 2.** (a) The total number of attacks per 10 minutes and (b) the probability of defending against a playful attack for WWCPs rats.

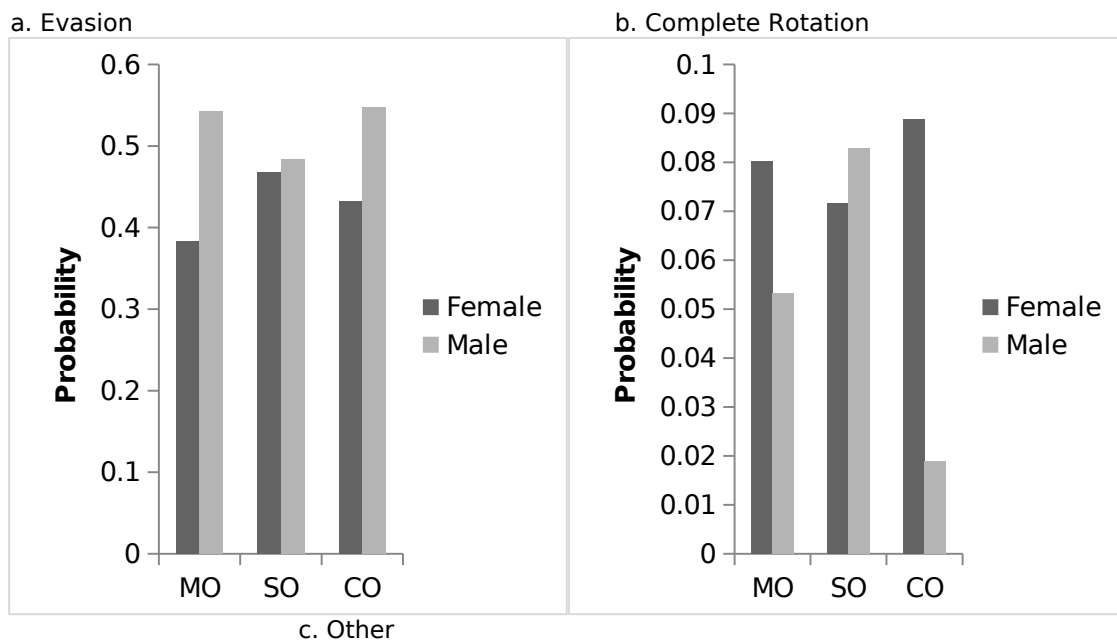
With regard to the execution of attacks, a Kruskal-Wallis test revealed a significant difference for *aim*,  $H(2) = 12.228$ ,  $p = 0.002$ , with the CO group scoring significantly higher than the SO and MO groups ( $p < 0.05$ ), but the MO and SO did not differ significantly from each other ( $p > 0.05$ ). There was no significant main effect for sex ( $p > 0.05$ ). For *vigor*, a Kruskal-Wallis test revealed a significant difference for group,  $H(2) = 7.605$ ,  $p = 0.022$  with the MO and SO groups scoring higher than the CO group ( $p < 0.05$ ), but the MO and SO did not differ significantly from each other ( $p > 0.05$ ). There was no significant main effect for sex ( $p > 0.05$ ). For *maintenance*, there were no significant main effects of group or sex ( $p > 0.05$ ) (Figure 3).

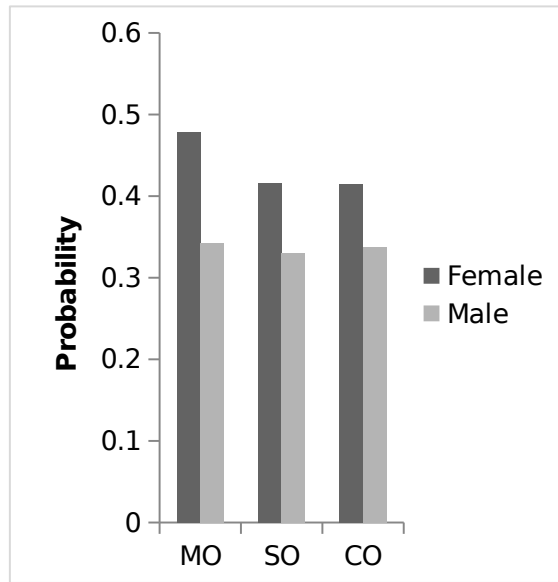


**Figure 3.** The median scores (and ranges) for the *aim*, *vigor*, and *maintenance* of playful attacks to the nape in all groups of WWCPs rats.

**Playful defense.** A 2 x 3 ANOVA for the probability of defending against a nape attack revealed no significant main effects or a significant interaction ( $p > 0.05$ ). All three groups defended their napes in  $\geq 90\%$  of cases. A 2 x 3 ANOVA for the probability of using evasive playful defense did not reveal significant group effect ( $p > 0.05$ ), but did reveal a significant main effect of sex,  $F(1, 26) = 8.474, p = 0.007$ , with males doing more than females ( $p < 0.05$ ). For facing defenses, there were no significant main effects or interactions for the probability of using complete rotation ( $p > 0.05$ ). However, there was a significant main effect for the probability of using other defenses for sex,  $F(1, 26) = 9.619, p = 0.005$ , with females using this defensive tactic more often ( $p < 0.05$ ). There was no significant main effect for group ( $p > 0.05$ ), nor a significant interaction between sex and group ( $p > 0.05$ ) (Figure 4).

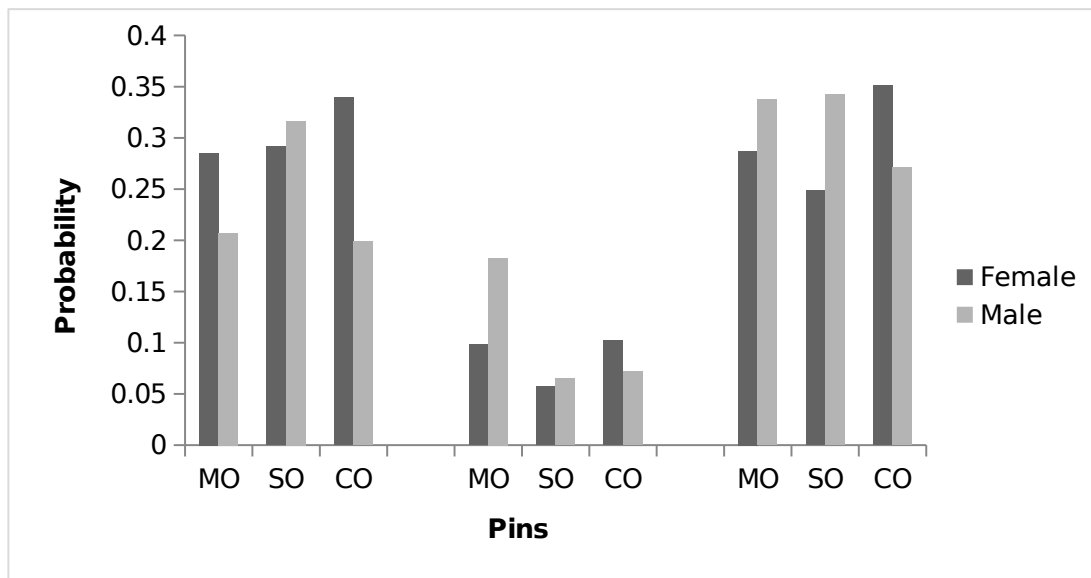
**Outcomes of play fighting.** A 2 x 3 ANOVA for the probability of defense involving rearing revealed a significant main effect for group,  $F(2, 26) = 4.027, p = 0.030$ , with the MO group having more rearing than the SO group ( $p < 0.05$ ), but neither group differed from the CO group (Figure 5). The probability that rearing led to boxing did not differ significantly among groups or between the sexes ( $p > 0.05$ ), but there was a significant interaction,  $F(2, 26) = 4.928, p = 0.015$ . Pair wise comparison revealed that the females in the SO group were more likely to engage in boxing than both the MO and CO groups ( $p < 0.05$ ) (Mean  $\pm$  SD: CO: Males =  $0.37 \pm 0.11$ ; Females =  $0.24 \pm 0.11$ ; MO: Males =  $0.29 \pm 0.14$ ; Females =  $0.18 \pm 0.14$ ; SO: Males =  $0.07 \pm 0.11$ ; Females =  $0.57 \pm 0.11$ ). A 2 x 3 ANOVA of pinning revealed no significant differences among the groups or between the sexes ( $p > 0.05$ ) (Figure 5).





**Figure 4.** The probability of using evasive tactics (a) the complete rotation tactic (b) and the ‘other’ tactic (c) in response to a playful attack for all groups of WWCPs rats.

For the probability of role reversals, there were no significant main effects for sex or group ( $p > 0.05$ ). However, there was a significant interaction,  $F(2, 26) = 4.665$ ,  $p = 0.019$ . Pair wise comparisons revealed role reversals were more common in females in the SO group compared to females in the MO and CO groups ( $p < 0.05$ ), although, within the SO group, they were more common in males as compared to females ( $p < 0.05$ ) (Figure 5).



**Figure 5.** The probability of defensive maneuvers resulting in a *pin*, a *rear*, or a *role reversal* for all groups of WWCPs rats.

## Discussion

The present study was designed to test the hypothesis derived from the findings by S. M. Himmler et al. (2014b) that peer-peer experience during the peri-weaning period is necessary for the development of juvenile-typical patterns of playful defense. In order to determine if peer experiences are needed, two experiments were conducted. The first experiment investigated the play of juvenile LE rats that were socially isolated for the week following weaning (i.e., matching the age at which the S. M. Himmler et al. (2014b) results were found) and the second experiment investigated the play of juvenile WWCPs rats that were reared as singletons with only the mother during the entire peri-weaning period. In both experiments, lack of play experiences with peers in the peri-weaning period did not affect the development of strain-typical preferences in the tactics of defense used during play in the juvenile period or for the consolidation of the nape as the target of playful attack. That is, peri-weaning play with peers is not necessary for the development of juvenile-typical patterns of attack and defense in play fighting, although the aim of nape attacks were less accurate and the vigor of their execution was increased in the experimental subjects from Experiment 2. However, given that these changes were present in both the MO group, which did not experience peer-peer play, and the SO group which did, it is likely that these may have resulted from underlying changes in excitability or motivation, rather than in the ability to play in the typical manner (see below).

A change that occurred in both experiments was in the motivation to play, as measured by the frequency of nape attacks (Panksepp, 1981; Pellis & McKenna, 1995; Thor & Holloway, 1984). There was a large increase in the frequency of nape attacks for the LE rats in the socially isolated group, which is consistent with other studies showing that rats that have been isolated for an extended period of time tend to play at a higher frequency (Byrd & Briner, 1999; Ikemoto & Panksepp, 1992; Panksepp & Beatty, 1980; Varlinskaya et al., 1999). An increase, albeit a smaller one, was also seen in the SO WWCPs rats. There may be two separate mechanisms involved in these two cases of increased of playfulness.

Following a period of social deprivation, rats will increase their initiation of playful contact with the nape, but this is not associated with a comparable increase in the frequency of social investigation (Panksepp, 1981; Panksepp & Beatty, 1980). This suggests that being deprived of peers is not simply producing an increase in the motivation for social contact, but a specific increase in the motivation to engage peers in play. This is supported by other studies which show that simply suppressing play without social isolation also produces a rebound in the frequency of play when the opportunity arises. For example, Baldwin and Baldwin (1976) showed that the frequency of social play in squirrel monkeys decreases when food is scarce, as more time is required to find food. However, once food is made readily available, the frequency of play increases to above normal baseline levels. These studies suggest that the motivation for play can be manipulated independently of other forms of social motivation. Such a selective increase in the motivation to play may account for the findings on the socially isolated LE rats from Experiment 1.

In the case of the SO WWCPs rats from Experiment 2, the increased play of the juveniles is consistent with findings from other studies showing that early weaning,

involving separation from the mother, tends to lead to increased playfulness (e.g., Brunelli et al., 1989; Janus, 1987). A possible avenue for this effect is that maternal contact involving licking, grooming and huddling, that is effective in altering juvenile play when received in the first two weeks after birth (e.g., Arnold & Sivi, 2002; Birke & Sadler, 1987; Karkow & Lucion, 2013; Moore & Power, 1992; Parent & Meaney, 2008; Veenema & Neumann, 2008), may continue to have some influence in the week preceding weaning. Whether this is the case has yet to be established, as is whether the change in playfulness arises from a specific influence on the motivation to play or from some more generic factors, such as changes in stress regulation (e.g., Caldji et al., 1998; Francis & Meaney, 1999), that may indirectly influence playfulness along with all social behavior.

That there were such potential stress-induced changes due to early rearing influences is the finding that, in the SO group, there was a significant increase in rearing. This suggests that, in the absence of the mother, there may be reduced regulatory control and an increase in aggression (see also Diamantopoulou et al., 2012). That both MO and SO reared subjects appeared more excitable (i.e., more vigorous nape attacks) and less accurate in their nape contacts, suggests that, in late infancy, both the presence of the mother and of siblings may contribute to the maturation of regulatory mechanisms that affect social behavior. That the changes in playfulness arising from atypical social environments in the peri-weaning period (Experiment 2) and from social isolation (Experiment 1) involve different mechanisms, is supported by the data on rearing in Experiment 1, in which the social isolates engaged in significantly less rearing. The reduced rearing typically results in more time engaged in contact promoting wrestling (Pellis & Pellis, 1987), suggesting that the increased frequency of launching playful attacks by the isolates is, indeed, a reflection of an increased motivation to engage in play.

## **The Prejuvenile Development of Playful Attack and Defense**

During the peri-weaning period, play is still developing, not becoming fully juvenile-typical until between 28-30 days of age (Pellis & Pellis, 1997). This continuing development suggests that the brain mechanisms involved in the regulation of play are also still maturing. The altered patterns of play induced over this period by the experience of play with peers of a different strain (S. M. Himmler et al., 2014b) further suggests that these brain mechanisms are sensitive to alteration by social play experiences. For these reasons, it is surprising that rats with no peer-peer play during the peri-weaning period still developed the juvenile-typical patterns of attack and defense.

The normal development of behavior patterns without prior experience with their performance has been categorized as prefunctional (Hogan, 2001). This label does not mean that no experience is necessary for the development of the behavior only that functional feedback from the performance of earlier forms of that behavior is not necessary. For example, dust bathing in fowl involves a sequence of movements, starting with the fowl pecking and scratching at the ground, dropping and spreading its wings, one at a time, rolling over to one side and then the other, and then finally standing and shaking its body. Dust bathing gradually matures in the young. Young

fowl will add elements of the dust bathing sequence to their unfolding repertoire, in the same order in which the complete sequence is performed. However, neither functional feedback from the incomplete versions of the dust bathing nor exposure to dust is necessary for the development of the complete dust bathing sequence (Vestergaard, Hogan, & Kruijt, 1990).

No deficits were found in the juvenile patterns of playful defense in rats that had been deprived of play with peers in either the post-weaning period alone or the whole peri-weaning period, extending from the beginning third week to the end of the fourth week after birth. The absence of practicing defensive tactics when attacked (the isolates in Experiment 1 and the MO rats in experiment 2) or of receiving slightly deviant attacks (the SO rats in Experiment 2) did not affect the achievement of juvenile-typical patterns of playful defense. That is, like the dust bathing in fowl, the development of juvenile-typical play in rats appears to be prefunctional in that it does not require experience from its performance during the peri-weaning period to develop into its mature form.

### **Why is the Development of Juvenile-Typical Play Fighting So Robust?**

Play and other social interactions during the juvenile period have been found to provide important experiences for developing and refining a variety of social, emotional and cognitive skills by modifying the brain mechanisms that regulate them (e.g., Arakawa, 2002, 2003, 2007a, b; Baarendse et al., 2013; Bell, Pellis, & Kolb, 2010; Delville, David, Taravosh-Lahn, & Wommack, 2003; Einon & Morgan, 1977; Einon et al., 1978; Hall, 1998; Himmler, Pellis, & Kolb, 2013b; Sivi, 2010; van den Berg, et al., 1999; Vanderschuren & Trezza, 2014; Von Frijtag et al., 2002). We hypothesize that, because play in the juvenile period is so critical in the development of these skills, its maturation is highly robust. That is, irrespective of small differences in experiences due to litter sizes, the sex-composition of those litters and the involvement of the mother, the form of the play expressed in the juvenile period converges onto the same pattern.

The increase in rearing and boxing, especially in the SO females, may suggest that they have become more aggressive; however, the finding that rats from all groups were able to maintain playful interactions as playful (i.e., all had similar levels of role reversals and pinning), suggests that this may not be the case. Therefore, even in the absence of earlier play experience with peers, juvenile rats must still be capable of using play signals or other cues which enable them to communicate these interactions as being playful (e.g., Bekoff, 1995; Himmler, Kisko, Euston, Kolb, & Pellis, 2014a; Kipper & Todt, 2002; Palagi, 2008; Pellis & Pellis, 1983) and must have the neural mechanisms in place to ensure that the interactions remain reciprocal (Pellis, Pellis, & Bell, 2010a).

### **Conclusion**

While social experiences with peers and the mother in the peri-weaning can influence the development of some aspects of play (e.g., level of motivation), the data from the present study converge in showing that playing with peers in the pre-juvenile

period is not necessary to develop juvenile-typical patterns of attack and defense. These findings apparently contradict those of S. M. Himmler et al. (2014b), which showed that housing with members of a different strain in the week following weaning alters the juvenile-typical pattern of playful defense. A possible resolution to these seemingly conflicting findings may be as follows. Under the normal range of variability in rearing experiences (e.g., different size of litter, sex ratio), playful attack and defense matures to its typical form. Moreover, as shown in the present paper, this maturation can proceed to its typical end-point without the need for functional feedback from playing with peers.

Thus, juvenile-typical play does not require to be reinforced by particular feedback from playing to emerge. However, encountering feedback that is discordant, as is provided by playing with a member of a strain with a marked difference in preference of particular defensive tactics, can reset the trajectory of development. Interestingly, once reset, the form of the play remains resilient and unchanging even when encountering rats of different strains with differing preferences in playful defense (S. M. Himmler et al., 2014b). Such resiliency suggests that the resetting involves changes to neural mechanisms that regulate play. This model suggests that there are bounds of experience within which juvenile-typical play develops unchanged, but that there are experiences that can alter that development. The question then becomes whether such pattern-altering experiences are within the naturally occurring range of variation likely experienced by some rats under natural conditions.

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