



## **Deficient Play-Derived Experiences in Juvenile Long Evans Rats Reared with a Fischer 344 Partner: A Deficiency Shared by Both Sexes**

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Play fighting during the juvenile period has been shown to be an important experience for the development of sociocognitive skills and the underlying neural mechanisms that support them. Various paradigms have been used to deprive rats of play while still providing social contact. We used the paradigm of rearing a playful rat with a low-playing Fischer 344 (F344) partner to limit the play experienced by Long Evans (LE) rats during the juvenile period. This rearing paradigm has previously been shown to cause sociocognitive impairments in adulthood. In the present paper, we examined the play of same-sex LE rats with LE or F344 partners at the peak juvenile period (around 35 days of age). F344 rats launched fewer playful attacks and, when attacked, defended atypically compared to how LE do in LE-LE pairs. Playing with an F344 partner afforded LE rats fewer opportunities to engage in prolonged wrestling and fewer opportunities to ward off counterattacks (in which the defending rat becomes the attacker). In addition, there were fewer vocalizations emitted during the encounters in LE-F344 pairs, and the types of calls most often emitted differed to those between LE-LE pairs. The altered play and communication experiences were equally present in male and female pairs. These findings are consistent with the hypothesis that, in such rearing paradigms, it is impoverished-play experiences in the juvenile period that lead to impaired sociocognitive skills in adulthood.

*Keywords:* executive functions, Fischer 344 rats, play deprivation, play fighting, reciprocity, sex differences

Rats live in large social groups, with the males forming complex dominance hierarchies that are reinforced by overt aggression as well as by affiliative interactions involving play and grooming (Adams & Boice, 1983; Blanchard et al., 1988; Pellis et al., 1993). While females do not form strict hierarchies, there is evidence for subtle, dominance relationships (Ziporyn & McClintock, 1991). Therefore, functioning within the group requires a repertoire of social behaviours that can be used in contextually appropriate ways (Barnett, 1975; Lore & Flannelly, 1977; Meaney & Stewart, 1979). The development of these social skills, which are mediated by the executive functions dependent on neural circuits in the medial prefrontal cortex (mPFC), has been linked to the social play experienced during the juvenile period (Omrani et al., 2020; Pellis et al., 2014; Schneider, Pätz, et al., 2016; Stark & Pellis, 2020; Van Den Berg et al., 1999; Vanderschuren & Trezza, 2014). For example, rats socially isolated during the juvenile period (postnatal day 21 – 42) have altered dopamine sensitivity of mPFC neurons and impoverished impulse control as adults (Baarendse et al., 2013). Similarly, rearing Wistar rats, a highly playful strain (Himmler, Modlinska, et al., 2014), during the juvenile period with a Fischer-344 partner, a low playing strain (Siviy, 2020; Siviy et al., 1997; Siviy et al., 2003), leads to impairments in social recognition in the Wistar as adults (Schneider, Bindila, et al., 2016). Moreover, when juvenile Long Evans rats, another highly playful strain (Himmler, Modlinska, et al., 2014), are only reared with adults, they experience impoverished play (Pellis et al., 2017) that appears to lead to atypical development in the dendritic morphology of their neurons in the mPFC (Bell et al., 2010; Himmler, Pellis, & Kolb, 2013; Himmler et al., 2018). Changes in the development of the mPFC, and social skills, resulting from inadequate play experiences have also been shown in other rodent species that have complex patterns of social play (Burlinson et al., 2016; Marks et al., 2017).

In rats, as in the young of many other mammal species, play fighting is the most common form of social play (Burghardt, 2005; Pellis & Pellis, 2009). Play fighting in juvenile rats involves competition for and defense of the nape of the neck, which, if contacted, is gently nuzzled with the tip of the snout (Pellis & Pellis, 1987; Siviy & Panksepp, 1987). Such play is highly rewarding (Trezza et al., 2010; Trezza et al., 2011; Vanderschuren, 2010). When defending the nape, rats use several tactics. They can evade by turning away from

their attacker and by swerving laterally, running or jumping, or they can turn to face their attacker, thereby using their body to block access to the nape. Facing defense can involve partial rotation, in which the rat rotates its upper torso while leaving at least one of its hind paws in contact with the floor, a complete rotation to supine, or a standing defense, in which the animal rears onto their its hind legs and engages in a pushing or boxing contest (Pellis & Pellis, 1987). Development of playful attacks directed at the nape begin at around 17 days after birth (Baenninger, 1967; Bolles & Woods, 1964), but defensive tactics, especially those involving complete rotation to supine, do not achieve their juvenile-typical form until around 28 days after birth (Pellis & Pellis, 1997). Thus, when play reaches its peak frequency between 30 and 40 days after birth, rats have a fully mature repertoire of playful actions (Meaney & Stewart, 1981; Panksepp, 1981; Pellis & Pellis, 1990; Thor & Holloway, 1984). This is the age at which the effects of play are most pronounced on the development of sociocognitive skills and the development of the neural circuitry of the mPFC (Pellis et al., 2014; Vanderschuren & Trezza, 2014).

There are several components of the play dynamic that influence the development of social competency and the mPFC. When a juvenile rat is reared with a partner that affords an impoverished play experience - such as an adult, a drugged peer, or a peer from a less playful strain, such as a Fischer 344 - one compensatory tactic employed by the impoverished rat is to increase the overall number of attacks it launches, thus increasing the overall number of play fights it experiences; however, despite such compensation, deficits still persist (Einson et al., 1978; Pellis et al., 2017; Schneider et al., 2014; Schneider, Bindila, et al., 2016; Schneider, Pätz, et al., 2016). Therefore, increasing playful nape attacks is not sufficient to overcome the sociocognitive deficits. Rather, it is the defensive tactics of one's partner that appear critical, especially as these affect the reciprocity of the interactions (Pellis, Pellis, Pelletier, & Leca, 2019; Pellis & Pellis, 2017; Schneider et al., 2014; Schneider, Bindila, et al., 2016; Schneider, Pätz, et al., 2016).

For play fighting to remain playful, some degree of reciprocity or turn-taking is needed (Altmann, 1962). Although the relative degree in the occurrence of turn-taking varies with the species, age, dominance relationship between the animals, and their sex, a degree of reciprocity is needed for play fighting to continue (Pellis & Pellis, 2017). In rats, as with many other species, this is achieved by showing restraint in the execution of the tactics of attack and defense in a way that makes it easier for one's partner to gain the advantage (Pellis & Pellis, 1998). For example, in rats, a common configuration adopted during play fighting is the pin, in which one animal stands over its supine partner (Panksepp, 1981). The rat standing on top typically holds its supine partner down with its forepaws while at the same time anchoring its body weight by standing on the ground with its hind paws. Sometimes, the rat on top will stand on its supine partner with all four of its paws, reducing its own stability as the supine animal squirms (Foroud & Pellis, 2003). Counterattacks by the supine partner are 2-3 times more likely to lead to a role reversal when the on-top rat does not have its hind paws anchored on the ground (Pellis et al., 2005). The unanchored position is most common between 30-40 days of age (Foroud & Pellis, 2002), the age at which play is most frequent (Thor & Holloway, 1984) and most likely to influence brain development (Vanderschuren & Trezza, 2014). The opportunity to reverse roles is a key component of play fighting (Pellis & Pellis, 2017), requiring the animals to track exchanges, so ensuring that they are reciprocal, and making decisions to respond to an attack appropriately, all of which engages executive functions related to the mPFC. It is likely that using these executive functions during play in the juvenile period finetunes the underlying mechanisms (Pellis et al., 2014; Pellis, Pellis, Himmler, et al., 2019). Reduced opportunity for reciprocity is not only present in Long Evans juveniles playing with adults (Pellis et al., 2017) but also occurs in Wistar juveniles when playing with Fischer 344 rats of the same age and sex (Schneider et al., 2014; Schneider, Bindila, et al., 2016; Schneider, Pätz, et al., 2016).

Male Long Evans rats that are reared with a Fischer 344 during the juvenile period have reduced social competence as adults (Stark & Pellis, 2020), as do female Wistar rats reared with Fischer 344 rats (Schneider, Bindila, et al., 2016; Schneider, Pätz, et al., 2016). In the present paper, we tested the hypothesis that the social deficits in adult Long Evans rats reared with Fischer 344 partners arise from their play experiences in the juvenile period. If this is true, the play of juvenile Long Evans rats with Fischer 344 peers should be impoverished relative to that of Long Evans juveniles playing with same strain partners. Based on previous research (Pellis et al., 2017; Pellis, Pellis, Himmler, et al., 2019; Schneider, Bindila, et al., 2016; Schneider, Pätz, et al., 2016), this impoverishment should be reflected in several ways.

We predicted (1) that Long Evans rats reared with a Fischer 344 peer will experience less play and compensate for the reduced frequency of attacks by their Fischer 344 partner by increasing their own frequency of attacks, and (2) that less of the play between the Long Evans and F344 rats will lead to wrestling and so result in fewer opportunities for them to gain experience with role reversals. Ultrasonic vocalizations (USV) seem to be important for coordinating behaviours during play, facilitating reciprocity, and avoiding escalation to aggression (Burke, Kisko, Pellis, et al., 2017; Kisko, Euston, & Pellis, 2015; Kisko, Himmler, et al., 2015). Given that Wistar-Fischer 344 pairs have reduced rates of emission of USV (Schneider, Pätz, et al., 2016), we predicted (3) that pairs of rats involving a Long Evans and a Fischer 344 will emit fewer USV, especially the types of calls most associated with coordinating play (Burke et al., 2018; Burke et al., 2020).

Because previous research using both the adult rearing paradigm (Bell et al., 2010; Himmler, Pellis, & Pellis, 2013; Pellis et al., 2017) and the Fischer 344 rearing paradigm (Schneider et al., 2014; Schneider, Bindila, et al., 2016; Schneider, Pätz, et al., 2016) have only used females, it was not known whether both sexes have comparable experiences during play in the juvenile period when reared with a partner that plays atypically. Therefore, in the present study, a second goal was to compare the play experienced by pairs of females with that of pairs of males. Based on previous findings (Himmler, Bell, et al., 2014; Pellis et al., 2017; Schneider, Bindila, et al., 2016), we predicted (4) that playing with an atypical play mate will affect the play experienced in both sexes, as, in both cases, sociocognitive skills are compromised (Schneider, Bindila, et al., 2016; Stark & Pellis, 2020). However, what cannot be predicted a priori is whether the magnitude of changed experiences is similar in both sexes.

## Method

### Subjects

A total of 72 weanling rats were purchased from Charles River Laboratories (Kingston, New York), and the rats arrived at the Canadian Centre for Behavioural Neuroscience at 24 days of age. Fifty-two (26 males, 26 females) were Long Evans (LE) rats and 20 (10 males, 10 females) were Fischer-344 (F344) rats. At 26 days of age, rats were housed in same-sex pairs, of either two LE rats (8 male pairs and 8 female pairs; LE-LE), or as pairs composed of a LE and a F344 (10 male pairs and 10 female pairs; LE-F344). All animals were housed in polyethylene cages (46 cm × 25 cm × 20 cm) with corncob bedding, in the vivarium colony room maintained at a constant temperature (21 – 23°C), and on a 12-hour light-dark cycle (lights off at 1900). Food and water were provided ad libitum. All care and procedures were approved by the University of Lethbridge Animal Welfare Committee in compliance with guidelines from the Canadian Council for Animal Care.

### Procedure

Play was tested twice between 33 and 38 days of age, which is within the peak period of play (Thor & Holloway, 1984). All pairs were habituated to the testing enclosure for 15 min each day for three days prior to testing. All habituation and testing trials occurred in complete darkness, which has been shown to increase play (Pellis & Pellis, 1997; Smith et al., 1998). After the third habituation session, pairs were isolated in separate cages for 24-hr before testing, a method that increases playfulness (Niesink & Van Ree, 1989; Panksepp & Beatty, 1980; Pellis & Pellis, 1990). Test sessions were 10 min in length, an amount of time found to be sufficient to capture most aspects of play (Pellis & Pellis, 1990). After the first test session, animals were pair housed again for 24-hr before the isolation and subsequent testing for trial two was performed. The second trial was to ensure adequate data for analysis in case of data loss. The rats were tested again as adults (Stark & Pellis, 2020) and were then sacrificed, with their brains removed for histological analysis for a follow-up study on the consequences of atypical play experience in the juvenile period (work in progress).

## Apparatus

Play trials were conducted in a 50 cm × 50 cm × 50 cm Plexiglas® box with 1-2 cm of CareFresh® bedding. The Plexiglas® box was housed in a sound-attenuating chamber lined with sound-attenuating foam (Primeacoustic, Port Coquitlam, British Columbia). Video was recorded through a small window of the chamber from a 45° angle with an ExmouRS 4K Sony Handycam. The camera was set to record in night-shot to film in the dark. Vocalizations were also recorded simultaneously using a microphone capable of recording in the ultrasonic range (Model 4939, Brüel & Kjaer, Denmark), which was 32-38 cm above the centre of the Plexiglas® box, and a red-light flash and audible sound was used to synchronize audio and video post filming. Sounds between 4-100 kHz were recorded. At the beginning of each habituation and test trial, three consecutive flash/beep signals were given to synchronize video and audio recordings.

## Behavioral Analysis

Only video files from the first test session were used for analysis, as there was no data loss during acquisition. Video was analyzed using a combination of normal speed and frame-by-frame inspection to score various aspects of playful attack and defense (Himmler, Pellis, & Pellis, 2013). The behavior of both partners in the dyad was scored. The play of the LE rat from the LE-F344 pairs was compared to the play of one of the rats from the LE-LE pairs (focal rats). The LE rat treated as the focal rat from the LE-LE pair was selected at random. Comparison of the focal rats provided a means of assessing the altered play experience of the LE rats reared with F344 partners. Comparison of the partners of the focal rats (the F344 partner and the other LE rat) provided a means of assessing the partners' contribution to the play experiences of the focal animals. Videos were scored in terms of the actions made by the focal animal toward their partner and those actions directed toward the focal animal by their partner. The pelage of the LE rats is white on the body and black on the head and has a black pattern extending down the back. During the trials, individual LE rats, especially those in the LE-LE pairs, were tracked by using their back patterns as well as by distinctive tail markings made with a Sharpie® before filming. The F344 rats have a fully white pelage and so could be easily tracked.

Playful attacks were scored when the snout of one rat either moved toward the nape or contacted the nape of the other rat. The recipient of an attack could either continue with its ongoing behavior or defend itself against the attack. The number of attacks defended divided by the total number of attacks gave the probability of defense. Defense involved two main types of tactics: evasion or a facing defense (Himmler, Pellis, & Pellis, 2013). When evading, the defending rat creates distance between the attacker's snout and its own nape by running, leaping, or swerving away. Facing defense involves the defending rat rotating towards its attacker, juxtaposing its face between its nape and the snout of its partner. The probability of a rat defending using a facing defense was calculated as the number of facing defenses divided by the total number of defenses. Three tactics of facing defense are used during play (Himmler, Pellis, & Pellis, 2013; Pellis & Pellis, 1990). The first facing defense is partial rotation, whereupon being attacked, the defending rat rotates its upper torso around its longitudinal axis to face its attacker, while keeping at least one of its hind paws on the ground. The second is complete rotation, whereupon being attacked, the defending rat rotates completely around its longitudinal axis to supine. The third facing defense is when, on being attacked, the defending rat rotates around its vertical axis at the level of its pelvis so that it remains prone when facing its attacker (Pellis et al., 1994). The vertical rotation tactic can lead to both rats rearing into a mutual upright position from which they push and box one another (Pellis & Pellis, 1987), although this is rarer in the juvenile period than it is later in development (Pellis & Pellis, 1990). The probability of each type of facing defense was calculated by dividing a particular type of defense (e.g., complete rotation) with the total number of facing defenses performed.

A common consequence of a facing defense is for one partner to end up lying on its back with its partner standing over it – referred to as a *pin* (Panksepp, 1981). Although for some strains, most pins arise from the defender performing a complete rotation, the partner can also be forced over onto its back (Himmler, Himmler, Styjek et al., 2016; Pellis et al., 2017; Pellis & Pellis, 1997). Irrespective of how the pin configuration arises, it is thought to be important in providing some of the rewards derived from play (Panksepp, 1998), so the frequency of pins was scored. However, given the predicted differences across groups in the total number of play fights (Schneider et al., 2014), the probability of pins was calculated by dividing the number of pins by the total number of facing defenses performed to standardize the comparison. Finally, because reciprocity is important for sustaining play fighting (Pellis & Pellis, 2017) and likely for the benefits derived from play in promoting the development of sociocognitive skills (Pellis et al., 2017; Pellis, Pellis, Himmler, et al., 2019; Schneider, Bindila, et al., 2016), a measure that assessed reciprocity was also scored. Playful encounters involving facing defense in which the defending animal was successful in launching a counterattack towards the nape of its attacker, thereby becoming the attacker, were scored. Again, because of the predicted group differences in the total amount of play fighting (Schneider et al., 2014), the probability of role reversals was used for comparison (Himmler, Himmler, Pellis, & Pellis, 2016). This was calculated by dividing the number of role reversals with the number of facing defenses performed.

In part, reduced reciprocity also leads to asymmetry in many aspects of the play between partners in a pair (Pellis et al., 2017). Therefore, to assess the asymmetry between the playmates in the different groups, the absolute difference between the scores of partners was calculated. By dividing this value with the total score of the pair, a score between 0 and 1 was produced. Values close to zero indicate little asymmetry in the behavior between partners and values close to 1 indicate a high degree of asymmetry between partners.

## Vocalization Analysis

Rats emit various vocalizations during which have been characterized into two broad categories based on the frequency they are emitted at and the context they are emitted in (see Portfors, 2007, for review). Fifty kilohertz (kHz) calls are emitted in appetitive situations, such as anticipating rewarding drugs, during sexual encounters, social play, and when tickled by an experimenter (Bialy et al., 2000; Burgdorf et al., 2008; Himmler, Kisko, et al., 2014; Knutson et al., 1998; Panksepp and Burgdorf, 2000). On the other hand, 22 kHz calls are emitted during negative situations, such as social defeat, foot shock, drug withdrawal, and when presented with predator odor (Blanchard et al., 1991; Thomas et al., 1983; Tonoue et al., 1986). Since it is known that juvenile emit various 50 kHz vocalizations during play, and no 22 kHz vocalizations were noted during analysis, we focused on this category. Additionally, in certain contexts, as a result to negative stimulations, such as foot shock, rats will emit audible calls which seem to vary amongst strain (Schwartz, 2018a, 2018b). Upon initial analysis, vocalizations were noted in the audible range and thus were included in the subsequent analysis (see below).

Acoustic data were analyzed using Raven Pro 1.4 software (Bioacoustics Research Program, Cornell Lab of Ornithology), which generates spectrograms with a 256-sample Hann window. A trained experimenter manually selected vocalizations for analysis. Spectral analysis was performed for all vocalizations emitted during the 10-min play session. As well as scoring the number of calls emitted in the pairs of rats from the different groups, the differential use of some of the types of calls was also scored. Although there are many different types of 50 kHz ultrasonic vocalizations (Wright et al., 2010), for present purposes, only four types were scored – flat calls, audible calls, trill and frequency modulated (FM) calls (Burgdorf et al., 2008; Burgdorf & Panksepp, 2006; Wöhr et al., 2008). Altogether, vocalizations were categorized into four types based on visual identification: (1) Trill calls, a form of FM ultrasonic call that are particularly common during play (Himmler, Kisko, et al., 2014), were identified based on their rapid frequency oscillations, (2) audible calls were identified based on the frequency at which they occur and were confirmed via audio playback detectable by the experimenter, (3) flat calls had a near constant frequency and occurred above 30 kHz, and (4) FM vocalizations included all nontrill ultrasonic vocalizations that involve frequency modulations. The total number of vocalizations included in the analysis is shown in Table 1. Trills are not only commonly emitted during play (Himmler, Kisko, et al., 2014), but are also highly correlated with nape attacks (Burke, Kisko, Swiftwolfe, et al., 2017; Burke et al., 2018). Similarly, some of the other FM calls are highly correlated with defensive actions that are likely to lead to role reversals (Burke et al., 2018; Burke et al., 2020).

**Table 1**

*Total Number of Vocalizations Included in the Analyses of Rates of Vocalizations and Types of Calls Emitted*

	LE-LE Pairs		LE-F344 Pairs	
	Male	Female	Male	Female
Number of ultrasonic vocalizations	7756	6012	4490	4986
Number of audible vocalizations	99	75	1246	1831

## Statistical Analysis

All statistics were performed using RStudio (R Core Team, 2018) and the packages *car* (Fox & Weisberg, 2019) and *lsmeans* (Length, 2016), as well as the package *ggplot2* (Wickham, 2016) to create the graphs. Comparisons of the proportion of playful attacks made by the focal and partner along with the probability of defense, facing defense, complete rotation, pin, and successful counterattacks (i.e., role reversals) were tested using a two-way ANOVA, with rearing condition and sex as the factors. Comparisons of the average number of vocalizations emitted during the 10-min play session, the average number of vocalizations emitted per play bout, as well as the proportion of call types emitted, were tested using a two-way ANOVA with the same parameters as above. As no significant interactions between sex and condition were found, post hoc analyses were not conducted. Differences were considered significant if the *p*-value was  $\leq .05$ . All graphs and tabular data are presented as the group means and standard error of the mean.

## Results

### Play Fighting: Focal Subjects

Compared to the matched juveniles from the same strain dyads, there was no difference in the number of nape attacks launched by the LE rats paired with the F344 rats,  $F(1, 32) = 0.11, p = .75$ , regardless of sex,  $F(1, 32) = 3.39, p = .08$  (Figure 1A). While there was no significant difference in the likelihood of defense,  $F(1, 32) = 0.13, p = .73$  (Figure 1B) or in the likelihood of using facing defense,  $F(1, 32) = 0.75, p = .39$  (Figure 1C), there was a significant difference in the likelihood of making a complete rotation,  $F(1, 32) = 67.68, p < .01$ , with the LE rats reared with an F344 rat less likely to rotate to supine (Figure 1D). There was a significant reduction in the likelihood of a facing defense, which resulted in the LE rats being pinned by the F344 rats,  $F(1, 32) = 17.71, p < .01$  (Figure 1E), and there was a significant increase in the probability of an LE rat reared with an F344 rat in launching successful counterattacks,  $F(1, 32) = 24.50, p < .01$  (Figure 1F). There were no significant sex differences for any of the measures.

### Play Fighting: Partners of the Focal Subjects

As seen in Figure 2A, compared to the matched juveniles from the same strain dyads, F344 rats launched fewer nape attacks,  $F(1, 32) = 44.47, p < .01$ , regardless of sex,  $F(1, 32) = 0.44, p = .51$ . While there were no differences found in the likelihood of defense between the F344 rats and the matched control LE rats,  $F(1, 32) = 1.00, p = .32$ , there was a sex difference with females more likely to defend themselves,  $F(1, 32) = 12.46, p < .01$  (Figure 2B). The F344 partners were more likely to respond with facing defense,  $F(1, 32) = 32.02, p < .01$  (Figure 2C), but not in the likelihood of using complete rotation,  $F(1, 32) = 0.91, p = .35$  (Figure 2D), or in the likelihood of a facing defense resulting in a pin,  $F(1, 32) = 0.53, p = .47$ . There was a significant difference in the probability of launching successful counterattacks,  $F(1, 32) = 54.93, p < .01$ , with F344 partners being less likely to produce role reversals (Figure 2F). Except for the sex difference in the probability of defense, no other measures differed significantly between the sexes.

### Asymmetry between Play Partners

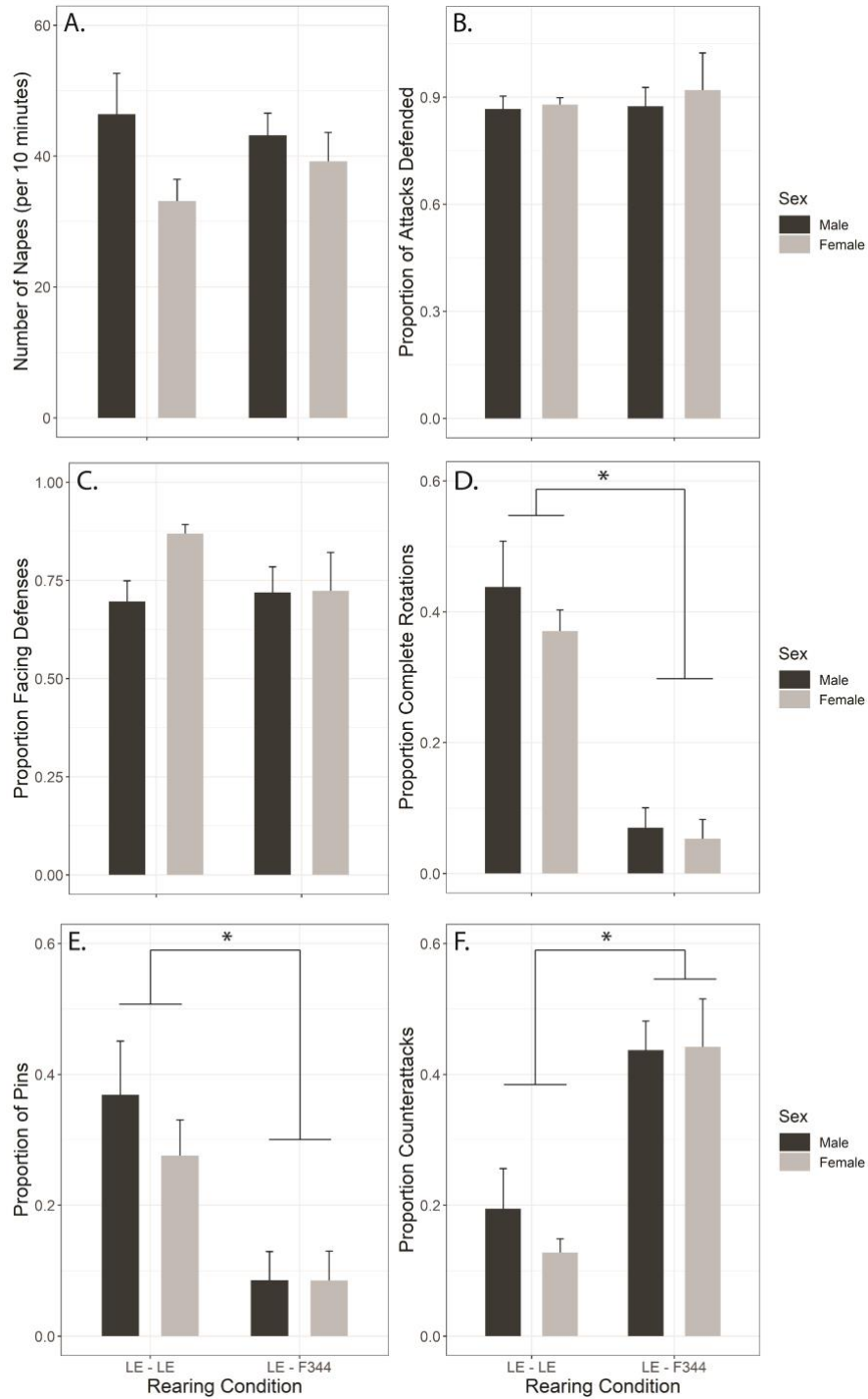
The mixed strain pairs exhibited more asymmetry than the same-strain control pairs in the number of nape attacks launched,  $F(1, 32) = 34.84, p < .01$  (Figure 3A), the likelihood of facing defense,  $F(1, 32) = 7.15, p = .01$  (Figure 3C), the likelihood of complete rotations,  $F(1, 32) = 15.07, p < .01$  (Figure 3D), the likelihood of pins,  $F(1, 32) = 4.17, p = .05$  (Figure 3E), and in the likelihood of launching successful counterattacks,  $F(1, 32) = 39.37, p < .01$ . There was a trend for defending against nape attacks being more variable in the mixed strain pairs (Figure 3B), but this was not significant,  $F(1, 32) = 2.79, p = .10$ . There were no significant sex differences for any of the measures.

### Vocalizations

There was a significant difference in the average number of vocalizations emitted during the 10-min play session,  $F(1, 32) = 6.91, p = .01$ , with mixed strain pairs emitting fewer calls (Figure 4A). However, there was no significant difference in the rate of calling per play bout,  $F(1, 32) < 0.01, p = .97$  (Figure 4B). There were also significant differences in the call types that were emitted. The LE-F344 dyads were less likely to emit trills,  $F(1, 32) = 29.19, p < .01$  (Figure 4C), and other FM calls,  $F(1, 32) = 6.77, p = .01$  (Figure 4F), as well as being more likely to emit audible calls,  $F(1, 32) = 50.27, p < .01$  (Figure 4D), but there were no significant differences in flat calls,  $F(1, 32) = 0.90, p = .35$  (Figure 4E). There were no significant sex differences for any of the measures.

# Figure 1

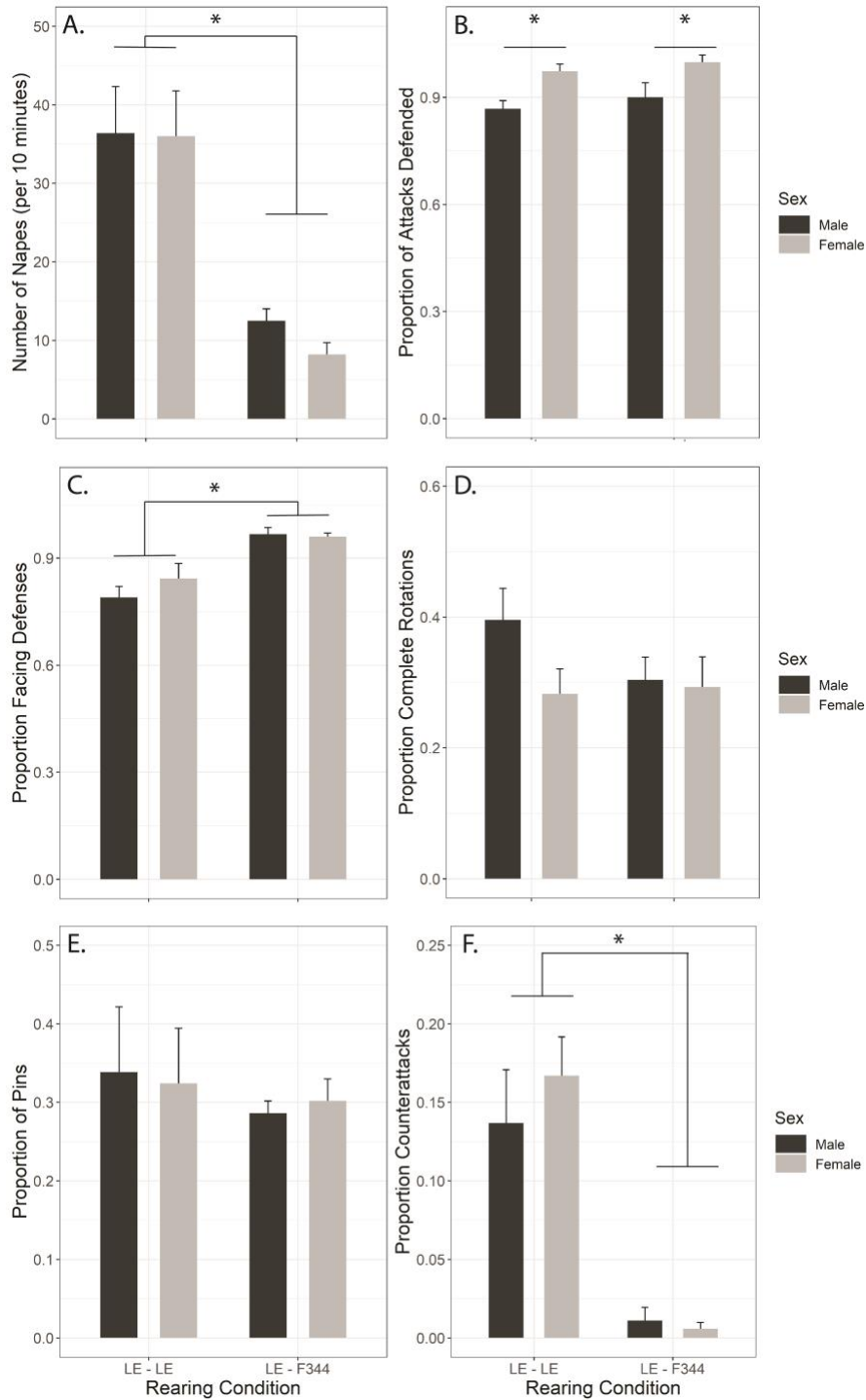
## Play Behaviors of the Male and Female Focal Rats



*Note.* The focal rat is the LE that was paired with the F344 (right) compared to its matched control, a LE reared with a LE (left), separated into males (dark grey) and females (light grey). A) shows the number of nape attacks made by the focal animal, while B - F) show the reactions made by the focal rat in response to being attack by its play partner. All measures are represented as the mean  $\pm$  the SEM, (\*) denotes significant  $p \leq .05$ .

**Figure 2**

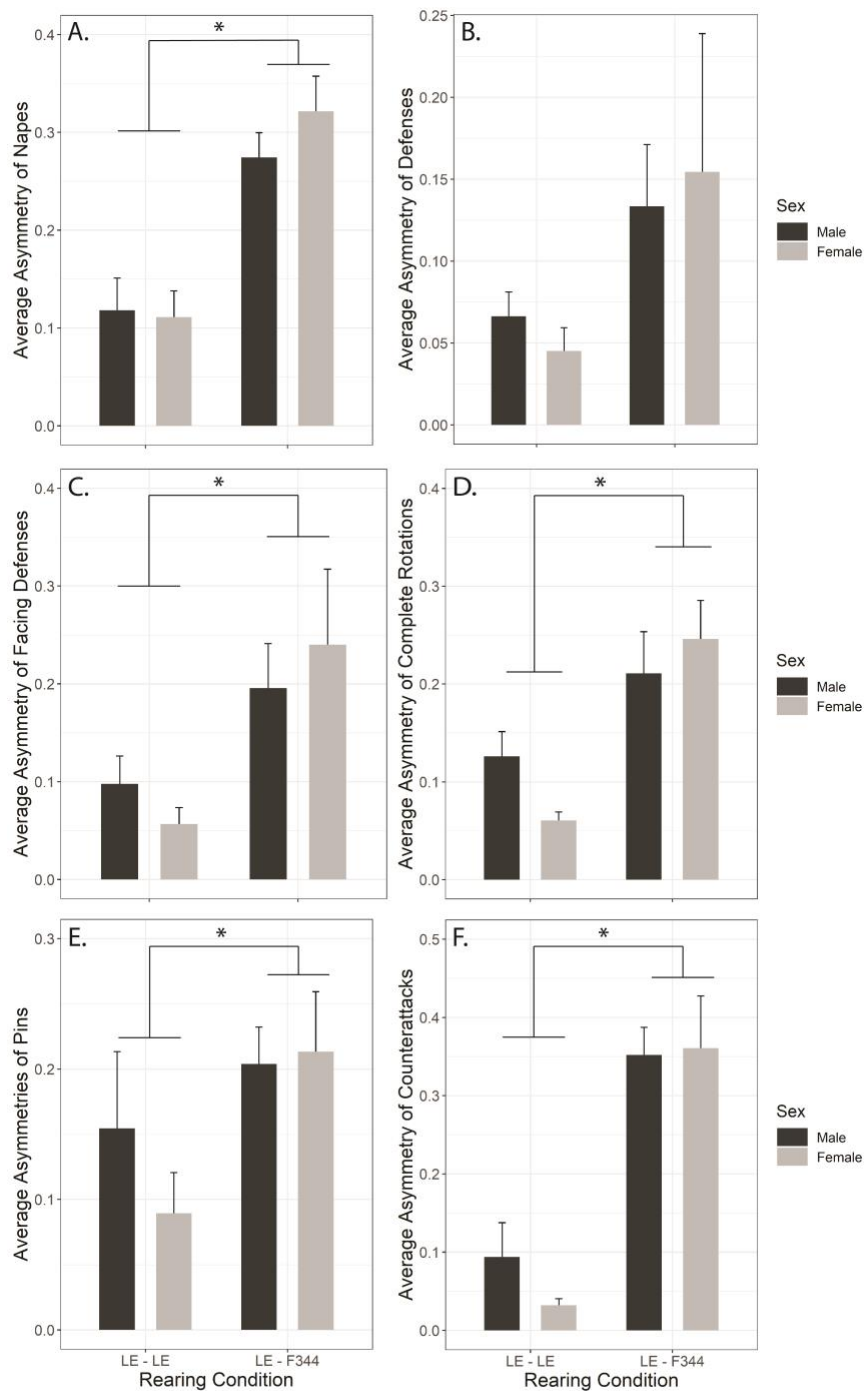
*Play Behaviors of the Male and Female Partners of the Focal Rats*



*Note.* The partner of the focal rat is the F344 in the experimental condition (right) and a matched LE rat in the control condition (left), separated into males (dark grey) and females (light grey). A) shows the number of nape attacks made by the partner, while B - F) show the reactions made by the partner in response to being attack by the focal rat. All measures are represented as the mean  $\pm$  the SEM, (\*) denotes significant  $p \leq .05$ .

**Figure 3**

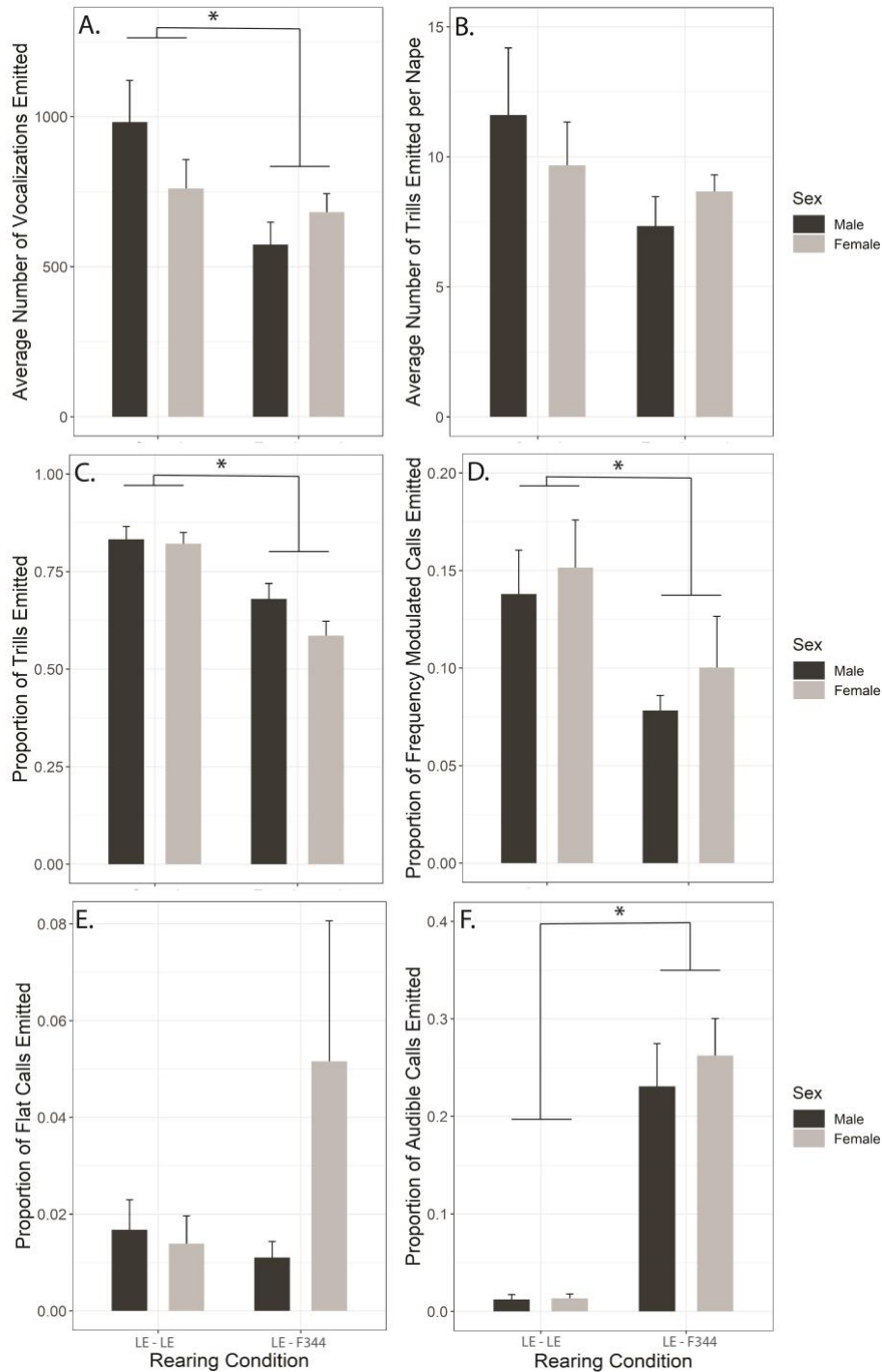
*Asymmetry Between the Experimental and Control Partners in Both Male and Female Rats*



*Note.* The experimental pairs are the LE rats paired with a F344 rat (right) and the controls are pairs of LE rats (left), separated into males (dark grey) and females (light grey). A) shows the asymmetry of nape attacks made, B) shows the asymmetry in defending an attack, C) shows the asymmetry in the use of facing defenses, D) shows the asymmetry in a facing defense leading to a complete rotation, E) shows the asymmetry in a facing defense leading to a pin, and F) shows the asymmetry in a facing defense leading to a counterattack. All measures are represented as the mean  $\pm$  the SEM, (\*) denotes significant  $p \leq .05$ .

**Figure 4**

*Average Number and Proportion of Vocalizations Emitted by Experimental and Control Female and Male Rats*



*Note.* The experimental pairs are the LE rats paired with a F344 rat (right) while the control conditions are pairs of LE rats (left), separated into males (dark grey) and females (light grey). A) shows the average number of vocalizations emitted per 10-min play session, B) shows the average number of vocalizations emitted per play bout, C) shows the proportion of trill call types emitted, D) shows the proportion of frequency modulated calls emitted, E) shows the proportion of flat calls emitted, and F) shows the proportion of audible calls emitted, on average per play session. All measures are represented as the mean  $\pm$  the SEM, (\*) denotes significant  $p \leq .05$ .

## Discussion

A LE rat reared with a F344 partner experienced a different play environment during the juvenile period than if it were reared with an LE partner. LE rats received fewer attacks from their F344 partners (Figure 2A), and, when the LE rats were attacked by their F344 partners, they were less likely to respond to a nape attack with a complete rotation (Figure 1D), leading to fewer instances of pins (Figure 1E). Even so, when attacked, the LE rats were more likely to launch successful counterattacks toward their F344 partners (Figure 1F). Conversely, LE rats compensated for the lack of nape attacks from their F344 partners by launching as many attacks as LE rats did with same strain dyads (Figure 1A). When attacked, F344s were more likely to defend with a facing defense (Figure 2C). Despite a comparable likelihood of defending an attack with a complete rotation (Figure 2D) and a similar frequency of pins (Figure 2E), F344s were less likely to launch successful counterattacks (Figure 2F).

There was greater asymmetry in the play between LE-F344 partners on virtually all measures of play, especially those reflecting reciprocity, such as pins and counterattacks. Given the importance of reciprocity in sustaining playful encounters (Palagi et al., 2016; Pellis & Pellis, 2017), the disparity in the contributions to the play fights between LE and F344 partners was most striking. Even though playfulness varies across individuals within strains of rats (Lampe et al., 2017; Pellis & McKenna, 1992), the degree of asymmetry we found in this study for LE-LE partners was around 5-15%, while the asymmetry between LE-F344 partners was around 20-30% (Figure 3). This clearly shows that an LE rat being reared with an F344 peer experiences a different pattern of play compared to an LE reared with an LE peer. Similarly, Wistar rats reared with F344 partners experience more asymmetry in their play compared to when reared with a Wistar partner (Schneider, Pätz, et al., 2016). Additionally, LE rats reared with F344 partners experienced a different vocal environment during play (Figure 4), as was the case of Wistar rats reared with F344 rats (Schneider, Pätz, et al., 2016).

Juvenile and adult rats emit ultrasonic vocalizations (USV) that can be grouped into two broad categories based on the frequency at which the calls are emitted: 50 kHz and 22 kHz. Whereas the latter are produced during aversive contexts (Portfors, 2007), the 50-kHz calls are associated with positive contexts, such as when the rat is anticipating the arrival of play partner and during play (Burgdorf et al., 2008; Knutson et al., 1998). During play, various 50-kHz call-types appear to promote play, which have been shown to facilitate reciprocity and deescalate aggressive situations (Burke, Kisko, Pellis, et al., 2017; Kisko, Euston, & Pellis, 2015; Kisko, Himmler, et al., 2015). As is the case of Wistar rats playing with F344 rats (Schneider, Pätz, et al., 2016), when the total number of calls per trial was compared, the LE-F344 pairs emitted fewer calls (Figure 4A), but, when adjusted for the number of play fights, the rate of calling per play fight did not differ with LE-LE pairs (Figure 4B). This suggests that the lower level of USV production in LE-F344 pairs arose from an overall reduction in the amount of play (Figures 1A, 2A). However, despite a similar number of vocalizations per nape attack, the types of calls used overall were different in their proportions in the LE-F344 pairs, which suggests that the calls most associated with promoting play and facilitating reciprocity, such as trills (Figure 4C) and other FM calls (Figure 4F) (Burke et al., 2018; Burke et al., 2020), are used more often in LE-LE pairs and could be influencing the increased symmetry in their play (Figure 3). Also potentially important is that audible calls, which are often used in threatening situations (Barnett, 1975), are more commonly emitted in the LE-F344 pairs (Figure 4D). This may reflect a greater aversion in the play between LE and F344 rats. Together with not detecting any 22 kHz calls in these encounters and an absence of any studies on how audible calls are used in play, caution should be exercised in interpreting this finding.

Nonetheless, the large difference in the audible calls present in LE-F344 pairs, accompanied with the differences in play behaviors, does suggest that differences in play tactics may differentially require the use of audible calls to communicate some aspects of the interaction. Although, because the calls by the LE and F344 rats could not be distinguished, it is possible that excessive calling in the audible range is a peculiarity of F344 rats. Given that there are strain differences in the profile of vocalizations used (Schwartz, 2018a, 2018b), a detailed study of calling, both with regard to USVs and audible calls, in F344 rats is needed to fully understand the altered pattern of calling in LE-F344 pairs. However, the evidence available (Figure 4) does suggest that, when reared with a F344 peer, LE rats experience a different acoustic environment compared to that when reared with other LE rats.

### **Potential Influences on the Development of Sociocognitive Skills**

The altered play experienced by LE rats reared with F344 peers may account for the sociocognitive deficits present in adult LE rats reared with F344 partners (Stark & Pellis, 2020). This is consistent with the findings on Wistar rats reared with F344 peers (Schneider, Bindila, et al., 2016; Schneider, Pätz, et al., 2016) and LE rats reared with adults (Bell et al., 2009; Himmler, Pellis, & Kolb, 2013). The impoverished play experience alters both the anatomy and physiology of the mPFC, and the development of sociocognitive skills has been shown in Lister hooded rats reared with social exposure but no opportunity for play (Baarendse et al., 2013; Baarendse & Vanderschuren, 2012; Omrani et al., 2020) and in hamsters, in which juveniles were reared with adults (Burlison et al., 2016). Furthermore, the finding that ground squirrels that engaged in more social play as juveniles have greater sociocognitive skills (Marks et al., 2017) adds to the growing body of evidence that play fighting in the juvenile period refines sociocognitive skills and does so by modifying the underlying neural mechanisms involved (Pellis & Pellis, 2017). The present study also sheds light on the experiences derived from play that may be important for this function.

The play of LE-F344 pairs is highly asymmetrical (Figure 3), as has also been found for Wistar-F344 pairs (Schneider, Bindila, et al., 2016; Schneider, Pätz, et al., 2016). Similarly, the play of pairs of LE rats comprising of a juvenile and an adult is also highly asymmetrical (Pellis et al., 2017). This common feature across strains and the type of play-deficient partner suggests that it is the experience of reciprocity derived from play that is critical. Three lines of evidence support this possibility. First, Wistar rats playing with F344 partners (Schneider, Bindila, et al., 2016; Schneider, Pätz, et al., 2016) and LE rats playing with adult partners (Pellis et al., 2017) compensate for the lack of playfulness in their partners by increasing the number of playful attacks that they launch. Although in the present study our LE rats did not show an absolute increase in playful attacks when interacting F344 partners (Figure 1A), there was a significant asymmetry with the LE launching more attacks than their F344 partners (Figure 3A). Even in the absence of an overt compensatory increase in launching attacks, these rats engaged in about the same amount of play as they did with same strain peers (Figure 1A), so it cannot be the physical actions performed during play that is crucial for training the mPFC and sociocognitive skills. Rather, it is likely that the engagement of executive functions that occur during play, such as short-term memory, impulse control, and decision making, allows the animals to be reciprocal in their interactions that train these skills (Pellis et al., 2014). For example, when two rats of playful strains (such as LE and Sprague Dawley) are paired together during the juvenile period, their play styles converge to be more like the other. While more research is needed to confirm, it could be that there is a level of plasticity in the behaviors performed in order to ensure adequate development; thus, we see an intermediary play style between strains with vastly different play tactics (Himmler, Lewis, & Pellis, 2014). Second, unlike LE rats reared with an adult (Bell et al., 2010; Himmler, Pellis, & Kolb, 2013), wild juvenile rats reared with an adult do not exhibit atypical development of the mPFC (Pellis et al., 2018), and, unlike their LE counterparts, the play of juvenile-adult pairs of wild rats is less asymmetrical (Pellis, Pellis, Himmler, et al., 2019). In particular, it is the opportunity for engaging in actions that lead to role reversals that is more symmetrical in the wild rats.

Third, while in juvenile-adult pairs, most of the counterattacks are by the adult (Pellis et al., 2017), in LE-F344 pairs, most of the counterattacks are by the LE partner (compare Figure 1E and Figure 2E). That is, in one context, the subject rat has too few opportunities to counterattack successfully and in the other, too many. So, it cannot be just performing the physical acts that are needed to counterattack that provides the essential experiences, but, rather, it must be the engagement of the psychological processes of tracking encounters and deciding when to self-handicap to allow the partner to reverse roles that provides the training of sociocognitive skills and their underlying neural circuitry. There must be an optimal level of asymmetry, and, when the asymmetry becomes too great, these experiences are insufficient (Baarendse et al., 2013; Bell et al., 2010; Burleson et al., 2016; Himmler, Pellis, & Kolb, 2013; Schneider, Bindila, et al., 2016; Schneider, Pätz, et al., 2016; Stark & Pellis, 2020). Indeed, the asymmetry can be much less than 50-50 for play to still have this protective effect on development (Pellis, Pellis, Himmler, et al., 2019). While highly asymmetrical play is detrimental, there is the possibility that a certain level of asymmetry can produce additive improvements (Marks et al., 2017). Identifying the minimal level necessary and characterizing the magnitude of the gains made by playing beyond that minimal level has important practical implications for both animal welfare (Ahloy-Dallaire et al., 2018) and for developing policies for human development (Yogman et al., 2018). The identification of asymmetry as a suitable index for characterizing adequate play experience (Figure 3) provides a tool for further research.

## **Play and Sex**

Studies using both the adult rearing paradigm and the F344 partner paradigm have only used females (Bell et al., 2010; Burleson et al., 2016; Himmler, Pellis, & Kolb, 2013; Pellis et al., 2017; Schneider et al., 2014; Schneider, Bindila, et al., 2016; Schneider, Pätz, et al., 2016). These studies revealed the importance of social play during the juvenile period in facilitating the development of the neuronal morphology of the mPFC and sociocognitive skills. Other studies, using different paradigms to deprive juveniles of play, have primarily used males, and these have shown impairments in impulse control, altered cognitive strategies, and altered neurotransmitter signaling in the mPFC and some connected neural systems (Baarendse et al., 2013; Baarendse & Vanderschuren, 2012; Omrani et al., 2020). One study used the paradigm in which pairs of rats were separated by a mesh barrier to prevent playing and compared the effects on both sexes (Pellis, Field & Whishaw, 1999). These play-deprived rats were then subjected to a food protection paradigm where a food item is given to one rat and protects it from being stolen by another rat by dodging laterally away (Whishaw, 1988). This paradigm revealed that, while species- and sex-typical food protection tactics emerged regardless of play experience, the coordination of the movements between partners in adulthood was reduced in both sexes in play-deprived rats (Pellis et al., 1999). Furthermore, damage to the mPFC in adult males showed that the rats could still execute the typical protective tactics to defend a food item but were less successful in doing so (Whishaw, 1988; Whishaw & Oddie, 1989), and this appears to be due to reduced ability to coordinate their movements with those of the rat trying to steal the food (Himmler, Bell, et al., 2014). These findings suggest that, in both sexes, juvenile play experiences affect the development of the mPFC and the sociocognitive skills dependent on this neural system. However, the stress of being fully or partially isolated from a social partner or being subjected to cohabitation with an adult may not be similar in both sexes (Boggiano et al., 2008; Brown & Grunberg, 1996; Burke, McCormick, et al., 2017). It is thus difficult to use these paradigms to compare the effects of play deprivation between the sexes without the confounding of differential stress effects. The rearing of a playful strain, such as the LE or Wistar rats, with a play deficient strain, such as the F344 strain, eliminates some of these confounds – the subject is reared in the physical presence of another rat with whom it can interact and that partner is an age- and sex-matched peer (Schneider, Bindila, et al., 2016; Schneider, Pätz, et al., 2016). The present study shows that LE rats, whether male or female, experienced the same distorted asymmetry in play when reared and tested with a familiar F344 peer. Therefore, in this rearing paradigm, the similar deficits in play experience in the juvenile period can be used to test for the effects of play in the development of the mPFC and sociocognitive skills between the sexes.

## **Conclusion**

LE rats reared with a F344 peer during the juvenile period experienced a vastly different play and vocal environment than LE rats reared with a same strain cage mate. Most striking is the increased asymmetry and the abundant use of audible vocalizations in LE-F344 partners. The asymmetry in play suggests that the LE rats reared with the F344 rats are not acquiring the necessary experiences to develop sociocognitive abilities later in life. Male LE rats that were reared with a F344 rat over the juvenile period were tested in the stranger test, in which adults unfamiliar with one another were introduced in a neutral arena (Stark & Pellis, 2020). In this context, the animals engage in a rough form of play that typically leads to one partner assuming the dominant status but occasionally can escalate to serious aggression, especially if communication skills are impaired (Kisko, Euston, & Pellis, 2015; Smith et al., 1999). In the stranger paradigm, the LE rats reared with a F344 partner were more likely to escalate these encounters to serious aggression (Stark & Pellis, 2020), indicating reduced sociocognitive skills. Females tested with other females in the stranger test have subtle differences, but the absence of overt dominance relationships in females as opposed to males (Adams & Boice, 1983; Blanchard et al., 1988; Ziporyn & McClintock, 1991), makes this test less useful for assessing social interaction skills in females (Stark & Pellis, 2021). Nonetheless, the finding that female Wistar rats reared with F344 partners have impaired social memory skills as adults (Schneider, Bindila, et al., 2016) suggests that the impoverished play experienced by females in the juvenile period impairs the development of sociocognitive skills as it does in males. Whether the magnitude of these play-induced effects are similar in both sexes will require testing them in the same sex-neutral, sociocognitive tests and evaluating the size of the changes in the anatomy and physiology of the mPFC. As an important starting point, however, the present paper shows that the altered play experience of being reared with a F344 peer is comparable between the sexes.

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