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Quality not quantity: Deficient juvenile play experiences lead to altered medial prefrontal cortex neurons and sociocognitive skill deficits

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Abstract

Reduced play experience over the juvenile period leads to adults with impoverished social skills and to anatomical and physiological aberrations of the neurons found in the medial prefrontal cortex (mPFC). Even rearing rats from high-playing strains with low-playing strains show these developmental consequences. In the present study, we evaluated whether low-playing rats benefit from being reared with higher playing peers. To test this, we reared male Fischer 344 rats (F344), typically thought to be a low-playing strain, with a Long–Evans (LE) peer, a relatively high-playing strain. As juveniles, F344 rats reared with LE rats experienced less play and lower quality play compared to those reared with another F344. As adults, the F344 rats reared with LE partners exhibited poorer social skills and the pyramidal neurons of their mPFC had larger dendritic arbors than F344 rats reared with same-strain peers. These findings show that being reared with a more playful partner does not improve developmental outcomes of F344 rats, rather the discordance in the play styles of F344 and LE rats leads to poorer outcomes.

KEYWORDS
Cg3 pyramidal neurons, development, executive function, plasticity, prelimbic cortex, rough-and-tumble play

1 | INTRODUCTION

Developing in social isolation during the juvenile period—from weaning to sexual maturity (Pagel & Harvey, 1993)—leads to adults with a wide range of behavioral, emotional, cognitive, and neural abnormalities (e.g., Arakawa, 2018; Baarendse et al., 2013; Da Silva et al., 1996; Fone & Porkess, 2008; Hall, 1998; Potegal & Einon, 1989; Von Frijtag et al., 2002). Social play peaks during the juvenile period (Thor & Holloway, 1984), so one implication of social isolation is that many of these deficits arise because of the lack of opportunity to engage peers in play (e.g., Einon & Morgan, 1977; van den Berg et al., 1999). However, as being reared in social isolation results in more than just being deprived of play (Bekoff, 1976), other ways to limit play without total social isolation have been developed (Pellis et al., 2023). For example, rearing rats (*Rattus norvegicus*) in the same cage but separated by a perforated partition that allows them to see, smell, hear, and, to a limited degree, touch one another provides some socialization but prevents social play. This paradigm leads to neural, cognitive, emotional, and behavioral deficits (Bell, 2014; Bijlsma et al., 2022, 2023; Marquardt et al., 2023; Pellis et al., 1999) in a similar fashion to total isolation,

indicating that social play in the juvenile period is an essential behavior for proper neurobehavioral development. Rearing a juvenile rat or hamster (*Mesocricetus auratus*) with an adult, which allows for a wider range of social behaviors, including some limited play (Pellis et al., 2017), also leads to neural and sociocognitive abnormalities (e.g., Bell et al., 2010; Burleson et al., 2016; Himmler, Pellis, & Kolb, 2013). This finding further emphasizes the importance of peer–peer social play during the juvenile period but may be confounded by stress effects; being reared with an adult can be stressful (Burke et al., 2010, 2013), which may contribute to the neural and sociocognitive abnormalities (Stark et al., 2023). To determine more precisely how the quality and quantity of social play during the juvenile period affect neurobehavioral development therefore requires a slightly more refined approach to limit potentially confounding effects.

Rat strains differ from one another behaviorally in several dimensions, including the amount that they play and the preferred tactics used during play (Himmler, Modlinska et al., 2014; Himmler, Stryjek et al., 2013; Keeley et al., 2015). One approach that has been used successfully to probe the effects of the quantity and quality of play on neurobehavioral development has been to rear rat strains that are more playful with strains that are less playful. Schneider and colleagues (Schneider et al., 2014; Schneider, Bindila et al., 2016; Schneider, Pätz et al., 2016) reared Wistar rats, a highly playful strain (Himmler, Modlinska et al., 2014), with Fischer 344 (F344) partners, a low-playing strain (Siviy et al., 1997, 2003). The Wistar rats reared with F344 partners experienced less and atypical play as juveniles and exhibited neural and pain threshold changes, as well as reduced social skills (Schneider et al., 2014; Schneider, Bindila et al., 2016; Schneider, Pätz et al., 2016). Following this paradigm, we reared Long–Evans (LE) rats, another highly playful strain (Himmler, Modlinska et al., 2014; Himmler, Stryjek et al., 2013), with F344 peers, with similar results: LE rats reared with F344 peers experienced atypical play as juveniles (Stark et al., 2021), as well as atypical neural architecture and deficient social skills as adults (Stark & Pellis, 2020, 2021; Stark et al., 2023).

Absent, reduced, or atypical play experience in the juvenile period leads to the development of impoverished sociocognitive skills and to abnormalities in the physiology and anatomy of the neurons of the medial prefrontal cortex (mPFC) (e.g., Baarendse et al., 2013; Bell et al., 2010; Bijlsma et al., 2022, 2023; Burleson et al., 2016; Himmler, Pellis, & Kolb, 2013; Schneider, Bindila et al., 2016; Stark & Pellis, 2020, 2021; Stark et al., 2023). This answers the question as to whether the deficits arising from being reared in social isolation (e.g., Fone & Porkess, 2008; Hall, 1998) result from the lack of play—at least some of the deficits can be attributed to the absence of social play. But if reducing play leads to impoverished development, can increasing play lead to improved outcomes?

Rearing Wistar and LE rats with F344 partners has the effect of significantly reducing the sociocognitive skills of those rats relative to their peers raised with Wistar and LE partners, respectively (Schneider, Bindila et al., 2016; Stark & Pellis, 2020, 2021). The logic behind this rearing paradigm was based on the premise that F344 rats are a low-playing strain (Siviy et al., 1997, 2003), and indeed, juvenile Wistar and LE rats reared with F344 partners play less and/or atypically compared to peers reared with members of the same strain (Schneider, Bindila et al., 2016; Stark et al., 2021). But it would be equally likely that F344 rats reared with Wistar or LE rats would experience more and/or qualitatively better play than F344 rats reared with F344 partners. If this were the case, it would be predicted that F344 rats reared with LE peers should have more refined sociocognitive skills and neural anatomy than F344 rats reared with F344 peers. However, there is an alternative possibility.

The social play of rats mostly involves play fighting, and play fighting involves competing for an advantage, such as biting or otherwise contacting a particular location of the partner's body (Aldis, 1975; Pellis, 1988; Symons, 1978). In the case of rats, the body target is the nape of the neck that is nuzzled with the snout if contacted (Pellis & Pellis, 1987; Siviy & Panksepp, 1987). However, unlike serious fighting, which also involves competing for an advantage (Blanchard & Blanchard, 1994; Geist, 1978; Pellis, 1997)—in rats, this involves biting the opponent's rump and lower dorsum (Blanchard & Blanchard, 1977; Pellis & Pellis, 1987)—play fighting involves an element of cooperation (Pellis & Pellis, 1998) that ensures turn-taking, making the interactions reciprocal (Palagi et al., 2016; Pellis & Pellis, 2017). Being paired with a mismatched partner, be it rats of different ages or different strains, leads to less turn-taking and so a reduction in reciprocity (Pellis et al., 2017, 2018; Schneider, Bindila et al., 2016; Schneider, Pätz et al., 2016). Moreover, most of the role reversals in pairs of LE–F344 rats are produced by the LE partner (Stark et al., 2021).

Given that role reversals seem to be particularly important experiences derived from play fighting that contribute to facilitating the development of sociocognitive skills (Pellis et al., 2019), even if the amount of play of F344 rats were increased by interacting with LE peers, the quality of the play may not. Rat strains such as LE, Wistar, and Sprague–Dawley seem to be particularly averse to interacting with F344 rats (Kiyokawa et al., 2022; Kogo et al., 2021). Perhaps, as the former three strains are more closely related to each other than any are to F344 (Lindsey & Baker, 2006), they may not be perceived as compatible partners. Indeed, LE and Wistar rats play more similarly to each other than they do to Sprague–Dawley rats (Himmler, Modlinska et al., 2014), and when reared with Sprague–Dawley peers, LE rats modify how they play (Himmler, Lewis et al., 2014), and Wistar rats reared with Sprague–Dawley peers show no long-term effects on sociocognitive skills as they do when reared with F344 peers (Schneider, Bindila et al., 2016). That is, the differences between the play of F344 rats and other strains, such as LE rats, may create a chasm in the quality of the exchanges that cannot be overcome by any increase in the amount of play. If so, whatever the intrinsic deficiencies in the play of F344 rats (Siviy, 2020), when reared with LE peers, they should have worse developmental outcomes than when reared with F344 peers.

In the present paper, male juvenile F344 rats were reared with either a same-sex F344 or LE peer and then compared as adults for sociocognitive skills and for the development of the neurons of the mPFC. Social competency was evaluated with the stranger test, in which two adult rats unfamiliar with one another are placed together in a neutral enclosure (Smith et al., 1999). If being reared with a higher

playing peer improves sociocognitive skill development, then F344 rats reared with LE peers should perform better, that is, more likely to play and less likely to escalate to aggression when tested with F344 strangers than those reared with F344 peers. In contrast, if the problem is in reduced coordination of play between peers of different strains, then the reverse should be the case.

As well as play deprivation resulting in pyramidal neurons of the mPFC with altered physiology (Baarendse et al., 2013; Bijlsma et al., 2022, 2023), LE rats reared with adult partners or with F344 peers have adult neurons that have a larger dendritic arbor than do LE rats reared with LE peers (Bell et al., 2010; Himmler, Pellis, & Kolb, 2013; Stark et al., 2023). Play in the juvenile period prunes the dendritic arbor of mPFC pyramidal neurons and this effect is present in both sexes (Stark et al., 2023). If being reared with a higher playing peer increases dendritic pruning, then F344 rats reared with LE peers should have a smaller dendritic arbor than those reared with F344 peers. In contrast, if the problem is in reduced coordination of play between peers of different strains, then the reverse should be the case.

2 | MATERIALS AND METHODS

2.1 | Subjects

Twenty-six F344 male rats and 10 LE male rats were purchased from Charles River Laboratories and arrived at the Canadian Centre for Behavioural Neuroscience (CCBN) at 24 days of age. At 26 days of age, the rats were housed in dyads composed of F344–F344 ($n = 8$) or F344–LE ($n = 10$) pairs. Eighteen additional F344 male rats were purchased at 75 days of age from Charles River Laboratories. These rats were housed in pairs and were used as the unfamiliar partners for the stranger test (see below) when all animals were 80 days of age. All animals were housed in polyethylene cages (46 × 25 × 20 cm) with corncob bedding. Food and water were available ad libitum. The rats were housed on a 12-h light–dark cycle (lights on between 7:30 a.m. and 7:30 p.m.) in a room maintained at a constant temperature of 21–23°C. All care and testing procedures were approved by the University of Lethbridge Animal Welfare Committee (Protocol #1809) in compliance with guidelines from the Canadian Council for Animal Care.

2.2 | Apparatus

Social play was tested when the rats were juveniles and again as adults in a sound-attenuating chamber with a clear Plexiglas enclosure (50 × 50 × 50 cm) placed inside. The floor of the Plexiglas enclosure was covered in 2–3 cm of CareFresh bedding. To dampen external noise, the chamber was lined with sound-attenuating foam (Primeacoustic). Interactions were filmed using an ExmourRS 4K Sony Handycam with night-shot capability, which was placed in a small window of the chamber at a 45° angle. Using only infrared lights, which were placed inside the chamber, the recordings were made using the night-shot capabilities of the camera.

2.3 | Procedure

For juveniles, play was tested between 33 and 38 days of age, the peak play period for rats (Thor & Holloway, 1984), and between 80 and 90 days, which corresponds to young adulthood after sexual maturity (Pellis & Pellis, 1990). Before their play was tested at both ages, the animals were habituated to the testing enclosure with their cage mates for 15 min a day, for four consecutive days. The only difference was that, as juveniles, they were tested with their cage mates for the play trials, but as adults they were tested with an unfamiliar same-strain, same-age, and same-sex partner (Figure 1). Both the play trials and habituation sessions took place in the dark, during the day, with only the infrared lights on. Prior to the play trials, the rats were socially isolated for a 24-h period to increase playfulness (Panksepp & Beatty, 1980; Pellis & Pellis, 1990; Pellis, Pellis, Burke et al., 2022). For the play trials, the pairs were placed into the test enclosure simultaneously, and left there for 10 min, before being retrieved and placed back in their home cages. Fresh bedding was replaced and the Plexiglas box was cleaned with Virkon after each trial to reduce any odors left from the previous rats.

2.4 | Behavioral analysis

Following both juvenile and adult play trials, the video files were analyzed using a combination of both normal speed and frame-by-frame analysis to score play behavior (Himmler, Pellis, & Pellis, 2013; Pellis, Pellis, Burke et al., 2022), and for the adult encounters, agonistic behavior (Kisko et al., 2015; Smith et al., 1999; Stark & Pellis, 2021). All videos were scored twice, once noting the actions initiated by the focal animal and directed toward the partner, and the second time noting the actions initiated by the partner and directed toward the focal animal. Playful attacks were scored when the snout of one rat was in contact with, or moved toward, the nape of the other rat (Pellis, Pellis, Burke et al., 2022). Once an attack was initiated, the recipient either continued with its ongoing behavior or defended itself, from which the probability of defense was calculated. If the recipient defends itself, it could do so by either evading (running away from the attacker) or by turning to face the attacker. There are several types of facing defense, and these typically lead to particular interanimal postural configurations (Pellis & Pellis, 1987). Two common configurations include the pin, in which the defender lies on its back, and the other stands on top, and the mutual upright position, in which the two partners rear on their hindfeet and grapple with their forepaws (Pellis, Pellis, Burke et al., 2022). Some defensive tactics, such as rotating cephalocaudally to supine, are likely to lead to pinning and some, such as rotating vertically around the pelvis to face an attacker approaching from the rear, are likely to lead to mutual uprights (for detailed descriptions, see Himmler, Pellis, & Pellis, 2013; Pellis, Pellis, Burke et al., 2022). Given that previous studies have reported that the distinguishing features of playful defense by F344 rates are low rates of pinning and high rates of evasion (Siviy et al., 1997, 2003), for the present study, it was this initiation of attacks and resulting defensive actions that were scored for statistical analysis.

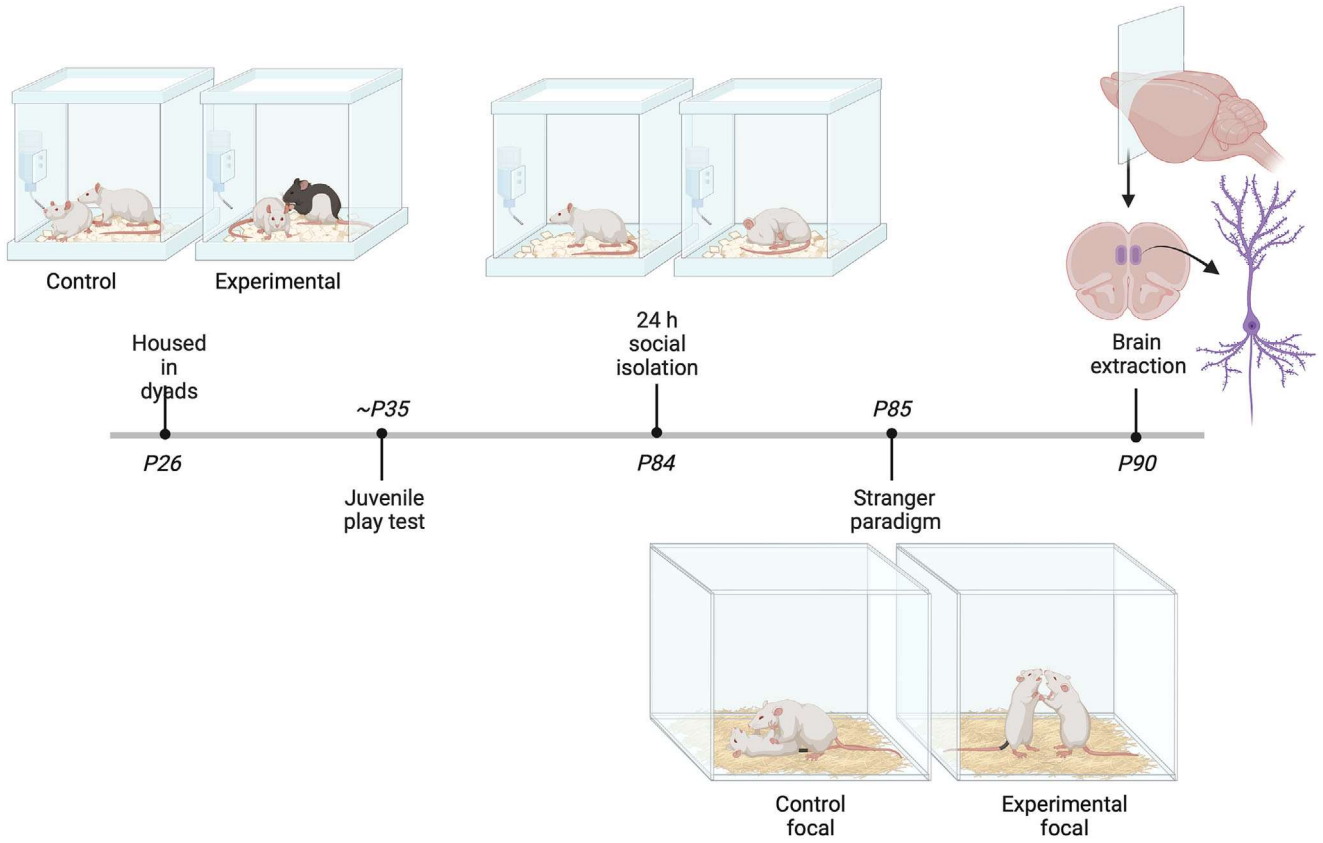

FIGURE 1 The diagram illustrates how the animals were housed in same- and mixed-strain pairings post natal day (P) P26, when juvenile play testing occurred (~P35), how animals were socially isolated and then tested with an unfamiliar animal (P84, P85), and when the animals were sacrificed (P90). Created with BioRender.com.

As noted above, an important feature of play fighting is that animals engage in actions that allow partners to reciprocate roles, or take turns in attacking and defending (Palagi et al., 2016; Pellis & Pellis, 2017). In rats, this often involves cooperative actions that ensure the partner can gain the advantage (Pellis et al., 2005). To assess and compare this cooperative aspect of play, the proportion of play fights that lead to a role reversal was scored (Himmler et al., 2016). Another good marker of reciprocity is the degree of symmetry in the playful actions of the partners in a pair (Stark et al., 2021). To assess the degree of symmetry in play, intrapair differences in launching nape attacks were scored. The number of attacks by one partner was subtracted from the other, and the absolute difference was divided by the total play score of the pair, resulting in values between 0 and 1. Values close to zero indicate the play was symmetrical, whereas values close to 1 indicate a high degree of asymmetry in the initiation of playful attacks between the partners (Pellis, Pellis, Burke et al., 2022).

The mean number of nape attacks per 10 min, the proportion of nape attacks being defended, and the proportion of nape attacks leading to evasion and pins were scored and calculated for both partners from each pair. As the subjects of this study were the F344 rats, in F344–LE pairs, it was the F344 partner that was selected as the focal animal, and for the F344–F344 pairs, one subject from each pair was randomly assigned as the focal animal for comparison between rearing conditions. Because the behavior of one partner can influence behavior of the other partner (Pellis, Pellis, Burke et al., 2022), not only the focal rats from the two conditions were compared, so were their partners. Although attack, defense, type of defense, and pins were scored for each pair mate, the likelihood of role reversals and the degree of symmetry were scored as pair measures and so it is the pair scores that were compared between the F344–F344 and F344–LE pairs.

When unfamiliar male adult rats play, it is rougher than the play in juveniles and among familiar adult males (Pellis & Pellis, 1987, 1992). Because of this increased roughness, the risk of escalating to serious aggression is greater (Smith et al., 1999). Indications of escalation can be identified by the rats using agonistic threat signals, such as piloerection, tail rattling, and the lateral posture and if the encounter escalates further, with bites, typically directed to a lower flanks or rump (Blanchard & Blanchard, 1977; Takahashi & Lore, 1983). Male rats with defective communication and poor social skills are less able to prevent playful encounters with strangers from escalating (Burke et al., 2017; Kisko et al., 2015; Stark & Pellis, 2020). Therefore, as well as the playful actions performed (see above), the occurrence and mean number of threat signals and bites per 10 min between pairs were scored for comparison between F344 rats reared with a F344 peer versus a LE peer when interacting with an unfamiliar adult male F344.

In previous studies, focusing on LE rats reared with F344 partners, both males and females were analyzed, revealing no sex differences (Stark & Pellis, 2020, 2021; Stark et al., 2021, 2023). Here, only

males were used as male rats form more clearly delineated, dominance hierarchies (Barnett, 1975; Ziporyn & McClinktock, 1991), and as a consequence, playful interactions among unfamiliar adult males (Smith et al., 1999) more readily escalate to aggression if there is a social deficiency (Kisko et al., 2015; Stark & Pellis, 2020). In contrast, female dominance relationships involve more subtle gestures (Ziporyn & McClinktock, 1991), making the stranger test less clear-cut to assess social deficiencies in females (Stark & Pellis, 2021). As shown in the present paper, adult male F344 rats are not as assertive as LE rats, making the stranger test still useful but weaker in this strain. Therefore, we concentrated our efforts on males, leaving the effects of cross-strain rearing on female F344 rats to be dealt with in a future study using tests of adult social cognition to which females are more sensitive (e.g., Schneider, Bindila et al., 2016).

2.5 | Histology

Animals were anesthetized at 90 days of age with 300 mg/kg of sodium pentobarbital and perfused, intracardially, with 0.9% saline. The brains from eight control animals and 10 experimental animals were extracted and weighed. Following a standard Golgi staining protocol (Gibb & Kolb, 1998), the brains were placed in Golgi–Cox solution for 14 days after which they were placed into a 30% sucrose solution for at least 5 days before sectioning. Brains were sectioned using a vibratome at a thickness of 200 μm. Sections were mounted onto slides coated in 3% gelatin and stored in a light-proof box, wrapped in a damp paper towel as to prevent drying. After 1–2 days, the slides were rinsed in distilled water and ammonium hydroxide and then placed in a 1:1 solution of Kodak Rapid Fix (Kodak) and distilled water. To dehydrate the slides, the brain tissue was moved through a graded ethanol series, cleared with a 1:1:1 solution of chloroform, Hemo De (Electron Microscopy Sciences catalog No. 23410-04), and 100% ethanol. Slides were placed in Hemo De before they were cover-slipped with Permount (Fisher Scientific, Catalog No. SP15-500). Slides were left to dry for at least l month or until they were dry to the touch.

2.6 | Anatomical analysis

We selected neurons from area Cg3 (prelimbic cortex) of the mPFC as defined by Zilles (1985), for imaging (Figure 2). Neurons selected were between Bregma 4.2 and 2.77 mm from the rostral–caudal plane (Zilles, 1985). Using an Olympus VS120 digital slide scanner, with a 40× oil objective (UPlanFL N, 40×/1.30 oil, ∞/0.17/FN26.5) and Olympus VS-ASE FL software, virtual slides of Cg3 were created (Brinkman et al., 2022; Stark et al., 2023). The virtual slides consisted of 147 z-stacked images spaced 0.68 μm apart throughout the section, yielding 99.96 μm of working distance. To reconstruct the neurons, the images of the slides were uploaded into Neurolucida 360 (MicroBrightfield). The apical and basilar dendrites from pyramidal neurons from layer III were then traced. Cells were only selected if (1) the cell was fully impregnated and not obscured by large truncations of debris, stain precipitation, or blood vessels and (2) the cell had to lie centered within the plane z-stack, ensuring that all dendritic branching could be traced. A minimum of three cells were traced from each hemisphere. A total of 172 neurons were traced across all animals.

Using Neurolucida Explorer, replicating the measurements taken of LE rats by Stark et al. (2023), the following measurements were extracted for statistical analysis: (1) the convex hull volume for both apical and basal projections; (2) the convex hull surface area for both apical and basal projections; (3) the sum of the total length of the apical and basal projections and the length of the cell body, which together obtained the total cell length; (4) the sum of the volume of the apical and basal projections and the volume of the cell body, which together provided a measure of the total volume of the cell; (5) the branch number for both apical and basal projections, which was summed for the total branch number; and finally, (6) the branch order for both apical and basal projections, which was summed for a total branch order.

2.7 | Statistical analyses

We compared the behavior and outcomes of the focal F344 rats reared in F344–F344 pairs with the focal F344 rats reared in F344–LE pairs. The data for juvenile play of the F344 focal rats from the F344–LE pairs are from a previous publication (Stark et al., 2021) and were scored by the same observers. All the analyzed data on adult stranger interactions and neuroanatomy are new for the present study.

As the behavioral measures involved specific items to be compared between two groups, a Welch *t*-test was used in each case. The play of focal rats paired from the two conditions were compared. The partners of the focal rats were similarly compared. Measures of Cg3 cortical neurons were assessed with a linear mixed model, using the *lme4* package (Bates et al., 2015), to compare the effects of rearing condition. Rearing condition was set as the independent fixed effect, and a random error term was assigned to account for the repeated measures of individual animals, with hemisphere nested within every subject. The significance level for all tests was set at $p < .05$. All statistics were performed using R Studio (R Core Team, 2020) using the *ggplot2* package (Wickham, 2016) to create graphs.

3 | RESULTS

3.1 | Juvenile behavior

F344 rats launched significantly more playful attacks toward F344 partners than toward LE partners ($t(9.57) = 14.21$, $p < .00001$) (Figure 3a) and were more likely to defend themselves when attacked by F344 partners (0.95 ± 0.01) than LE partners (0.72 ± 0.07) ($t(15.39) = 8.48$, $p > .0001$). Additionally, the proportion of playful attacks that resulted in pins was significantly greater for focal rats playing with F344 partners (0.35 ± 0.02) than with LE partners (0.09 ± 0.04) ($t(13.74) = 5.22$, $p = .0001$). With regard to type of defense, focal F344 rats were just as likely to evade attacks by F344 rats (0.48 ± 0.03) or LE rats (0.52 ± 0.16) ($t(9.54) = -0.23$, $p = .82$).

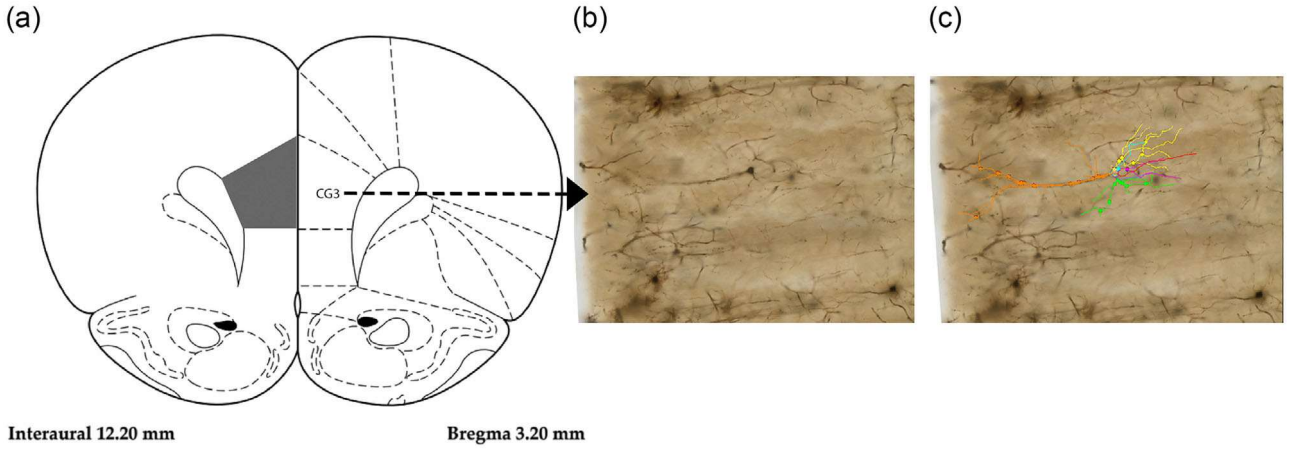

FIGURE 2 A diagram of a rat brain section through the medial prefrontal cortex (a) (from Paxinos & Watson, 1986). After digitally scanning the slides (b), neurons were traced digitally (c).

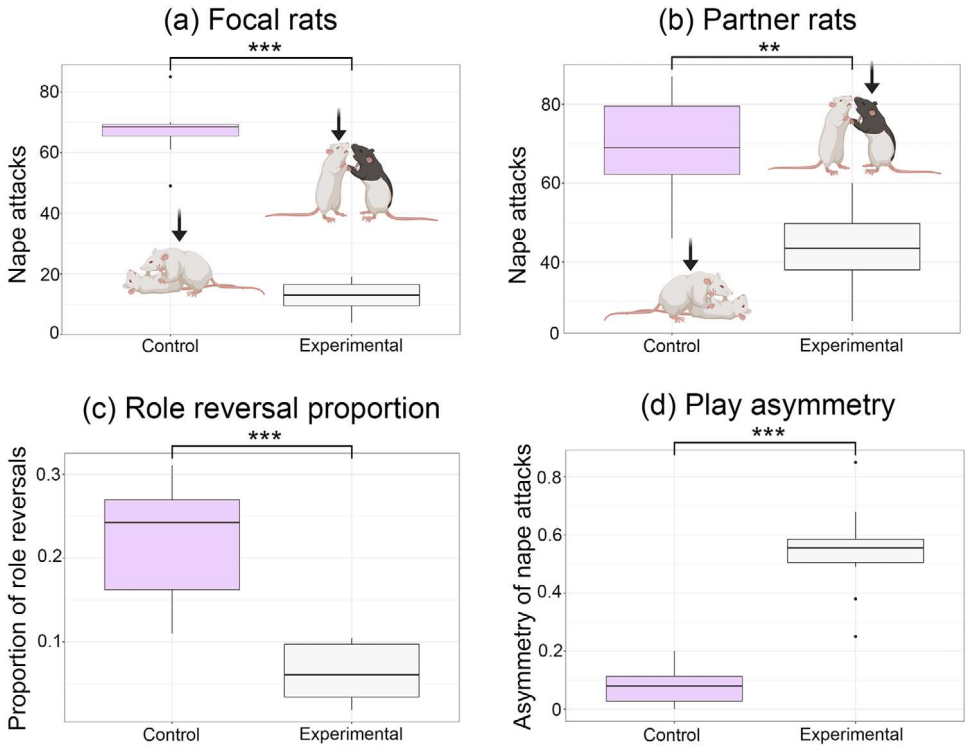

FIGURE 3 The number of nape attacks initiated by the focal rats in control (F344–F344 pairs) and experimental (F344–LE pairs) rearing conditions was significantly different (a). The number of nape attacks initiated by the focal animal's partner was also significantly different (b). The proportion of playful attacks between pairs that led to a role reversal was significantly different when comparing the two rearing conditions (c). As a symmetry score closer to zero indicates a more symmetrical relationship, panel (d) illustrates that control partners had significantly more symmetrical play relationships compared to experimental pairs. Rat images were made with BioRender.com.

The F344 partners launched significantly more nape attacks than did LE partners ($t(12.61) = 4.16$, $p = .001$; Figure 3b) and were more likely to defend themselves (0.95 ± 0.008) than were LE partners (0.86 ± 0.04) ($t(13.19) = 4.48$, $p = .0006$). Additionally, the proportion of playful attacks that resulted in pins was greater for F344 partners (0.41 ± 0.04) than LE partners (0.29 ± 0.02) ($t(9.06) = 2.86$, $p = .02$). With regard to type of defense, the proportion of playful attacks that resulted in evasions was significantly greater for F344 partners (0.46 ± 0.03) than LE partners (0.05 ± 0.02) ($t(12.94) = 12.81$, $p < .00001$).

Play between F334–F334 pairs was significantly more likely to lead to role reversals than F344–LE pairs ($t(9.54) = 5.79$, $p = .0002$; Figure 3c) and playful nape attacks were significantly more symmetrical in F344–F344 pairs than F344–LE pairs ($t(13.11) = -8.13$, $p < .00001$; Figure 3d).

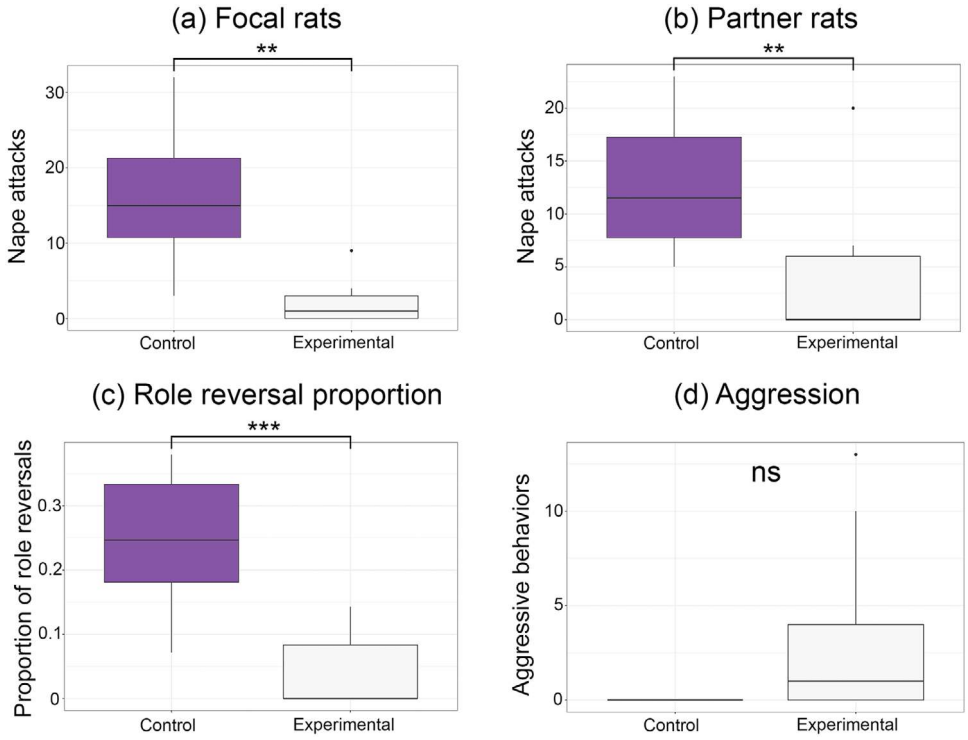

FIGURE 4 The number of nape attacks initiated by the focal rats interacting with an unfamiliar animal was significantly different when comparing animals reared in the two conditions (a). The number of nape attacks initiated by the focal animal's unfamiliar partner was also significantly different (b). The proportion of playful attacks between the focal rats and unfamiliar rats from that led to a role reversal was significantly different when comparing the two rearing conditions (c). Although not significant, while some pairs of rats escalated to aggression, only experimental animals (i.e., the F344 reared in F344–LE pairs) interacting with a stranger escalated to aggression, whereas control rats (i.e., F344 reared in F344 in F344–F344) never did (d).

3.2 | Adult behavior

Focal rats reared with a F344 partner launched significantly more playful nape attacks against the F344 "stranger" than did those reared with a LE partner ($t(8.01) = 3.77$, $p = .005$) (Figure 4a). Focal rats from both rearing conditions were equally likely to defend themselves when playfully attacked by the stranger ($p > .05$). F344 rats from both rearing conditions were equally likely to be pinned ($p > .05$), but focal rats reared with a F344 rat were more likely to evade playful attacks compared to focal rats reared with a LE rat (0.85 ± 2.21 vs. 0.33 ± 2.21, respectively) ($t(14.24) = 2.50$, $p = .03$).

The "stranger" partners of the focal rats launched significantly more playful nape attacks toward focal rats that had been reared with F344 than with LE partners ($t(14.96) = 2.84$, $p = .01$) (Figure 4b). When stranger rats were playfully attacked by a focal rat, they were equally likely to defend themselves, regardless of their attackers rearing condition ($p > .05$). Additionally, regardless of the focal rats' rearing condition, when defending themselves, stranger partner rats were equally likely to be pinned ($p > .05$) or use an evasion tactic ($p > .05$).

Role reversals were significantly more likely in pairs in which the focal rat had been reared with a F344 partner (Figure 4c), and although not significantly different ($p > .05$), aggressive behaviors were only observed in pairs of strangers in which the focal rat had been reared with LE partners (Figure 4d). Moreover, the escalation that did occur in these pairs was equally likely to be initiated by the focal rat (1.8 ± 1.01) or the partner (1.4 ± 0.73) ($p > .05$).

3.3 | Neuronal morphology

There were significant effects of rearing condition on both convex hull volume ($F(1,16) = 33.34$, $p < .00001$) and surface area ($F(1,16) = 24.50$, $p = .0001$). This significant effect of rearing condition also extended to the convex hull volumes and surface areas of both the apical ($F(1,16) = 41.08$, $p < .00001$ and $F(1,16) = 33.88$, $p < .00001$, respectively) and basilar dendritic projections ($F(1,16) = 17.85$, $p = .0006$ and $F(1,16) = 14.24$, $p = .002$, respectively) (Table 1; Figure 5a,b). For all convex hull volume and surface area measures, F344 rats reared with F344 partners had neurons with reduced dendritic arbors compared to F344 rats reared with LE partners (Figure 5c).

Similarly, there was a significant effect of rearing condition on total branch number ($F(1,16) = 8.90$, $p = .009$), apical branch number ($F(1,16) = 11.75$, $p = .003$), and basilar branch number ($F(1,16) = 5.81$, $p = .03$). Additionally, there were significant effects on the total branch order ($F(1,16) = 9.84$, $p = .006$), apical branch order ($F(1,16) = 12.45$, $p = .003$), and basilar branch order ($F(1,16) = 7.02$, $p = .02$) (Table 1).

TABLE 1 The average measurements (± standard error of the mean) of pyramidal neurons found in Cg3, layer III, of male rats that were either reared with a same-strain F344 individual or a mixed-strain LE individual.

Measurement	Control (F334 from F344–F344 pairs)	Experimental (F334 from F344–LE pairs)	% difference
Convex hull volume (cm^3)	342.62 ± 26.70	757.55 ± 39.94	57.52*
Apical convex hull volume (cm^3)	66.77 ± 9.08	207.62 ± 16.04	82.57*
Basilar convex hull volume (cm^3)	33.43 ± 4.30	86.69 ± 7.84	69.37*
Convex hull surface area (cm^2)	18.04 ± 0.94	28.85 ± 0.98	33.31*
Apical convex hull surface area (cm^2)	6.84 ± 0.49	12.88 ± 0.61	45.45*
Basilar convex hull surface area (cm^2)	5.55 ± 0.47	10.39 ± 0.64	45.05*
Length (mm)	3.37 ± 0.11	4.47 ± 0.13	22.84
Apical length (mm)	0.85 ± 0.04	1.38 ± 0.05	33.81*
Basilar length (mm)	1.39 ± 0.08	2.19 ± 0.10	32.22*
Cell body length (mm)	1.12 ± 0.03	1.10 ± 0.03	1.22
Volume (mm^3)	13.25 ± 0.50	13.35 ± 0.45	0.51
Apical volume (mm^3)	2.60 ± 0.15	2.95 ± 0.16	8.67
Basilar volume (mm^3)	1.53 ± 0.10	2.12 ± 0.15	23.00
Cell body volume (mm^3)	9.13 ± 0.40	8.28 ± 0.35	6.38
Branch number	15.62 ± 0.85	25.30 ± 1.10	34.25*
Apical branch number	5.92 ± 0.45	11.04 ± 0.55	44.75*
Basilar branch number	9.70 ± 0.63	14.26 ± 0.72	27.12*
Branch order	23.89 ± 0.99	35.06 ± 1.23	26.96*
Apical branch order	6.95 ± 0.45	12.25 ± 0.56	40.56*
Basilar branch order	16.95 ± 0.78	22.81 ± 0.87	20.69*

Note: The percent difference in the various measures was calculated for cases when significant differences were found. An asterisk (*) denotes a significant difference ($p < .05$); actual p values are reported in text.

For all three measurements, the controls had fewer branches than the rats reared with LE partners.

Although there was no significant effect of rearing condition on the total length of neurons ($F(1,16) = 18.80$, $p = .29$), there was a significant effect on both the apical length ($F(1,16) = 17.64$, $p = .0007$) and basilar length ($F(1,16) = 13.55$, $p = .002$). No significant effect was found for the length of the cell body ($F(1,16) = 0.04$, $p = .84$). For both the length of the apical and basilar projections, F344 rats reared with F344 partners had shorter projections compared to the F344 rats reared with LE partners (Table 1).

In contrast to the convex hull volumes and areas, branch numbers, and apical and basilar dendrite lengths, no significant effect of rearing condition was detected for the total volume of the cells ($F(1,16) = 0.01$, $p = .91$), apical projection volumes ($F(1,16) = 0.76$, $p = .40$), basilar projection volumes ($F(1,16) = 3.60$, $p = .08$), or cell body volumes ($F(1,16) = 0.76$, $p = .40$) (Table 1).

4 | DISCUSSION

When juveniles of high-playing strains of rats are reared over the juvenile period with peers from the low-playing F344 strain, the play they experience is deficient, and the developmental consequences include poorer social skills and mPFC neurons with a dendritic arbor that is less pruned than when such rats are reared with same-strain partners or partners from other highly playful strains (Schneider, Bindila et al., 2016; Schneider, Pätz et al., 2016; Stark & Pellis, 2020, 2021; Stark et al., 2021, 2023). The present study evaluated the effects of this cross-rearing on the F344 partners. Two hypotheses with opposing predictions were tested. The first hypothesis was that being reared with a peer from the more playful LE strain, F344 rats should experience more play and that this should improve developmental outcomes (e.g., greater social competence, mPFC neurons with greater dendritic pruning). In contrast, the second hypothesis posited that it is the quality, not the quantity, of play that is important. That is, the play of F344 rats is so discordant with that of LE rats, and other high-playing strains, the play experiences of F344 rats reared with LE peers would be more impoverished than those reared with F344 peers. This in turn would lead to worse developmental outcomes. Our findings were consistent with the predictions of the second hypothesis.

4.1 | Differences in play behavior

Juvenile F344 rats reared with LE peers not only engaged in less play, but that play was also qualitatively different. Both the F344 rats

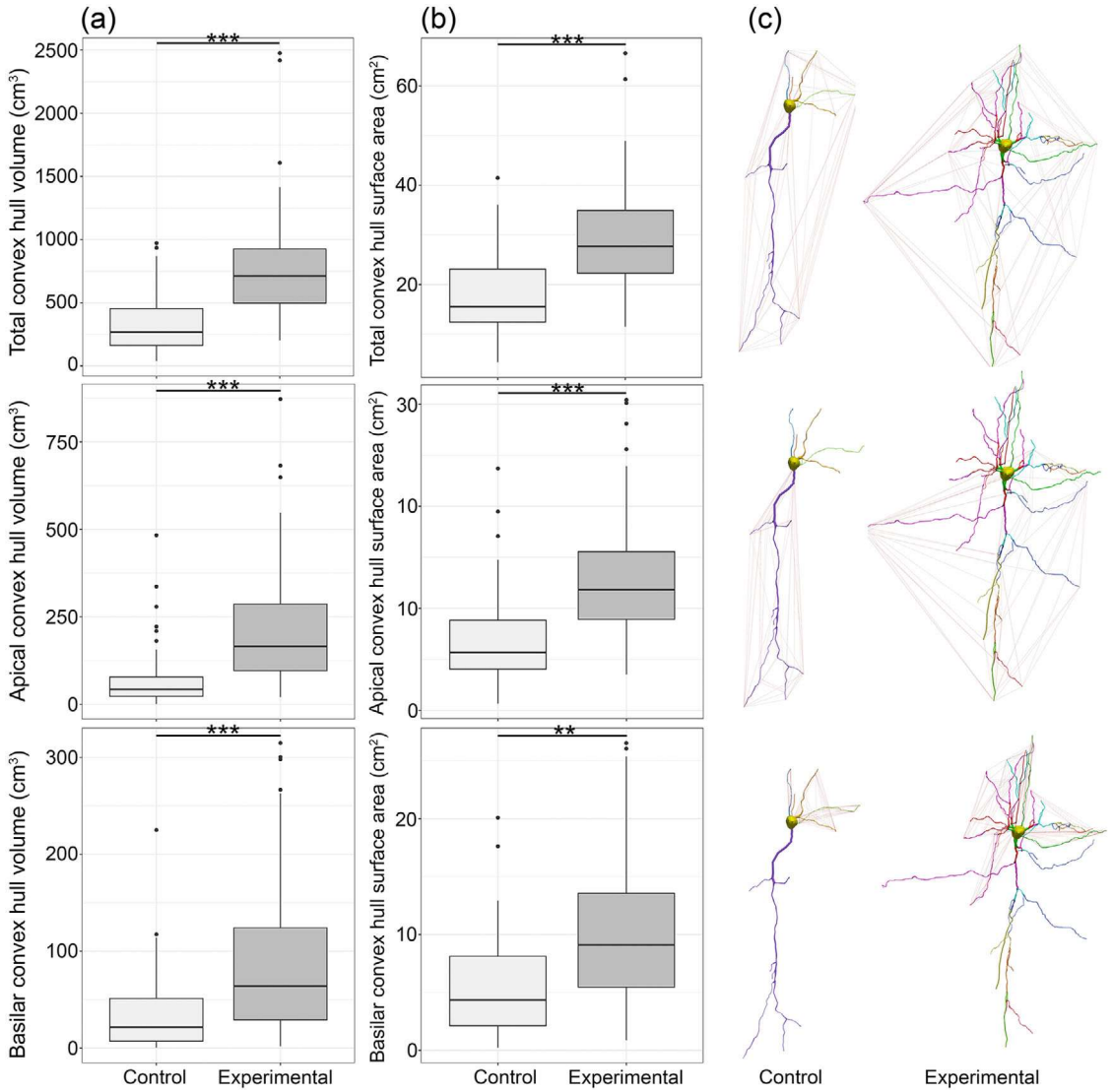

FIGURE 5 The boxplots illustrate the results for the total convex hull, apical convex hull, and basilar convex hull analyses, for each neuron measured, showing both the volume (a) and surface area measures (b). Digital reconstructions illustrate the size differences of the neurons, with webbing showing the region of the cells being scored (c). Control = F344 from F344–F344 pairs; experimental = F344 from F344–LE pairs.

themselves launched fewer playful attacks against LE rats than against F344 peers, and their LE partners launched fewer attacks than did F344 partners (Figure 3a,b). The style of juvenile play was also different, with mixed-strain pairs engaging in less pinning, so less close-quarter wrestling, and their play led to fewer role reversals and was more asymmetrical (Figure 3c,d). Given that it is the ability to sustain reciprocal interactions during play fighting that refines social skills during juvenile play (Pellis & Pellis, 2017; Pellis et al., 2023), the opportunity for such training is reduced, not improved, by rearing F344 rats with LE rats.

As adults, the F344 rats reared in mixed-strain pairs launched significantly fewer playful attacks, and received significantly fewer attacks, when interacting with an unfamiliar same-strain partner (Figure 4a,b). The interactions with strangers also led to significantly fewer role reversals in pairs with F344 rats reared with LE peers. Although not significant, given the low rate of escalation to aggression by F344 rats, it is noteworthy that only interactions involving mixed-strain reared rats led to aggression. The reduction in initiating play and the inability of at least some of the mixed-strain-reared rats to mitigate aggression suggest their sociocognitive skills were impaired relative to F344 rats reared with a F344 peer. In some strains, like LE rats (Kisko et al., 2015; Smith et al., 1999), strangers interacting jockey for dominance. However, the pairs of unfamiliar F344 rats encountering one another did not. Nonetheless, the diminished levels of play between partners where one animal was derived from mixed-strain rearing indicate that their conspecifics recognized them as less attractive partners. This is consistent with other findings that rats can detect something odd about potential partners, which may be outside the capacity of humans to detect and measure (Pellis, Pellis, Burke et al., 2022), and reflect this detection by avoiding playing with them (e.g., Holloway & Suter, 2004; Pellis et al., 2006; Stark & Pellis, 2021).

4.2 | Differences in dendritic pruning

Using different manipulations and species, it has been repeatedly shown that play experience in the juvenile period has a pruning effect on the dendritic arbor of the pyramidal neurons of the mPFC (Bell et al., 2010; Burleson et al., 2016; Himmler, Pellis, & Kolb, 2013; Stark et al., 2023). A possible explanation for the importance of such pruning was originally proposed by Cajal (1899). The idea is that neurons will be arranged and connected to minimize cost: the "wiring economy principle." That is, fewer connections and smaller neurons should be favored over larger, more expansive neurons if they are capable of performing the same task. Indeed, too many connections may lead to less precise signaling and control of action potentials (Stark et al., 2023). If this is true, pruning of the mPFC, influenced by social play behavior, may create more stream-lined and efficient circuits, ultimately improving control over behavior (Pellis et al., 2023).

The dendritic arbors of pyramidal neurons in Cg3 (prelimbic cortex) of the mPFC in rats reared in mixed-strain pairs were significantly larger in the current study. That is, the male rats in mixed-strain rearing conditions did not go through the same pruning process of the apical and basilar neuronal arbors as did the F344 rats reared in the same-strain pairs. These differences were greatest in the convex hull volumes/areas but were also present in dendritic lengths and branching. The reduced pruning is not only consistent with LE rats and hamsters reared with low-playing adults (Bell et al., 2010; Burleson et al., 2016; Himmler, Pellis, & Kolb, 2013), but also with LE rats reared with low-playing F344 peers (Stark et al., 2023). Indeed, the magnitude of the effect is greater in F344 than in LE rats. For example, the convex hull volume measure was 52.5% larger and the convex hull surface area measure was 33.3% larger in F344 reared in mixed-strain pairs compared to F344 rats reared in same-strain pairs (Table 1), whereas for LE rats reared with F344 peers compared to same-strain peers was 22.7% and 15.2%, respectively (Stark et al., 2023). Moreover, while for F344 rats reared with LE peers, the pruning affected both the apical and basilar dendrites (Table 1), for LE rats reared with F344 rats, only the apical dendrites were significantly affected (Stark et al., 2023).

While the dendritic arbor of the mPFC neurons of both F344 rats (present study) and LE rats (Stark et al., 2023) was affected by cross-strain rearing, there were several, notable strain differences. First, the magnitude of the effects of being reared with a same-strain versus cross-strain peer was greater in F344 rats than LE rats (see above). Second, although only the apical dendrites were significantly affected in the LE rats (Stark et al., 2023), both the apical and basilar dendrites of F344 rats were affected (present study). As changes in the dendritic arbor of mPFC neurons following the manipulation play in the juvenile period have only been studied in two strains of rats, LE (Bell et al., 2010; Himmler, Pellis, & Kolb, 2013; Stark et al., 2023) and F344 (present study), it is possible that the neural sensitivity to this experience varies across strains. Quite simply, while most or all strains may show an effect in the same direction, the magnitude of that effect may vary (Pellis et al., 2018). A fuller comparative survey across domestic strains of rats is needed to determine the degree of such variation, but that play experience in the juvenile period has the effect of pruning the dendritic arbor of mPFC neurons has now been demonstrated in two strains of rats and in a different species, the Syrian hamster (Burleson et al., 2016), suggests that this causal influence of play on the development of mPFC neurons is likely a general phenomenon.

Alternatively, abnormal play experiences may increase dendritic size through dendritic overgrowth that pruning does not reverse. During the juvenile period, neurons are pruned, but dendritic and synaptic growth also occurs. Synapses in the mPFC increase between postnatal day 35 and 45 in rats (Drzewiecki et al., 2016). Indeed, if rats are deprived of play during the juvenile period, they show altered development of inhibitory synapses in the mPFC (Bijlsma et al., 2022, 2023). To determine whether the differences in dendritic arborization are due to overgrowth or less pruning, dendrites need to be sampled at various timepoints throughout the juvenile period. In addition, such an analysis would help further characterize the mechanism by which abnormal play alters neuronal development, as it would determine the critical period during adolescence when the mPFC neurons are most sensitive to the influence of play experiences. Regardless of the mechanism involved, there is mounting evidence that juvenile play experiences alter the dendritic arbors of the mPFC (Pellis et al., 2023).

Rats have considerable variability in their play behavior, varying the amount of play, the partners with which they play, and the style in which they play (Achterberg et al., 2023; Ham & Pellis, 2023; Lampe et al., 2019; Lesscher et al., 2021; Melotti et al., 2014; Pellis, Pellis, Burke et al., 2022; Pellis, Pellis, Ham et al., 2022; Poole & Fish, 1976); however, it remains unknown if individual variation in play experience is sufficient to result in differences in sociocognitive skills and altered dendritic pruning. It may be that, irrespective of individual variation, all rats reared with same-strain peers may achieve the threshold level of experience to impact neural and sociocognitive development equally. Alternatively, while most rats may achieve that threshold, some may not. While we did not test how individual variation interacts with development, animals reared in the experimental condition (i.e., F344–LE pairs) had greater variability in convex hull volume and surface area measurements as adults than did the rats in the control condition (i.e., F344–F344 pairs) (see the error bars in Figure 5). This suggests that F344 partners may provide F344 rats with a relatively narrower range of variation in play experiences than did LE partners. A similar pattern is present for LE rats reared with F344 peers compared to LE peers (Stark et al., 2023). Even so, both rats reared with same-strain and mixed-strain peers show considerable variation in the amount of pruning present (Stark et al., 2023; present paper), suggesting that the magnitude of pruning may depend on the degree of play reciprocation they experienced as juveniles. Indeed, while the mixed-strain-reared animals were more likely to escalate encounters with strangers to aggression, not all subjects did so (Stark & Pellis, 2020; present study), again supporting the possibility that rats differ in their social competence and that this difference may be, in part, dependent on their play experiences as juveniles. Preliminary data from a large sample of LE rats suggest that some individuals fail to gain the requisite play experiences as juveniles even when the opportunity to do so is present,

and this appears to have a negative impact on dendritic pruning of mPFC pyramidal neurons and on the development of sociocognitive skills (Ham et al., 2023). If supported by more detailed analyses, this would support the view that not all normally reared rats benefit equally from juvenile play.

4.3 | Are F344 rats a high playing strain?

An unexpected finding was that the F344–F344 pairs played more than expected for this strain (Siviy, 2020). Our juvenile males initiated around 70 playful attacks during a 10-min play session, which is around 2.5 times higher than what was expected based on previous studies (Siviy et al., 1997, 2003). However, most analyses of play in F344 rats have involved testing them in dyads with partners from other strains, and even though still relatively low, in the cases in which F344–F344 pairs were tested, the number of playful attacks was greater (Siviy et al., 1997, 2003, 2011, 2017, 2023), suggesting that F344 rats play more together than when playing with partners from different strains. Indeed, as adults, LE, Sprague–Dawley, and Wistar rats prefer their own strain but will readily associate with one of the other strains before settling for a F344 partner if that is the only option available (Kiyokawa et al., 2022; Kogo et al., 2021). These findings suggest that F344 rats are so different from other strains that they are unattractive as play partners, as other strains are to them. Consistent with this is that LE and Sprague–Dawley can adapt their play to the idiosyncacies of each others' strain-typical pattern of play (Himmler, Lewis et al., 2014), and Wistar rats reared with Sprague–Dawley rats do not exhibit the long-term developmental effects that Wistar rats reared with F344 rats do (Schneider, Bindila et al., 2016).

The reason for why our F344 rats initiated more play than expected still needs to be resolved. One possibility is that the original studies from Siviy's laboratory used F344 rats from a different breeding company than the one from which we obtained ours (see Siviy et al., 1997, 2003; Stark et al., 2021; present study). Indeed, when Siviy obtained F344 rats from a different breeder, he found that they were more playful than those previously tested (Siviy, personal communication, 2021). A detailed comparison of the play of F344 rats obtained from different breeders is needed. Importantly, though, irrespective of their origin and whether tested with partners from the same or different strains, F344 rats are more likely to evade nape attacks and less likely to roll over to supine, and so being pinned, when nape contact is successful (Siviy et al., 1997, 2003, 2011, 2023; Stark et al., 2021; present paper), suggesting that they have a strain-typical style of play that is different to that of many other commonly used laboratory strains of rats (Himmler, Modlinska et al., 2014; Himmler, Stryjek et al., 2013). Failure to significantly alter that style even when cross-fostered with another strain (Siviy et al., 2017) is unlike the modification present in juvenile LE and Sprague–Dawley rats even if only housed together for a few days shortly after weaning (Himmler, Lewis et al., 2014). We intended to pair F344 rats with a high-playing partner to boost the play experience, but instead we found the LE rats were less playful than F344 rats. Nonetheless, the play styles of F344 and LE rats seem to be so incongruent that both LE and F344 rats reared together in cross-strain pairs end up having impoverished sociocognitive skills and less pruned Cg3 neuronal morphology (Stark & Pellis, 2020, 2021; Stark et al., 2023; present study). Therefore, it is the quality of the experiences derived from playing, not the overall quantity of play, that is critical to gain the sociocognitive benefits from playing in the juvenile period (Pellis et al., 2023).

5 | CONCLUSION

Our findings show that, like the case for LE rats (Bell et al., 2010; Himmler, Pellis, & Kolb, 2013; Stark & Pellis, 2020, 2021; Stark et al., 2023), Lister hooded rats (Baarendse et al., 2013; Bijlsma et al., 2022, 2023), and Wistar rats (Schneider et al., 2014; Schneider, Bindila et al., 2016; Schneider, Pätz et al., 2016), diminished play experience in the juvenile period of F344 rats results in both neuroanatomical changes and reduced sociocognitive skills in adulthood (present paper). In addition to rats, hamsters seem to show a similar effect (Burleson et al., 2016; Cooper et al., 2023). Studies of wild ground squirrels (*Urocitellus beldingi*) have also demonstrated that juvenile play experience influences adult behavior, refining the development of motor skills and social behaviors, in addition to temperament (Hurst-Hopf et al., 2023; Marks et al., 2017; Nunes, 2014; Nunes & Monroy Montemayor et al., 2023; Nunes, Muecke, Lancaster et al., 2004; Nunes, Muecke, Sanchez et al., 2004). Relevant to the present findings is that, for the ground squirrels, it is social, not solitary, play that specifically influences the development of social behavior (Marks et al., 2017). What remains unclear, however, is the mechanism by which play can produce these effects. The present findings, along with the results from the LE reared with F344 (Stark & Pellis, 2020, 2021; Stark et al., 2021, 2023), suggest that it is not the amount of play an individual experiences but the quality of the play that provides the critical experiences for these developmental effects. That is, it is the ability to negotiate interactions with the partner to make play reciprocal (as measured by role reversals and symmetry) that seems to provide the experiences derived from play critical for refining sociocognitive skills and their underlying neural substrates (Pellis et al., 2017, 2019, 2023; Schneider, Bindila et al., 2016). Juvenile F344 rats reared with LE partners not only played less but also less reciprocally than their counterparts reared with F344 partners (Figure 3), and most of the role reversals between mix-strain pairs were initiated by the LE and not the F344 (Stark et al., 2021). Thus, F344 rats reared in LE–F344 pairs may gain fewer of the critical experiences than do the F344 rats reared in such mixed-strain pairs, and this might explain why the magnitude of the effects on dendritic pruning was greater in F344 rats than LE rats in mixed-strain pairs.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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