

The Goldilocks Principle: Balancing Familiarity and Novelty in the Selection of Play Partners in Groups of Juvenile Male Rats

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Abstract – Like many mammals, rats frequently engage in play fighting as juveniles, an activity that influences the development of socio-cognitive skills. Most studies that assess play are based on staged dyadic encounters, implying that some average quantity and quality of play are sufficient to produce these developmental effects. However, there are individual differences, with some rats not only preferring to play more, but also to have more physical contact than others. Given that rats have individual differences in play, it raises the possibility that rats might express these preferences when playing in groups. To determine whether rats form partner preferences, trials were conducted in which a focal rat was given the opportunity to play with three partners of varying familiarity. One partner was a cage mate, another was housed on the other side of a transparent and perforated divider and so familiar, but not a prior play partner, and the third was a stranger from another cage. A total of 36 focal rats, between 30-36 days of age, were tested and video recorded in 20-minute trials following 2.5 h of social isolation. Focal rats expressed a preference for neighbors over both strangers and cage mates, indicating that balancing between familiarity and novelty influences social play partner preferences, and congruency of play style, were investigated, but none were correlated with the preferences expressed. This group dynamics perspective provides a novel approach to studying play, and more generally, provides insights into social exploration and decision-making.

Keywords - Dominance, Partner choice, Partner preferences, Play fighting, Rat group play, Rough-and-tumble play

Social play has been reported in a wide array of mammals (Burghardt, 2005; Fagen, 1981), with play fighting, or rough-and-tumble play, being one of the most commonly reported forms (Pellis & Pellis, 1998). During play fighting, partners compete for an advantage, such as biting or striking a particular body target (Aldis, 1975). For many species, the targets competed over are the same as those in serious fighting, but for many others, the targets can be the same as those contacted during sex, greeting or other amicable interactions, and predation (Pellis, 1988; Pellis & Pellis, 2018; Pellis et al., 2022). Whatever the advantage sought in play fighting, attack and defense is attenuated, allowing both partners the opportunity to at least sometimes gain the advantage, thus creating a degree of reciprocity (Palagi et al., 2016; Pellis & Pellis, 2017).

Although most common in the juvenile period, for many species, play fighting can continue into adulthood (Palagi, 2011; Pellis & Iwaniuk, 2000) and in post-pubescent animals, it is often used to assess and manipulate social relationships (e.g., Antonacci et al., 2010; Mills, 1990; Pellegrini, 1995; Pellis et al., 1993), so providing an immediate benefit. In contrast, play fighting in juveniles most likely has delayed benefits, whereby the play experience alters future socio-cognitive performance or other skills (Palagi, 2018; Pellis & Pellis, 2009; Smith, 1982, as discussed in Pisula & Modlinska, 2023). It has been the play fighting of juveniles that has received the most intense scrutiny regarding the mechanisms that regulate it

and the benefits it may confer (Sharpe, 2019; Vanderschuren et al., 2016), but determining the critical experiences derived from such play can be challenging. An important influence that needs to be taken into account is the social setting in which juveniles live.

For species that are typically reared alone with the mother, such as in giant pandas (*Ailuropoda melanoleuca*), play fighting may be preferentially directed to the mother (Kleiman, 1983; Pellis & Pellis, 2009; Snyder et al., 2003), whereas for species living in social groups, not only do juveniles tend to play with other juveniles but they also exhibit preferences as to which juveniles in the group are played with the most (e.g., Cheney, 1978; Ham et al., 2022, 2023; Lilley et al., 2020; Lutz & Judge, 2017; Shimada & Sueur, 2014, 2017; Turner et al., 2020). Several relevant factors have been identified as influencing which partners are preferred. Dominance status influences play in some non-human primates, with animals more likely to play with partners of similar rank (e.g., Biben, 1986; Lutz et al., 2019), although dominance can be less of a factor for species with less rigid dominance hierarchies (Petit et al., 2008; Reinhart et al., 2010). For many species, play is preferred with partners of similar age and sex (e.g., Biben, 1998; Cheney, 1978; Thompson, 1996), although for some, like beluga whales (*Delphinapterus leucas*), these preferences can change with age (Ham et al., 2022; Lilley et al., 2020). In contrast, for some species, play partner preference can remain stable for long periods of time (e.g., Ward et al., 2008), even into adulthood (e.g., Mann, 2006). Given such diversity in play partner preference, it is important to understand the preferences of the species being studied and how these preferences can influence play experiences during the juvenile period.

Laboratory rats (*Rattus norvegicus*) have been key subjects for studying the neurobehavioral mechanisms that regulate play fighting (Siviy, 2016; Vanderschuren et al., 2016), and the delayed functions of this behavior (Pellis et al., 2014; Vanderschuren & Trezza, 2014). In rats, play peaks in occurrence from days 30-40 after birth (Meaney & Stewart, 1981; Panksepp, 1981; Pellis, Pellis, Burke, et al., 2022; Pellis & Pellis, 1990, 1992, 1997; Thor & Holloway, 1984). Play fighting during the juvenile period improves the development of socio-cognitive skills and alters the neural circuits associated with those skills (e.g., Baarendse et al., 2013; Bell et al., 2010; Bijlsma et al., 2022; Himmler, Pellis, & Kolb, 2013; Schneider et al., 2016; Stark & Pellis, 2020, 2021; Stark et al. 2023; van Kerkhof et al., 2013). As these consequences of play have been reported in some other rodents (e.g., Burleson et al., 2016; Marks et al., 2017), it is likely that the findings on rats may be generalized to many other species that engage in play fighting as juveniles (Pellis et al., 2014; Pellis & Pellis, 2017).

In both male and female rats, play fighting mostly involves competing to access the partner's nape of the neck, which is nuzzled with the snout if contacted (Pellis & Pellis, 1987; Siviy & Panksepp, 1987). The most common way to evaluate play fighting in rats is the 'dyadic test,' in which rats are socially isolated, from a few hours to several days, and then introduced into a test enclosure to which they have been habituated (Pellis, Pellis, Burke, et al., 2022). Trials typically last for 5-30 min and play usually commences within the first minute (Burke et al., 2022). The period of social isolation, even when brief, increases the rats' motivation to engage in play, ensuring a high frequency of encounters to score and evaluate. Different scoring schemes allow differing degrees of details to be recorded from the play that occurs (Himmler et al., 2013; Pellis, Pellis, Burke, et al., 2022), but a key measure to ascertain how much an individual is inclined to play is how many nape attacks it initiates (Pellis & Pellis, 1990; Thor & Holloway, 1983).

Juvenile rats can be matched in the dyadic test with either familiar partners, ones with which they share a home cage (Lampe et al., 2017; Pellis & Pellis, 1990), or with strangers, animals they have never met before (e.g., Achterberg et al., 2015; Achterberg & Vanderschuren, 2020). The number of nape attacks are sometimes reported to be higher when play is between strangers than cage mates (e.g., Panksepp, 1981 cf. Pellis & Pellis, 1990), but this is often confounded by the duration of the pre-test social isolation period, as longer periods of separation increase the amount of play (Pellis et al., 1997). Nonetheless, when a rat in a dyadic test is satiated as evidenced by a decline in launching nape attacks, its level of initiating nape attacks rebounds if a novel, unfamiliar partner is introduced (Reinhart et al., 2006), suggesting that strangers may be more attractive play partners. Indeed, in some non-play test paradigms, adult rats tend to have a preference for approaching and socializing with strangers (e.g., Cirulli et al., 1996; Hackenberg et al., 2021; Rogers-Carter et al., 2018; Schweinfurth & Taborsky, 2020).

Additionally, while adult rats may direct prosocial behaviors to both familiar and unfamiliar individuals, they are less likely to interact with strangers of a different strain (Ben-Ami Bartal et al., 2014).

This preference for same-strain individuals in prosocial tasks, however, is not present in juveniles (Breton et al., 2022) or in adults that were cross-fostered with the other strain, suggesting that familiarity is important in making social decisions (Ben-Ami Bartal et al., 2014). Given that, in some contexts, adult rats prefer strangers, it is possible that the observations suggesting that juveniles play more with strangers are correct. If so, we predicted that, if rats were given a choice, they would launch more nape attacks toward unfamiliar partners than toward cage mates.

However, observations to date confound two factors: when presenting a subject rat with a play partner in the dyadic test paradigm, an unfamiliar partner is both a stranger and peer with which the subject has never played. That is, it could be either the novelty of the partner's identity or the novel play provided by the stranger that generates increased play. To overcome this confound between familiarity and play experience, groups of three male rats each were housed in cages with a clear, perforated partition between them and three rats on the other side of the barrier. The perforated partition enables the rats across the barrier to see, hear, and smell each other, but not interact physically (Stryjek & Modlinska, 2022). In this way, rats on the same side of the partition are both play partners and familiar, whereas the rats across the barrier are familiar but have never been play partners. To provide a choice, a focal rat from one side of the barrier was placed in a large test enclosure with a cage mate, a rat from across the barrier, and a rat from another cage, so a true stranger. Play within a group, rather than in a dyad, allow rats to initiate play with preferred partners differentially, so permitting a choice to be made and measured (Pellis, Pellis, Burke, et al., 2022; Pellis, Pellis, Ham, et al., 2022). If playing with a novel animal is the main reason for choosing a partner, then the focal rat should direct more nape attacks toward the stranger, as it is the least familiar option. Whatever partners are preferred, if there is a preference, the issue is that of how those partners are identified. Different mechanisms are likely important if it is either the novel identity or the novel playfulness of the partner that is the basis for selection (hypotheses and predictions are summarized in Table 1).

If the novelty of potential play partners is their unfamiliarity, then they need to be identified as novel before being selected as a play partner. Like many mammals, rats use scent to identify the sex, dominance status, kinship, and individual identity of other rats (Barnett, 1975; Brown, 1979; Clemens et al., 2020; Hepper, 1987). Although juvenile rats can play in the absence of olfaction (Siviy & Panksepp, 1987; Thor & Holloway, 1982), when first introduced into a test enclosure, rats will engage in vigorous anogenital sniffing in the first minute before they start engaging in play fighting (Panksepp, 1981; Pellis & Pellis, 1990). If scent is the means by which to determine which partner is which, it should take longer to distinguish between familiar and unfamiliar rats in a group setting: this should lead to different latencies for the focal rat to begin to play with different categories of rats.

Whether identified by smell or some other sensory modality, if focal rats play more with strangers it could be because they are more socially attractive (e.g., Cirulli et al., 1996; Schweinfurth & Taborsky, 2020), and so, focal rats may spend more time in close proximity with them. In turn, this would create greater opportunity to engage in play with strangers. It would thus be expected that the preferred play partners are the ones that the focal animals spend more time with in close proximity, including when not playing. Even though there is no proximity-driven association between play and play partner preferences in groups of familiar rats (Pellis, Pellis, Burke, et al., 2022), the greater attractiveness of strangers could change this pattern (Hepper, 1987).

If the novelty of unfamiliar partners is driven by the different playful feedback they provide, then focal rats should play with all the rats available in the test enclosure before focusing on the one providing the preferred play experience. Indeed, even within a litter or group setting, in which the animals are familiar with one another and all play together, some are preferred as play partners over others (Meaney & Stewart, 1981; Pellis, Pellis, Burke, et al., 2022). This raises the possibility that rats will sample all members of the group for their play, but then focus their play with the preferred members. If this is so, it would be expected that, early in the trial, focal rats should playfully attack all group members, but then increasingly limit their **Table 1**

Summary of the Hypotheses and Predictions Tested

	individual
As strangers must be identified, probably by olfactory inspection, the onset of play with stranger partners will be delayed as compared to when encountering familiar individuals	The latency to laun partners would be a familiar individu
As novel animals are generally more attractive, rats should	The time spent in s

- As novel animals are generally more attractive, rats should spend more time with strangers, even when not playing with them, as compared to when with familiar individuals
- A reason for why strangers are more attractive play partners is that they play in a way that is more rewarding as compared to play with familiar individuals
- Whether due to recognition or playful feedback, the individual properties of potential partners need to be assessed before play can be focused on strangers
- Rats preferentially direct more nape attacks towards dominant animals, therefore, when placed in a group of known and unknown rats, the most dominant rat in the test trail should receive the most attacks
- Dominance among rats is positively correlated with size, therefore, as a corollary to the previous hypothesis, the largest rats in the trial should receive the most playful attacks

frequently directed towards the 'stranger' than a familiar individual

- The latency to launch playful attacks toward all potential partners would be longer with strangers as compared to with a familiar individual
- The time spent in social proximity to strangers, when not playing, is predicted to be longer than time spent in proximity to familiar individuals
- Play with strangers should involve more close-quarter wrestling and more role reversals than play with familiar individuals
- Focal rats would play with all partners early in the trial then mainly play with strangers later in the trial
- The partner with which the focal rat had the greatest dominance asymmetry would receive the most playful attacks
- The partner with which the focal rat had the greatest weight asymmetry would receive the most playful attacks

play to the preferred partners, leading to a different distribution of play with the different group members. Similarly, the style of play should differ among the play bouts with familiar versus unfamiliar partners.

When attacked, a rat can either defend its nape or simply not respond. If the recipient defends the attack, the rat can do so by either fleeing/evading or by turning to face its attacker. In turn, facing defenses can either lead to the defender wrestling on the ground or warding off the attacker by remaining standing, with the former involving more close bodily contact (Pellis, Pellis, Burke, et al., 2022). Moreover, once the nape is successfully defended, the defender can launch a counterattack leading to a role reversal, in which the original attacker becomes the defender (Himmler et al., 2016). Role reversals are important for ensuring that play fighting is reciprocal and lead to more prolonged encounters (Palagi et al., 2016; Pellis & Pellis, 2017). There are individual differences in how rats play (Pellis & Pellis, 1987; Poole & Fish, 1976), and these differences can lead to different experiences during play depending on the combination of players (Pellis, Pellis, Burke, et al., 2022). Consequently, the differing play experienced with familiar versus unfamiliar partners could be reflected in the behavior performed (e.g., more wrestling versus more evasion, more role reversals versus no response). In this way, partners could be selected based on whether they play in a preferred style (Pellis, Pellis, Ham, et al., 2022).

A final mechanism examined would combine both the identity and behavior of the strangers. Although dominance relationships among cohabiting male rats are not fully established until after sexual maturity (Smith et al., 1996; Takahashi, 1986; Takahashi & Lore, 1983), they do begin to emerge in the juvenile period (Pellis & Pellis, 1991), and this can affect how they play together (Panksepp et al., 1985; Pellis & McKenna, 1992; Smith et al., 1998). Rats in the home cage will launch more playful attacks toward the dominant male both as juveniles and adults (Pellis & McKenna, 1992; Pellis & Pellis, 1991; Pellis et al., 1993). Given that the focal rats have not had the opportunity to meet the stranger, this rat's dominance status may be the most ambiguous, stimulating more play with them to assess their potential dominance (Smith et al., 1996). Once the relative dominance of the rats in the play trial were identified, if dominance influences the way that play occurs in established groups, then it would be expected that the focal rats would launch more playful attacks toward the most dominant group member, unless the focal rat is the most dominant. In addition, as dominance is positively correlated with body size (Pellis & Pellis, 1991; Smith et al., 1996), we also used differences in body weights as a proxy measure of dominance, to assess the partner preferences of focal rats. If consistent with dominance relationships, then the focal rat should direct the most playful attacks to the heaviest member of the test trial. It should be noted that, as male rats form more clearly delineated dominance hierarchies than do females (Barnett, 1975; Ziporyn & McClinktock, 1991), and dominance relationships can influence how rats play (Pellis & McKenna, 1992), for the present study

only males were used. No matter which, if any, of the above mechanisms may be used, all reflect that, in a situation in which an animal can choose among potential play partners, some degree of social exploration is required.

Method

Ethics Statement

All care and testing procedures were reviewed and approved by the University of Lethbridge Animal Welfare Committee (protocol #1809) in compliance with guidelines from the Canadian Council for Animal Care.

Subjects

Thirty-six weanling Long Evans (LE) male rats were purchased from Charles River Laboratories (Kingston, NY, USA) and arrived at the Canadian Centre for Behavioural Neuroscience at 22 days of age. Upon arrival, the animals were moved into Tecniplast[®] GR1800 double decker cages (46.2cm x 40.3cm x 40.4cm), which had a Tecniplast[®] cage divider placed in the middle of the cage creating two spaces to house two, separate groups of rats. The weanling rats were placed on either side of the divider in groups of three, resulting in 12 groups. The home cages had corncob bedding on the floor, and food and water were available *ad libitum*. Animals were housed on a 12-hour light-dark cycle and maintained at a constant temperature of 21° C - 23° C.

Apparatus

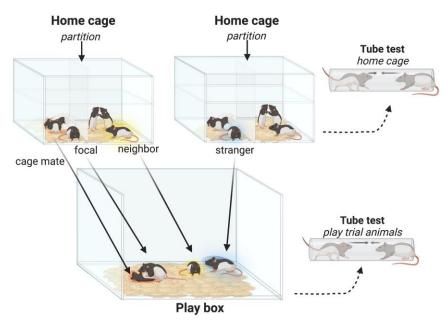
Animals were tested in a large Plexiglas[®] play enclosure (80cm x 80cm x 50cm). The play enclosure was filled with a layer of corncob bedding which was around 1.5 cm thick. An ExmourRS 4K Sony Handycam was used for filming the play sessions and was placed over the top of the enclosure, giving a top-down view. Rats were tested in a room illuminated with red lights (Himmler et al., 2013).

Procedure

Starting at 28 days of age, rats from the same side of the divider were habituated to the test enclosure for 30 min in red light over two consecutive days. At 30 days of age, the rats were tested in groups of four: two from one side of the divider ('cage mates'), one from the other side of the partition ('neighbor'), and one animal from another cage ('stranger'), with one of the cage mates serving as the focal animal (Figure 1). Each of the 36 rats were designated as the focal animal on one of the test days. Once designated, their cage mate partner was determined randomly. The neighbors and strangers were picked at random with the only selection criteria being that they could not have previously interacted. Different groups were formed over the course of the six days of testing, ensuring that each of the 36 rats served as the focal animal once.

Figure 1

Housing and Test Paradigm



Note. The diagram illustrates how the animals were housed in the partitioned home cage (top), how the partners were combined for testing in the play trials (bottom), and how dominance was determined using the tube test in both the home cage (top right) and the play trial groups (bottom right). Created with BioRender.com.

Each group was placed in the enclosure, in red light, for 20 min and filmed. The bedding was replaced, and the test enclosure was cleaned with Virkon[®] after each trial to reduce any odors left from the previous rats. To identify individual rats, their tails were marked, using different patterns, with a permanent marker pen (Sharpie[®]). Prior to testing, the rats were weighed and also socially isolated for 2.5 h to increase playfulness (Pellis, Pellis, Burke, et al., 2022). After testing, animals were rehoused in their respective groups for around 24 h before being socially isolated again for the following play trial.

Even as juveniles, male rats can differ in their dominance relationships, which can affect how they play together (Panksepp et al., 1985; Pellis & Pellis, 1991), so we assessed the dominance relationships among the cage mates. To do so, we used the tube test (Fan et al., 2019; Fulenwider et al., 2021) at 29 days of age (the day before testing began) and at 35 days of age (on the final day of testing). In addition, to assess dominance among the individuals in the groups used in the trials, the tube test was employed at the end of every play trial, which tested the dominance among the focal, cage mate, neighbor, and stranger. Pairs of rats from within the home cage group (e.g., focal + cage mate) and among the play groups (e.g., neighbor + stranger, focal + neighbor, cage mate + neighbor) were placed into a Plexiglas® tube (19.5 cm in length and 4.5 cm in diameter) headfirst at opposing ends. The tube was just large enough to allow one rat through, with the second rat unable to squeeze past its opponent. The 'loser' was thus designated as the rat pushed out of the tube, the 'winner' the rat that remained in the tube. The winning rat was given a point for that round. If neither rat was pushed out, this was considered a tie, and no point was given for that encounter. Each pair was tested five times, and the sum of the points was then used to determine which rat was the most dominant. After testing was completed for a pair, the tube was cleaned with Virkon®, and the next pair was tested.

Behavioral Analysis

The 20-min video recordings were analyzed using a combination of normal speed and frame-byframe analysis to score various aspects of the rats' playful attack and defense strategies (Himmler et al., 2013; Pellis, Pellis, Burke, et al., 2022). Each video was scored in terms of the actions performed by the focal animal. For the present study, we focused on comparing nape attacks as this provided information about the play initiated with each of the potential partners by the focal rat. A playful attack was scored when the snout of one rat contacted the nape of another rat as this is the target in around 90% of playful attacks (Himmler et al., 2013). Additionally, if a playful attack was directed towards the nape but the defender evaded before it could be contacted, this was also scored as a playful attack. After an attack was launched, the partner could either continue its ongoing behavior (e.g., digging, exploring) or defend against the attack. To defend its nape, the partner could either evade (e.g., swerve or run away from the attacker) or engage in a facing defense, in which the defending rat turns to face its attacker. Depending on the tactics used, a facing defense can lead to wrestling that involves rolling over onto the ground (i.e., supine defense) or remain standing while warding off its opponent (i.e., standing defense) (Himmler et al., 2013). A simple measure of supine defense is to score the configuration of the pair when one partner is lying on its back and the other is standing over it (i.e., a pin), and a simple measure of standing defense is when members of the pair both stand up and face one another and hold one another with their forepaws (i.e., mutual upright). In addition, the number of role reversals, in which the focal animal is attacked, and launches a counter attack that leads to the attacker becoming the defender, were recorded (Pellis, Pellis, Burke, et al., 2022).

In addition to play, the social proximity of the focal animal to the other rats was calculated. This was done to determine whether the focal rat just played with whichever rat was closest to them or instead seek out a particular play partner within the play box. Animals were considered to be in social proximity if they were within one body length of each other. As some of that time was spent playing together, the total time two individuals spent playing with one another was subtracted from the total time they were in proximity to each other.

Statistical Analysis

All plots were created using R (R Core Team, 2020) using *ggplot2* (Wickham, 2016) or *ggpubr* (Kassambara, 2019), except for Figure 5 and Figure 6b, c, which were constructed manually. In addition, all statistical tests were run in R. Post-hoc Tukey tests were done using the base R functions while Dunn tests were done using the *FSA* package (Ogle et al., 2021). Different types of data also required using different types of statistical analyses.

Frequency of Play Directed Across Partner Categories

Nape attack frequencies were plotted, and the data were not normally distributed (Shapiro Wilks p < .05), so a Kruskal-Wallis test was used to compare if rats directed more nape attacks toward each available partner. For *post hoc* comparisons, the Dunn test was used. For data that were normally distributed, an ANOVA was used, with the Tukey test used for *post hoc* pairwise comparisons.

Latency, Temporal Distribution, and Order of Nape Attacks

Latency to the first nape attack with each potential play partner was assessed using a Kruskal-Wallis test as the data were not normally distributed (Shapiro Wilks p < .05). To determine if nape attacks were directed to each play partner equally across the 20-min play trial, nape attacks initiated by the focal animal were plotted in 2-min time bins. Nape attack events were plotted illustrating which partner they were directed to, in the order in which they occurred. These data show both the number of nape attacks initiated by each focal animal, as well as who the rats first played with, who they played with the most, and the order in which they played with each potential partner. A Chi-square test was used to determine if the partner attacked first differed. A Student's *t*-test was used to compare the first half of the nape attacks, for each focal rat, with the second half of the nape attacks to determine if certain partners were preferred during the first half of the play session over the second half. Clusters of nape attacks (three or more nape attacks directed to one partner) were summed and compared among play partners with a Kruskal-Wallis test after finding the data were not normally distributed (Shapiro Wilks p < .05). Additionally, the cluster with the most nape attacks directed toward each partner was compared using a Kruskal-Wallis test, after finding the

data were not normally distributed (Shapiro Wilks p < .05), to determine if the clusters of nape attacks were greater for some partners over others.

Dominance, Weight, Play Style, and Proximity

To determine if weight influences partner preferences, the difference in weight between the focal animal and each play partner was calculated and plotted against the percentage of nape attacks initiated between the pair. Similarly, dominance scores of both the focal animal and their play partners (dominance was assessed in both the home cage grouping and the play trial group) were plotted against the percentage of nape attacks to determine if dominance influences how much the focal animals play with each partner. Pearson correlations were used to determine if there was a significant relationship between the amount of play and weight differences and dominance. The play style, or how individuals defended a playful attack, as well as the number of role reversals, were compared using a Kruskal-Wallis test among the three partners to determine if partners responded differently, depending on their familiarity.

Finally, the social proximity of the focal animal to other group members when not playing together during the trials was calculated. An ANOVA was used to determine if the focal rats spent more time with an individual rat, as these data were normally distributed (Shapiro Wilks p > .05). In addition, social network analyses (Farine & Whitehead, 2015) were conducted to compare the pattern of association when playing and not playing.

Results

Partner Preferences

A Kruskal-Wallis test revealed that focal rats differed significantly in how many nape attacks they directed toward different partners (Figure 2a; H(2) = 9.16, p = .01), with more nape attacks launched toward neighbors than toward cage mates (p = .003) or strangers (p = .036). Given the considerable variation across individuals in the total number of nape attacks they launched (see Figure 4), the pattern of attacks across partner categories (Figure 2a) could have been biased by some overly playful outliers. Therefore, the distribution across partner categories for each focal rat was recalculated as a percentage of its total attacks, and these percentage scores were used to calculate group means (Figure 2b). An ANOVA revealed that there was a significant difference across categories of potential partners (F(2, 105) = 25.97, p < .001), with focal rats launching a significantly greater percentage of attacks toward neighbors than either cage mates (p < .001) or strangers (p < .001). In addition, focal rats launched a significantly greater percentage of nape attacks toward strangers than cage mates (p = .034).

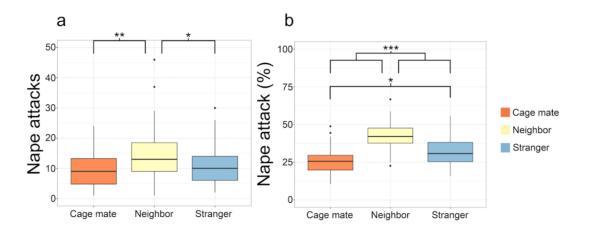
Mechanisms Influencing Partner Preferences

Latency and Temporal Distribution

A Kruskal-Wallis test revealed that there were no significant differences in latency to the first bout of play across partner categories (H(2) = 0.32, p = .85). In addition, descriptively, no difference was observed in the temporal distribution of play, with play peaking in the first five minutes of the trials in all cases (Figure 3).

Figure 2

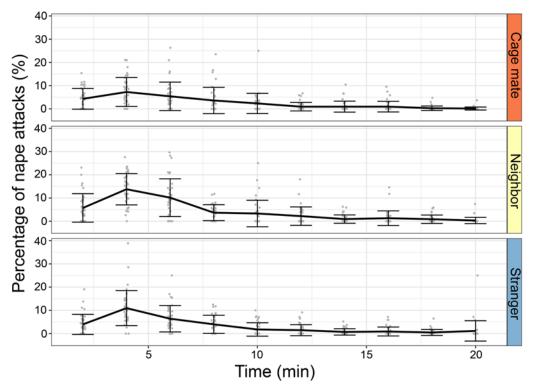
Frequency and Percent of Nape Attacks



Note. The frequency of nape attacks (a) and the percent of nape attacks (b) by the focal animal towards the cage mate, neighbor, or stranger play partner are shown. Statistical significance is indicated by: * p < .05; ** p < .01; *** p < .001.

Figure 3

Temporal Distribution of Play



Note. The distribution of play between the focal rat and each category of partner is shown over the 20-min play trial.

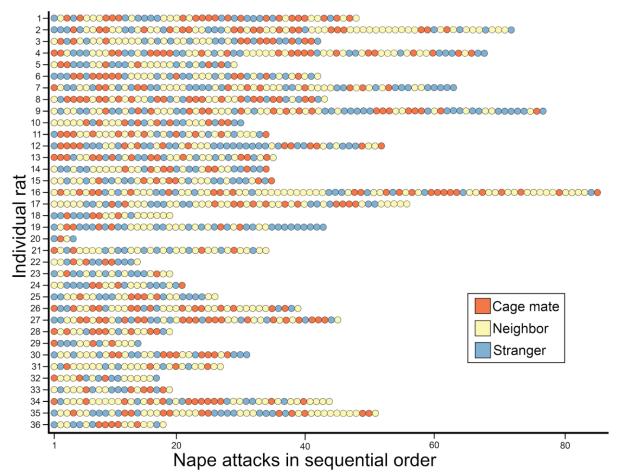
Order of Nape Attacks

The order of attacks directed at different types of partners is shown in Figure 4. A Chi-square test revealed that the target of the first attack did not significantly differ among the partners available ($X^2 = 1.16$, df = 2, p > .05). Comparing the first half with the second half of the nape attacks per trial for all individuals, using a Student's *t*-test, except rat #20, which only launched four attacks, revealed no difference in the percentage of attacks directed at the three categories of potential partners (cage mate: t(34) = 0.11, p = .46;

neighbor: t(34) = 0.17, p = .43; stranger: t(34) = -0.39, p = .35). So, partners of all three categories were attacked repeatedly over the course of the trial, although, in many cases, there were clusters, with focal rats repeatedly attacking one partner before moving on to the next. These clusters, of three or more nape attacks in succession, occurred differently among play partners, as revealed by a Kruskal-Wallis test (H(2) = 9.01, p = .01), with clustered nape attacks occurring toward neighbors significantly more often than cage mates (p = .008). Clustered nape attacks did not occur significantly more between cage mates and strangers nor neighbors and strangers. In addition, a Kruskal-Wallis test revealed there was a significant difference in the length of the clusters of nape attacks directed at each partner (H(2) = 11.57, p = .003), with neighbors having longer sequences of repeated nape attacks compared to cage mates (p = .004) and strangers (p = .03). However, there was no difference between the length of clusters for cage mates and strangers.

Figure 4

Sequential Order of Nape Attacks



Note. The sequential order of nape attacks initiated by each focal rat with all categories of partners is shown for each trial. *Weight and Dominance Asymmetries*

When tested with a Pearson correlation there was no significant relationship between the weight difference between partners and percentage of nape attacks directed toward cage mates (r(34) = -0.095, p = .58) or neighbors (r(34) = -0.23, p = .18). However, there was a significant positive correlation between weight difference and nape attacks directed toward strangers (r(34) = 0.38, p = .023), with heavier, focal rats initiating more play with lighter, stranger rats. Focal rats that weighed around 30-20g less than the

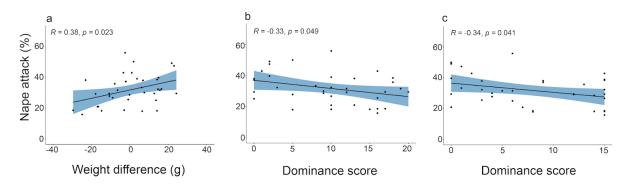
stranger initiated far fewer nape attacks with the stranger (Figure 5a), a trend not observed with the cage mates or the neighbors.

Based on Pearson correlation tests, the dominance level of the focal animal in the home cage was not significantly correlated with the percentage of nape attacks directed toward cage mates (r(34) = -0.042, p = .81), neighbors (r(34) = -0.18, p = .3), or strangers (r(34) = 0.2, p = .23). The dominance level of the focal animal in the play trial was not significantly correlated with the percentage of nape attacks directed toward cage mates (r(34) = -0.035, p = .84), however, there was a negative trend with neighbors (r(34) = -0.32, p = .06), and a positive trend with strangers (r(34) = 0.33, p = .052), indicating that more dominant focal animals were more likely to launch attacks toward strangers but not toward neighbors.

Based on Pearson correlation tests, the dominance level of the partners in the home cage was not significantly correlated with either the cage mates (r(34) = -0.16, p = .35) or neighbors (r(34) = 0.23, p = .18), however, there was a significant negative correlation with strangers (r(34) = -0.33, p = .049), whereby focal rats were less likely to attack strangers who were dominant in their home cages (Figure 5b). The dominance level of the partners in the play trial was not significantly correlated with the percentage of nape attacks directed toward cage mates (r(34) = -0.23, p = .18) or neighbors (r(34) = -0.039, p = .82), however, there was a significant negative correlation with strangers (r(34) = -0.34, p = .041), whereby focal rats were less likely to attack strangers who were dominant in the play group (Figure 5c).

Figure 5

The Significant Measures Contributing to Play Preferences



Note. These measures were only significantly correlated with interactions involving strangers. The percentage of nape attacks directed toward strangers is plotted against the weight difference between the focal and the stranger partners (a), the dominance scores of the strangers in their home cage (b), and the dominance scores of the strangers in the groups used in the play trials (c).

Play Style and Quality

Kruskal-Wallis tests did not reveal significant differences in the percentage of attacks defended that led to pins, evasions, mutual uprights or to role reversals (Table 2). That is, the pattern of play fighting appeared to be similar, regardless of the partner.

Table 2

Results of a Kruskal-Wallis Test for Several Measures of Play

Behavior	Home cage	Neighbor	Stranger	Kruskal-Wallis test
Percentage of attacks directed to each partner that were defended	58.78 ± 4.19	61.48 ± 3.54	61.51 ± 3.76	H(2) = 0.19, <i>p</i> = .91

30.83 ± 3.43	24.77 ± 3.12	25.78 ± 3.14	H(2) = 1.61, p = .45
21.73 ± 3.03	27.72 ± 3.01	30.15 ± 3.58	H(2) = 3.00, p = .22
0.56 ± 0.19	1.89 ± 0.50	2.20 ± 0.79	H(2) = 4.29, <i>p</i> = .12
2.67 ± 0.50	4.09 ± 0.94	3.14 ± 0.77	H(2) = 1.02, <i>p</i> = .60
	21.73 ± 3.03 0.56 ± 0.19	21.73 ± 3.03 27.72 ± 3.01 0.56 ± 0.19 1.89 ± 0.50	21.73 ± 3.03 27.72 ± 3.01 30.15 ± 3.58 0.56 ± 0.19 1.89 ± 0.50 2.20 ± 0.79

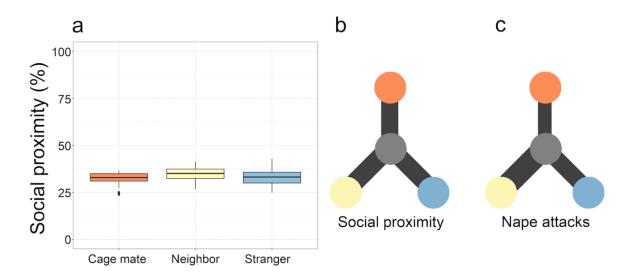
Note. Several behavioral measures of play between the focal rat and each category of partner are shown. Each behavior is represented by the mean and standard error of the mean, and for each the results of a Kruskal-Wallis test is shown.

Social Proximity

An ANOVA revealed that the time spent in non-playful proximity did not significantly differ (F(2, 105) = 2.89, p = .06) across categories of partners (Figure 6a). Thus, focal rats spent around 33% of their time in social proximity during the trials with each partner, but, on average, directed 45% of their play toward the neighbor, 30% of their play to the stranger, and only 25% of their play to the home cage mate (Figure 6b, c).

Figure 6

The Percentage of Time Spent in Social Proximity



Note. The percentage of time spent in social proximity when not playing between the focal rat and each category of partner is shown (a). Egocentric unidirectional social networks are presented to compare the time spent in social proximity (b) and playing (c) by the focal rat with each category of partner. There was no significant difference in social proximity but there was for play (see text).

Discussion

Given that in tests of social preference adult rats tend to attend more to strangers than familiar partners (e.g., Cirulli et al., 1996; Hackenberg et al., 2021; Rogers-Carter et al., 2018; Schweinfurth & Taborsky, 2020), and that, in dyadic tests, juvenile rats seem to play more when paired with strangers than cage mates (Achterberg et al., 2015; Panksepp, 1981; Pellis & Pellis, 1990; Reinhart et al., 2006), we predicted that, when given the choice, juveniles would initiate more play with strangers than cage mates. To test this, we devised a paradigm that removed three confounds. First, rats were tested in groups, so that the focal rat would have a choice between strangers and cage mates. Second, only a modest amount of pre-

test social isolation was imposed on the rats to avoid elevating their motivation to play so high as to produce a ceiling effect that could potentially eliminate preferences. Third, two types of strangers were offered, ones that lived across a perforated transparent barrier, allowing some degree of familiarization, but no play experience (Peartree et al., 2012), and ones with which they were completely unfamiliar. When so tested, focal rats did show a preference for initiating play with strangers, but while we expected the preference to be stranger>neighbor>cage mate, the data revealed the preference to be neighbor>stranger>cage mate (Figure 2 and Table 3). Indeed, while focal rats initiated play with all partners during the trials, consecutive play bouts, in which the rats continued to play with one partner repeatedly, occurred more frequently with neighbors than with home cage mates and strangers (Figure 4).

Table 3

Prediction	Supported	Result
Rats will prefer the most novel partner	No	Neighbor>stranger>cage mate
Latency to play will be longer with novel partners	No	No difference in the latency to play
Novel partners will be more socially attractive	No	No difference in social proximity
Playful feedback will influence preferences	No	Play measures did not differ among partners
Distribution of play will be biased for novel partners	Partially	Focal rats did not play with one rat over another at the beginning of the trial, but did engage in more bout repetitions, and longer sequences of repeated bouts with neighbors
Rats will prefer to play with the most dominant animal in the group	Partially	Dominance influences play preferences with strangers but not cage mates and neighbors
Rats will prefer to play with the heaviest animal in the group	Partially	Weight differences only influenced play preferences with strangers but not cage mates and neighbors

Summary of the Predictions and Results

While excessive novelty or stress dampens the occurrence of non-social play (e.g., play with objects, self-directed locomotor play), more moderate levels of novelty or stress, as induced by novel objects placed in the cage, may stimulate play (see review in Pellis & Burghardt, 2017). Similarly, mild to moderate social stress in some animals, including rats, increases play fighting (Darwish et al., 2001; Norscia & Palagi, 2011; Palagi, 2006; Von Frijtag et al., 2002), whereas more severe stress dampens play (Siviv et al., 2006; Siviy & Harrison, 2008). The neighbor may thus present the right balance between excessive familiarity and excessive novelty and so is the 'Goldilocks' partner-the partner that is 'just right.' Consistent with this possibility is that the weight and dominance of strangers influenced the degree to which play was initiated with them (Figures 5). Essentially, an excessive difference in dominance or weight with a stranger increases its novelty-induced stress to a level that play is avoided. Indeed, for the four focal rats that preferred their cage mate over novel partners, both the neighbor and stranger available were more dominant. However, it should be noted that, at this young age, dominance rarely leads to aggression (Pellis & Pellis, 1987; Takahashi, 1986; Takahashi & Lore, 1983), and aggression was never observed in this study. The 'threat' is not one of physical attack, but of novelty and strangeness, which leads to stress. Interestingly, in the home cage, with known social partners, greater dominance of a partner attracts more playful contact (Pellis & McKenna, 1992; Pellis & Pellis, 1991), whereas, with strangers, play is avoided if they are more dominant (present study). Clearly, while dominance may be important in influencing partner choice, context may be critical.

Direct measures of changes in stress hormones (Blanchard et al., 1993; Reinhart et al., 2006; Takahashi et al., 1992), depending on the quality of the unfamiliar partners presented to rats are needed to test this hypothesis, but the present findings reveal a strong preference for novel play partners, although ones that are not too threatening. Even so, novelty seems to trump excessive familiarity, as generally rats

initiated more play with strangers than with cage mates. While rats preferred novel, but not too novel partners, they initiated play with all members of the group (Figures 2 and 4), raising the problem of how the rats identified who is a stranger, who is a familiar, and who constituted the 'best' play partner.

Social exploration

In the paradigm that we used, the focal rat had never played with two of the potential partners and had never met one of them, so several mechanisms were examined that could be used to identify and focus playful interactions differentially across partners. Since rats engage in anogenital sniffing and other social exploration before they begin to play (e.g., Burke et al., 2022; Panksepp, 1981), we expected that there would be a longer delay in playing with strangers. This was not the case. The onset of play had the same latency for all types of partners and followed the same timeline over the course of the trial, with most focal rats initiating play with all three partners, primarily over the first 3-6 min of the trial (Figure 3). That the focal rats did not appear to use odor cues to identify and preferentially target neighbors and strangers appears to be consistent with experiments showing that rats play as normal when olfaction is suppressed or ablated (e.g., Beatty & Costello, 1983; Thor & Holloway, 1982). However, those experiments involved rats being tested in dyads in which rats had no choice but to play with the partner provided. Therefore, while olfactory information is not required for rats to engage in play, in a test context in which rats choose to play with some partners more than others, as in the present experiment, olfactory cues cannot be discounted. It could be that the measures we used to infer differences in olfactory information gathering were insufficiently sensitive. After all, rats are not insensitive to odors encountered during play as juveniles. In one experiment, the choice of male sex partner by adult females was influenced by the odor associated with the play they experienced as juveniles (Paredes-Ramos et al., 2011). Given that rats are able to identify individuals, and their relative dominance, based on their scent (Gheusi et al., 1997; Wesson, 2013), to discount that our rats did not use odor to target the less familiar partners, olfaction needs to be blocked in a group play paradigm.

In whatever way the preferred partners are recognized, a possible way to produce a context resulting in more play with some partners than others, is for the focal rats to have spent more time with the strangers, increasing the opportunity to initiate play. Several studies have shown that for adults, unfamiliar conspecifics attract experimental rats to remain in closer proximity (Hackenberg et al., 2021; Smith et al., 2015). However, our data show that the focal rats spent equal time, when not playing, with all three test mates (Figure 6a, b), even though they played more with neighbors and strangers than with cage mates (Figure 1, Figure 6c). This is the same pattern that occurs in groups of familiar rats when tested together (Pellis, Pellis, Burke, et al., 2022). In both cases, a rat often left the vicinity of one rat to traverse across the cage to initiate play with a more distant rat. These findings suggest that distance sensors are used to orient toward and target preferred partners. As noted above, the indirect measures we used cannot discount olfaction being involved, but other distance sensors need also to be considered. Two possible sensory modalities are vision and audition.

By itself, blocking vision is insufficient to prevent pairs of rats from playing at typical frequencies in the dyadic test (Bierley et al., 1986; Pellis et al., 1996), and indeed, rats tested under red light or in complete darkness play as much if not more than when tested under lighted conditions (Himmler, Modlinska, et al., 2014; Pellis & Pellis, 1987, 1990; Smith et al., 1998). Thus, while it is possible that the visual acuity of rats, especially pigmented ones like the Long Evans hooded rats we used, may be sufficient to identify partners at the inter-individual distances afforded by the test enclosure (Prusky et al., 2002), given that our trials were conducted under red light, visual cues would not have been available. Small predatory mammals can use auditory cues, such as footsteps taken in a leaf litter strewn substrate to detect and track prey (Goerlitz & Siemers, 2007; Langley, 1988; Siemers et al., 2007), and similarly, the location of potential partners in the test enclosure could be detected by the sounds made when stepping on the corncob bedding. Masking such sounds with white noise does not prevent play from occurring in dyadic tests, but it does decrease the likelihood of close quarter wrestling leading to pins (Siviy & Panksepp, 1987), suggesting another auditory cue may be used. When playing, rats emit ultrasonic vocalizations, especially frequency modulated 50 kHz calls (Burgdorf et al., 2008; Himmler, Kisko, et al., 2014), and when devocalized pairs of rats are matched, both the frequency of playful attacks and the incidence of role reversals is halved (Kisko et al., 2015). Moreover, different calls are associated with performing different actions during play (Burke et al., 2018), with some calls being able to attract other rats (Wöhr & Schwarting, 2007), providing the possibility that such calls could be used to target preferred partners. Nonetheless, when triads of rats were tested in play trials, in which one of the three was devocalized, the focal vocalizing rat launched just as many nape attacks to the devocalized partner as the vocal partner (Kisko et al., 2015). These findings do not discount the possibility that ultrasonic calls are used to localize partners, but this may only be detectable when other, more salient cues, such as olfactory ones are not available. Regardless of how they are detected, why are some partners preferred?

Some rats are more playful than others (Achterberg et al., 2023; Lampe et al., 2019; Lesscher et al., 2021; Pellis & McKenna, 1992) which can lead to different patterns of play when rats with different preferences play together (Pellis, Pellis, Burke et al., 2022; Poole & Fish, 1976). Therefore, it is possible that different partners provide different feedback during play. This led us to make two predictions. First, all potential partners should be sampled in the first few minutes of the trial and then, play should mostly be with the preferred partner in the later phases of the trial. Second, the pattern of play gained between preferred and less preferred partners should be different. In particular, the play with preferred partners should provide more close quarter wrestling and role reversals, the features of play that make it most rewarding (Pellis & Pellis, 2017; Vanderschuren et al., 2016). Neither prediction was supported by the data. The focal rats had the same temporal distribution of play with all three partners (Figure 3) and interspersed play bouts with all three across the entire span of the trial (Figure 4). Moreover, the pattern of play by the focal rat was the same when playing with all three partners (Table 2). While sampling and feedback may not account for why neighbors come to be preferred over cage mates and strangers, the lack of difference in the play experienced may account for why, despite significant preferences being present (Figure 2), the focal rats continue playing with all three partners. The finding that there was a significant difference in the length of the string of consecutive play fights initiated with preferred partners (Figure 4) suggests that there may be some subtle differences, not detected by our measurements, that make play with preferred partners more rewarding. It could be as simple as the neighbor providing a more exciting balance between novelty and familiarity to make play more stimulating (Pellis & Burghardt, 2017). The role of partner novelty, however, may differ across species.

Unlike the preference for strangers shown by rats, adult mice (*Mus musculus*), spiny mice (*Acomys cahirinus*), and female degus (*Octadon degus*) do not express a preference for either unfamiliar or familiar animals (Beery, 2021; Beery & Shambaugh, 2021; Fricker et al., 2022; Insel et al., 2020), and prairie voles (*Microtus ochrogaster*) and meadow voles (*M. pennsylvanicus*) prefer familiar conspecifics over strangers (Beery et al., 2018; Beery & Shambaugh, 2021). These differences in preference among rodent species may depend on differences in their mating and social systems (Beery & Shambaugh, 2021). While differences in social systems appear to influence variation in styles of play fighting across rodent species (Pellis & Iwaniuk, 1999a), there are no comparative studies to ascertain whether they also influence partner preferences. One comparison suggests that if they do, the effects may be quite subtle and idiosyncratic.

Rats live in colonies in which multiple females, rearing litters of young, live in proximity to each other (Schweinfurth, 2020). Infants mostly interact with their mothers and each other until weaned (Cramer et al., 1990). Once weaned, young rats have the option to play with littermates or with peers from neighboring litters. If our experimental design mimics real-life choices, juvenile rats should prefer to play with neighbors over siblings. Free living Belding's ground squirrels (*Urocitellus beldingi*) also live in colonies, but with females maintaining individual territories around their burrow in which they raise litters. When the young emerge from their burrows and begin to play, they have a choice between their littermates and the young from neighboring burrows (Nunes, 2014; Nunes, Muecke, Lancaster, et al., 2004). Unlike rats in the present study, juvenile ground squirrels play with littermates twice as much together as they do with non-littermates (Holmes, 1994; Nunes et al., 2015; Nunes, Muecke, Sanchez, et al., 2004). As in our study (Figure 6), these preferences are not accounted for by the physical distance between littermates and non-littermates (Holmes, 1994). This suggests that, for the ground squirrels, partner novelty does not influence play partner preference as it does in rats (Figure 2). Interestingly though, preliminary data on rats indicate that there are partner preferences when playing with littermates (Pellis, Pellis, Burke et al., 2022), a pattern also reported for the ground squirrels (Nunes, Muecke, Sanchez, et al., 2004).

In non-rodent species, partner play preferences are often influenced by kin relationships (e.g., Cappiello et al., 2018; Thompson, 1996), dominance hierarchies (e.g., Biben, 1986), and familiarity (e.g., Antonacci et al., 2010; Walker et al., 2015). While the juveniles of some non-rodent species only play with familiar animals or siblings (e.g., Antonevich et al., 2020; Drea et al., 1996; Pfeifer, 1978), as adults they may use playful social interactions to assess and manage encounters with strangers (Pellis & Iwaniuk, 1999b). For example, wild male sifakas (*Propithecus verreauxi*) play more with outgroup members than with ingroup members, seemingly to manage and test social relations with unknown individuals (Antonacci et al., 2010). When adult male grizzly bears (*Ursus arctos*) encounter unfamiliar, adult females, they too engage in play to seemingly familiarize themselves with one another (Herrero & Hamer, 1977). In this regard, rats are not atypical, as adult rats similarly use play fighting to familiarize themselves with unfamiliar adults (Stark & Pellis, 2020, 2021). It remains unknown as to how wild juvenile rats associate with peers in large colonies, and some laboratories studies routinely test juveniles with unfamiliar peers (e.g., Achterberg et al., 2015; Achterberg & Vanderschuren, 2020). Indeed, our results suggest that rats are not neophobic when young, and even prefer somewhat novel partners. Why different patterns of preferences are expressed across contexts in different species remains to be determined.

Future directions

While only males were used in this study, it is likely that females would express a similar pattern of partner preference. Males were selected for this study as dominance hierarchies, which are more prevalent in males (reviewed by Schweinfurth, 2020), influence playful interactions (Pellis & McKenna, 1992). However, because the relative dominance only significantly affected preferences with strangers, and this accounted for very little of the variance, females would likely express a similar partner preference pattern, as relative dominance likely would not influence females. With that said, juvenile female rats are more sensitive to the familiarity of their play partner when playing in dyads, playing more with familiar animals than strangers (Argue & McCarthy, 2015). However, the rats in this study were not afforded a choice and they were not presented with a 'somewhat' strange partner (i.e., a neighbor). Nonetheless, now that we have found juvenile male rats express partner preferences in a group play testing paradigm, we plan on performing the same experiment in females. Using this paradigm, we also plan to explore how mixedsex groups interact to determine if juvenile rats express a preference for their own sex in rough-and-tumble play (Argue & McCarthy, 2015) when given a choice. As the amount and roughness of play changes with age, the sex of the preferred partner may also change (Meaney & Stewart, 1981). Again, the group play paradigm used in the current paper could prove useful in discerning developmental changes in partner preferences.

Additionally, this paradigm may also prove useful in understanding how mixed-strain groups play. Mixed-strain experiments have become increasingly popular and have been used to explore rat prosocial behavior (e.g., Ben-Ami Bartal et al., 2014, 2021), the development of play behaviors (e.g., Schneider et al., 2016; Siviy et al., 2017; Stark et al., 2021), and social preferences (e.g., Mauri et al., 2022; Kiyokawa et al., 2014; Kogo et al., 2021), to name just a few areas of research. The paradigm employed here could explore how groups of mixed-strain juveniles interact with one another and determine if they prefer their own strain or not. In such a study, the preference for same-strain individuals could be due to dissimilarities in play style between strains (Himmler et al., 2014; Siviy et al., 1997) or other factors, such as discordant olfactory cues (Kogo et al., 2021; Nakamura et al., 2016). This is another experiment we plan on conducting in the future.

Conclusion

Our results demonstrate that juvenile male rats express partner preferences when playing in a group. When given the choice by allowing juveniles to play in groups rather than dyads, rats prefer novel partners, but partners that are not too novel (the Goldilocks principle). None of the possible mechanisms used to form partner preferences that we assessed accounted for how these preferences were formed. Whether partner preferences emerge in groups of complete strangers remains to be tested, but if they do, that could be a better paradigm with which to discern how preferences are formed. Although in the current study

relative dominance among partners did not account for partner preferences, dominance involving a stranger did influence play. Focal rats were less likely to initiate play with more dominant strangers (Figures 5b, c). A potential application of this finding concerns dyadic test paradigms in which strangers are paired together (Pellis, Pellis, Burke et al., 2022). Rats of markedly different dominance should not be matched. To avoid pre-test or post-test evaluation of each rat's dominance in its home cage or with the test trial partner, body weight, which is correlated with play preferences in the same way as dominance (Figure 5a), can be capitalized on by matching rats with as little weight asymmetry as possible (Achterberg et al., 2015; Achterberg & Vanderschuren, 2020). Play partner preference is an understudied feature of play, especially in key animals such as rats that have served as major laboratory models for the study of play (Siviy, 2016; Vanderschuren et al., 2016; VanRyzin et al., 2020), but may yield important new insights into the mechanisms of social cognition and exploration (Pellis, Pellis, Ham, et al., 2022; Pellis et al., 2023).

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