

When the individual comes into play: The role of self and the partner in the dyadic play fighting of rats

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ABSTRACT

Social play in rats is rewarding and important for the development of brain and social skills. There are differences in the amount of play behavior displayed among individuals, with earlier studies suggesting that, despite variation across trials, individual differences tend to be consistent. In the present study, juvenile Lister-hooded rats were paired with a different, unfamiliar same-sex partner on three days and based on the amount of play each individual initiated, it was characterized as a high, medium or low player. Using this categorization, we explored three features related to individual differences. First, we show that by increasing the number of test days from two, as was done in a previous study (Lesscher et al., 2021), to three, characterization was effectively improved. Secondly, while the earlier study only used males, the present study showed that both sexes exhibit a similar pattern of individual differences in the degree of playfulness. Even though low players consistently initiated less play than medium and high players, all rats varied in how much play they initiated from one trial to the next. Thirdly, we assessed two potential mechanisms by which the playfulness of one rat can modify the level of playfulness of the other rat (i.e., emotional contagion vs homeostasis). Analyses of individuals' contribution to the play of dyads suggest that rats consistently adjust their play behavior depending on the behavior displayed by the partner. Since this adjustment can be positive or negative, our data support a homeostatic mechanism, whereby individuals increase or decrease the amount of play they initiate, which results in the experience of an overall stable pattern of play across trials. Future research will investigate the neural bases for individual differences in play and how rats maintain a preferred level of play.

1. Introduction

Rough-and-tumble play or play fighting is widespread across many mammals, birds and even in some other taxa (Burghardt, 2005; Fagen, 1981). While some species routinely engage in play fighting involving multiple partners (Reinhart et al., 2010), typically, play fighting is dyadic in that two partners compete to gain some advantage over one another (Aldis, 1975). Unlike serious fighting, play fighting involves a delicate balance between competition and cooperation ensuring that the interaction remains playful (Palagi et al., 2016b; Pellis and Pellis, 2017), therefore it can serve as a model for studying the dynamics of social interactions (Palagi et al., 2016a; Pellis et al., 2022a, 2022b).

For studying the neural, developmental and psychological mechanisms that regulate social play, the laboratory rat has been the most used

species (Pellis and Pellis, 2009; Siviy, 2016; Vanderschuren et al., 1997, 2016). Pioneering research by Jaak Panksepp and colleagues established a general paradigm that has become a standard approach to studying play in rats – juveniles are socially isolated for some period and then tested as dyads (Panksepp, 1981; Panksepp and Beatty, 1980). This is considered a robust way to increase the motivation to engage in play (for review see Vanderschuren et al., 2016).

Importantly, studies of rats with multiple littermates in their home cage (e.g., Meaney and Stewart, 1981; Pellis and Pellis, 1997) have shown that the basic form of play fighting is the same as that performed in the isolation-dyadic paradigm (e.g., Pellis and Pellis, 1987, 1990). In both contexts, rats compete for access to the nape of their partner's neck, which is nuzzled with the snout if contacted (a pounce), (Pellis and Pellis, 1987; Siviy and Panksepp, 1987), and use a common set of

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behavior patterns to attack and defend the nape (Pellis et al., 2022a, 2022b). However, while increasing the period of isolation can increase the frequency of playful attacks, it can also affect the likelihood as to which defensive tactics are used (Pellis et al., 1997; Siviý et al., 1997; Siviý et al., 2003). This is an important caveat to consider as how the rats defend themselves can differentially affect the two most used measures to score play, pouncing and pinning. For example, a rat less motivated to play may be more inclined to move away before contact is made, reducing the number of pounces, and less inclined to roll over to supine, reducing the occurrence of pins (Pellis et al., 1997; Varlinskaya et al., 1999), a common postural configuration in the play of juvenile rats in which one rat lies on its back and the partner stands on top (Panksepp, 1981).

Applying a consistent within experiment pre-test isolation period and type of partner, the isolation-dyad paradigm has revealed that some rats are more playful than others (Pellis and McKenna, 1992). Moreover, even when tested as a group, some individuals initiate more play than others (Ham and Pellis, 2023), and individuals found to initiate more play in the dyadic test also initiate more play in their home cage (Lampe et al., 2017; Melotti et al., 2014), suggesting that individual differences in playfulness are not an artifact of testing.

Irrespective of the combination of factors leading to some individuals being more playful than others, a recent study showed a degree of consistency over two, consecutive dyadic trials that enabled the researchers to classify the rats as high (HP), medium (MP) and low (LP) players, and then, tested their consumption of alcohol. While the higher playing rats drank more, the low players were impaired in their ability for conditioned suppression of alcohol intake (Lesscher et al., 2021). Clearly, individual differences in juvenile playfulness can be indicators of underlying mechanisms that have broader implications for other behaviors. However, the robustness of such individual differences in play may vary with the identity of the partner. For example, in the method for selecting playfulness used by Lesscher and colleagues (2021), some rats deviated more than 35% in their measures of play from one trial to the next and so could not be categorized with certainty, and since the partners used for pairing were randomly selected strangers, variation in the playfulness of the partner could have been a major factor in the volatility of the play between trials.

It is known that the playfulness of one partner can influence the amount of play expressed by the other. Strains with a lower propensity to initiate play may initiate more play when paired with a partner from a strain with a higher propensity to initiate play (Reinhart et al., 2006). Similarly, untreated partners of male rats with increased propensity to initiate play due to neonatal testosterone exposure, also initiate more play (Pellis et al., 1992). Alternatively, a rat from a high playing strain matched with a partner from a low playing strain may initiate more play, seemingly compensating for the lower number of initiations received from its partner (Schneider et al., 2016). To make matters more complicated, while pairs of high players may play more than pairs of low players, mixed pairs may play more than pairs of high players (Lampe et al., 2019). That is, the playfulness of one partner can influence the playfulness of its partner thus masking the baseline differences in the play of individuals. The unpredictable effect of the partner could have accounted for the high number of uncategorizable rats in the Lesscher et al. (2021) study.

Therefore, in the present study, we expanded on this method by testing rats on three days with a different, unfamiliar, same sex, but weight-matched, partner on each of those days. The extra test day is predicted to better identify overall trends rather than a one-off change in play in a particular trial. The first goal of the present paper, using males, was to replicate the findings of Lesscher et al. (2021) regarding individual differences in playfulness. The second goal was to determine if females exhibited a similar pattern of individual differences in playfulness. The third goal was to test and so discriminate between two potential mechanisms by which the playfulness of one rat can modify the level of playfulness of the other rat (i.e., emotional contagion vs

homeostasis). The contagion hypothesis would predict that if matched with a more playful partner, a less playful partner would exhibit more play (Pellis and McKenna, 1992; Reinhart et al., 2006). In contrast, since the homeostasis hypothesis views individuals as having a preferred level of playfulness (Baldwin and Baldwin, 1976), it would predict that an individual's playfulness could increase or decrease depending on whether the partner plays more or plays less. By regulating the amount of play initiated depending on context, this could explain why some rats decrease their play when confronted with hyper-playful partners (Lampe et al., 2019; Pellis and McKenna, 1992).

2. Methods

2.1. Subjects

One hundred and twenty-six male and 30 female Lister Hooded rats (Charles River, Sulzfeld, Germany) arrived in 6 batches at postnatal day 21–23 in the facility and were group housed with 3 or 4 animals per cage under controlled conditions (i.e., temperature 20–21 °C, 55–65% relative humidity and 12/12 h light cycle with lights on at 7.00 a.m.) and had ad libitum access to water and rat chow. The rats were acclimatized to the facility for 5 days upon arrival and were handled four days prior to testing. Animals were weighed the day before each test to assign animals to a test pair.

In batch 1, play behavior of 30 male rats was assessed to replicate and expand the findings of individual differences in play reported in Lesscher et al. (2021). Batch 2 consisted of 30 females and was used to assess whether the patterns of individual differences found in the males were also present in females. Male and female behavior was not statistically compared because they belonged to different batches of animals.

To robustly test whether contagion or homeostatic regulation modulates social play, batch 1 was supplemented with batches 3–6 to a total of 126 male rats. All the rats were housed and tested under the same conditions as described above. All experiments were approved by the Animal Ethics Committee of Utrecht University and conducted in agreement with Dutch laws (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

2.2. Experimental procedures

In total, all animals were subjected to 3 characterization days (postnatal days (PND) 26, 28 and 30 to assess their tendency to engage in social play behavior to categorize them into player types (see Fig. 1). In these three 15-minute trials used for categorization, animals were paired with an unfamiliar (i.e., not a cage mate), weight matched (maximal difference of 10 g to prevent dominance effects), same-sex partner on every test-day (i.e., animals play with 3 different, similarly treated, unfamiliar animals). Experiments were performed as previously described (Achterberg et al., 2014; Trezza and Vanderschuren, 2008) in a sound attenuated chamber under red light conditions. The testing arena was a Plexiglas cage (40 × 40 × 60 cm; 1 x w x h) with approximately 2 cm of cellulose fibers mixed with wood shavings as bedding (ALCarefresh®). Animals were habituated to the test-cage for 10 min on PND 22 and 23 with their cage mates.

On the characterization days, each rat was isolated for 2.5 h prior to testing, in a Eurostandard type III cage in a room different from the housing room, to increase their motivation to play as was done previously (Achterberg et al., 2014; Lesscher et al., 2021). By only increasing social play behavior to half as much as it could be by longer periods of social isolation, a ceiling effect is prevented (Vanderschuren et al., 2008; Niesink and Van Ree, 1989). Play characterization consisted of placing a pair of rats in the play cage for 15 min. Each play session was recorded using a digital camera (Logitech C922 pro stream webcam, Lausanne, Switzerland). The behavior of both rats was assessed live by a trained observer from a computer in an adjacent room using Observer XT15 software (Noldus Information Technology, Wageningen, The

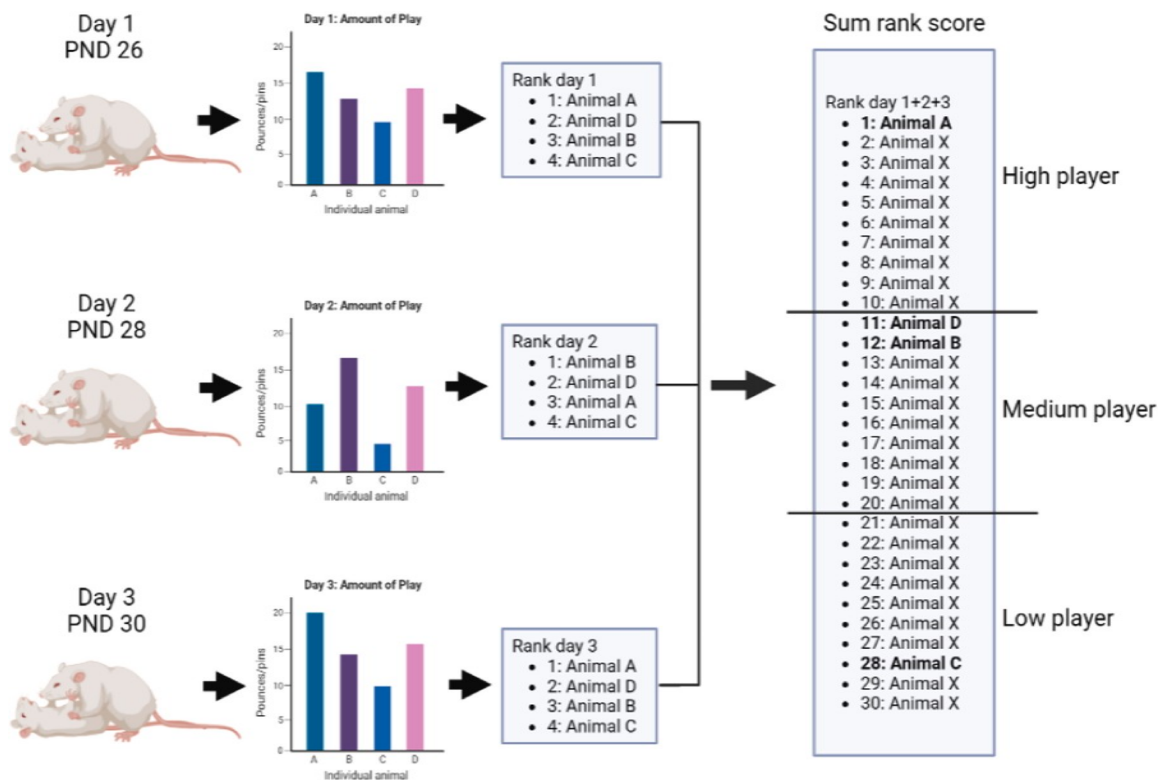


Fig. 1. Visual representation of the classification into playertypes. Across three characterization days (postnatal day (PND) 26, 28 and 30), the tendency of young rats to engage in social play behavior was assessed to categorize them in playertypes. In these three 15-minute characterization trials, rats were paired with an unfamiliar, weight matched, same-sex partner on every characterization-day, i.e., animals play with three different, similarly treated, unfamiliar animals. The amount of play (pounces: play initiations and pins: one animal standing over its supine partner) was ranked and the total rank score of the three characterization days was divided using a tertile split, resulting in high- (HP), medium- (MP) and low players (LP). Figure was created with BioRender.com.

Netherlands).

2.3. Behavioral analysis

2.3.1. Assessment of social play to determine playertypes

The following behavioral parameters were scored (Vanderschuren et al., 1997; Panksepp et al., 1984; Trezza et al., 2010; Pellis et al., 1989) for the animals over the three characterization days. Index of play solicitation: the frequency of pouncing, in which one animal attempts to nose/rub the nape of the neck of their partner. Frequency of pinning: one animal stands over its supine partner.

Some rats appear to attract more playful attacks than others (Ham and Pellis, 2023; Pellis and Pellis, 1990; Varlinskaya et al., 1999), which in turn, may stimulate them to initiate more play, i.e. they are more attractive play partners. To assess whether rats that initiate more play also receive more play, the number of times rats were pounced on ('being pounced') and were pinned ('being pinned') was also scored.

A potential confound is that greater playfulness may be a by-product of a subset of rats being more active or more motivated to engage in social behavior. A period of social isolation preceding the dyadic test leads to an increase in social play, not an increase in social exploration (Panksepp, 1981), which suggests that play is under independent control from general social interest. There are neural differences among individuals varying in boldness (Cools et al., 1990), and relative boldness could indirectly affect individual differences in playfulness (Pellis and McKenna, 1992). Therefore, as well as scoring the number of pounces and pins performed by individuals in each pair, the amount of social and non-social exploration was tracked. Social exploration, and so general social interest, was assessed by the total duration of one animal sniffed or groomed any part of their partner's body. Non-social exploration, and so general activity, was measured by the total duration of one animal

explored the test cage.

2.3.2. Characterization of playertypes

The first goal is to replicate and expand the findings of Lesscher et al. (2021). Rats that had a low, medium or high tendency to engage in social play behavior were selected on the basis of the sum of ranking scores for the number of active pounces and pins on each of the three characterization days (e.g., pouncing rank 22 out of 30 and pinning rank 25 out of 30 results in a sum ranking score of 47 out of 60 for a given day). Based on the total rank scores of these three test days, the population was subdivided into low, medium and high playing rats (LP, MP and HP, respectively) using a tertile split. This resulted in 10 animals in each group (10 LP, 10 MP and 10 HP) for batch 1 (males) and batch 2 (females), and these were used to address our second goal, that of determining whether comparable individual differences could be determined in both sexes. The animals of batches 3–6 were similarly categorized in player types.

2.3.3. In-depth day-to-day analyses

The third goal was to distinguish between the play contagion or play homeostasis hypotheses which involved different analyses rats from batches 1 and 2, as well as combining rats from batches 1 and 3–6. Using the data from batch 1 (males) and batch 2 (females), a more in-depth analysis of the data was performed. First, based on the three-day playertype classification, play levels on the separate categorization days were investigated to determine consistency of the classification into playertypes. Second, it was determined which of the two most characteristic play behavior postures, the pounce or the pin, was most prominent in the characterization into playertypes. To that end, correlation analyses were performed on play behavior measures within pairs. Third, coefficients of variation in the data between individuals and pairs over

days were analyzed to determine how behavior varies over days both on the individual as well as the pair level. Using batch 1, 3–6, in our fourth analysis, the relative and absolute contribution to the total amount of play of an animal of a certain playertype within a pair of playertypes was determined.

2.4. Statistics

Statistics were performed on data from batches 1 and 2 unless otherwise indicated. Social play and exploratory behavior data were analyzed using univariate ANOVAs with player type as a between-subject factor. *Post hoc* paired or unpaired Student's *t*-tests were performed with Bonferroni correction when appropriate. Male and female data were analyzed separately because the animals belonged to different batches. Patterns that were comparable in these datasets are indicated.

Correlations were made using Pearson's *r* correlation coefficient. The proportion of pounces of a certain animal in a particular play dyad was tested against equal contribution (a proportion of 0.5) using one-sample *t*-tests. The proportion and absolute frequencies of pounces within a playertype with a particular focal animal (e.g., LP in LP-HP pair) were compared using univariate ANOVAs followed by *post hoc* unpaired Student's *t*-tests (corrected for multiple comparisons) when appropriate (for this statistical test, batches 1, 3–6, were used). Statistical analyses were conducted using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). All data are presented as mean + SEM.

3. Results

3.1. Play behavior

3.1.1. Characterization of playertypes: frequency of play behavior

Juvenile rats were categorized into playertypes (LP, MP and HP) based on a tertile split of their amount of social play behavior during three characterization days (Fig. 2). The playertypes, both males and

females, based on batches 1 and 2, differed significantly in their amounts of play initiation, i.e., pounces (males: $F(2,27) = 41.72$, $p < 0.001$; females: $F(2,27) = 32.95$, $p < 0.001$) and pins (males: $F(2,27) = 37.47$, $p < 0.001$; females: $F(2,27) = 32.76$, $p < 0.001$, Fig. 2a and b). Rats of both sexes characterized as HP pounce and pin more than MP (HP vs MP pounce: $t_{\text{males}}(19) = 10.67$, $p < 0.001$, $t_{\text{females}}(19) = 14.13$, $p = 0.002$; pin: $t_{\text{males}}(19) = 6.90$, $p = 0.001$, $t_{\text{females}}(19) = 5.40$, $p = 0.004$) and LP (HP vs LP pounce: $t_{\text{males}}(19) = 22.70$, $p < 0.001$, $t_{\text{females}}(19) = 30.40$, $p < 0.001$; pin: $t_{\text{males}}(19) = 14.23$, $p < 0.001$, $t_{\text{females}}(19) = 6.90$, $p = 0.001$). MP pounce and pin more than LP (pounce: $t_{\text{males}}(19) = 12.03$, $p < 0.001$, $t_{\text{females}}(19) = 16.27$, $p = 0.001$; pin: $t_{\text{males}}(19) = 7.33$, $p < 0.001$, $t_{\text{females}}(19) = 8.67$, $p < 0.001$).

Whereas actively performing the characteristic play configurations (pouncing and pinning) was significant, being pounced on or being pinned did not differ between the playertypes in males ($F(2,27) = 1.52$, $p = 0.24$; $F(2,27) = 1.83$, $p = 0.18$, Fig. 2a). In females, being pounced on did differ significantly between the playertypes ($F(2,27) = 4.41$, $p = 0.02$, Fig. 2b) with LP being pounced on more compared to HP ($t(19) = 15.90$, $p < 0.001$), but there were no other differences between the playertypes (LP-MP: $t(19) = 4.83$, $p = 0.99$; MP-HP: $t(19) = 11.07$, $p = 0.16$). The playertypes in females did not differ in being pinned ($F(2,27) = 2.55$, $p = 0.10$).

3.1.2. Characterization of playertypes: duration of social and non-social exploration

General social interest, measured as social exploration, did not differ between the player types of either sex (males: $F(2,27) = 1.83$, $p = 0.18$; females: $F(2,27) = 1.68$, $p = 0.21$, Fig. 2c and d). Non-social exploration differed between the player types in males ($F(2,27) = 6.28$, $p = 0.006$), where LP spend more time exploring the cage compared to HP (HP-LP $t(19) = 58.48$, $p = 0.005$) but not MP (MP-LP $t(19) = 21.53$, $p = 0.62$) and MP did not differ from HP ($t(19) = 36.95$, $p = 0.11$, Fig. 2c). Females did not differ in cage exploration between the player types (F

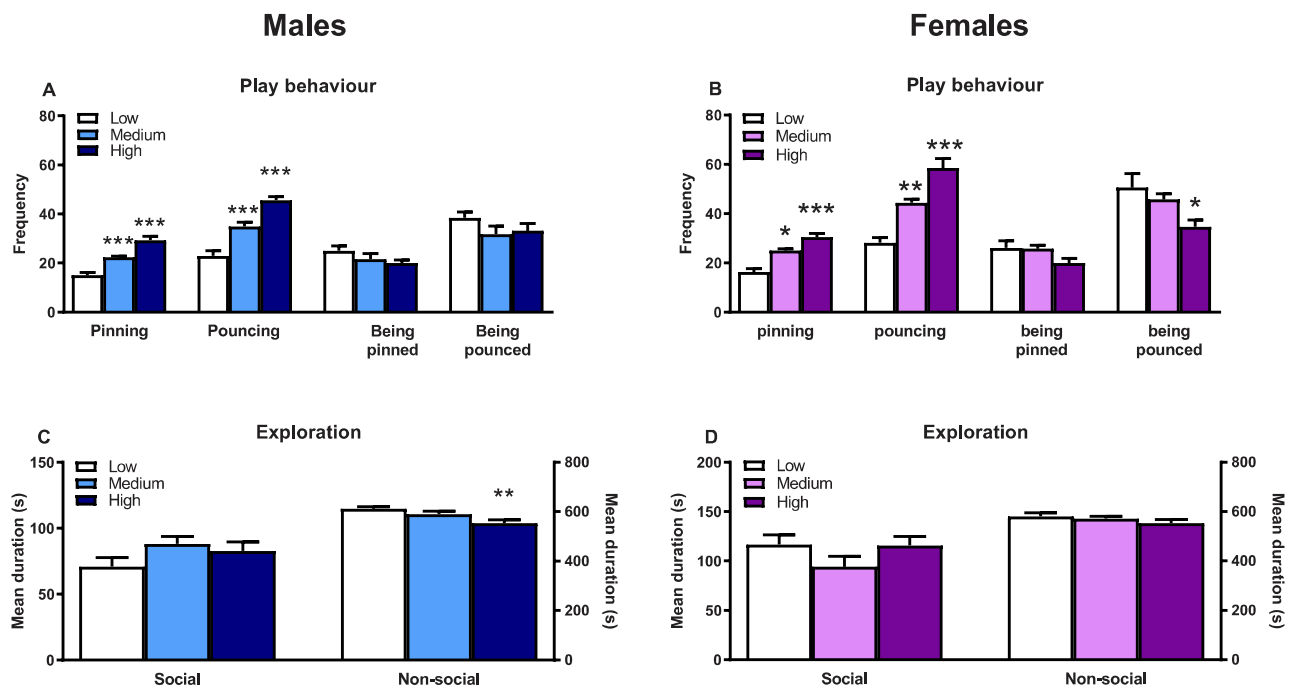


Fig. 2. Characterization of individual variation in social play behavior. A-B. Animals can be characterized as low, medium and high players (LP, MP and HP) based on the mean of three characterization days and a tertile split. HP (males: dark blue bars, females: dark purple bars) display significantly more pins and pounces compared to medium (males: mid blue bars, females: mid purple bars) and low (white bars) players. Being pinned or pounced upon does not differ between the player types. C-D. Social exploration does not differ between the player types in both sexes. HP males spent significantly less time on exploration of the test-cage (non social exploration), whereas there were no differences in females. Data are represented as mean + SEM, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

(2,27) = 1.23, $p = 0.31$, Fig. 2d).

3.2. Consistency in level of play

When taking the three test days together, three distinct player types can be reliably discerned (Fig. 2). Next, we determined whether this pattern is consistent across test days. When rats are confronted with three different unfamiliar play partners, individual animals of both sexes show variability in play (Fig. 3). Overall, compared to the grand mean of the batch, for LP rats, pounce (Fig. 3a and b) and pin (Fig. 3c and d), two or more days fell below the grand mean, whereas, for HP rats, both pounce and pin, were above the grand mean for two or more days. MP rats exhibited an intermediate pattern, with pounce and pin having one or two days above or below the grand mean.

When comparing day-to-day play levels, the frequency of pounces fluctuated significantly over days in both sexes, whereas pinning levels were more stable (Table 1). Importantly, no interaction effects of playertype and characterization day were found for either pouncing or pinning for either sex (Table 1). This indicates that play may fluctuate over days but that the playertype differences in the two play behaviors is a consistent pattern and not the result of the performance on one particular day (Table 1 and Fig. 4).

Receiving a pounce or a pin in males fluctuated over days (Table 1) whereas in females, these measures of play were more constant over days (Table 1). Together, these results suggest that being the initiator of the pounce and the pin, in contrast to being receiver, measured over three test days with an unfamiliar partner, shows consistent player type

differences in both sexes.

3.3. The play of pairs and the contribution of pair mates

Before focusing on the contribution of individuals to the amount of play evident in pairs, the most discriminating scores need to be identified. Pounces and pins were scored as independent measures, but detailed descriptive analyses of how pins arise indicate that the two are correlated (Pellis and Pellis, 1987). A pin most commonly arises from the recipient of a pounce maneuvering to avoid nape contact, with turning over to supine being the most frequently used tactic by juveniles of many strains of rats (Himmler et al., 2016b).

Since pins by one animal are contingent on the number of pounces by the other, it would be predicted that the number of times one rat is pinned should be positively correlated with the number of pounces by its partner. This was found to be the case across the three characterization days for both males and females (Table 2, row 1). Consequently, pins are not an independent measure, but rather, are contingent on the number of pounces received.

Taking that contingency into account, pins were re-scored as the percentage of pounces leading to pins. The analysis showed that the likelihood of partners being pinned was not correlated (Table 2, row 2), indicating that most of the variance in the absolute number of pins across individuals was due to the variance in the number of pounces by their partners. Therefore, for more detailed analyses of inter-individual differences, pounces were used.

If play is contagious (Pellis and McKenna, 1992), then it would be

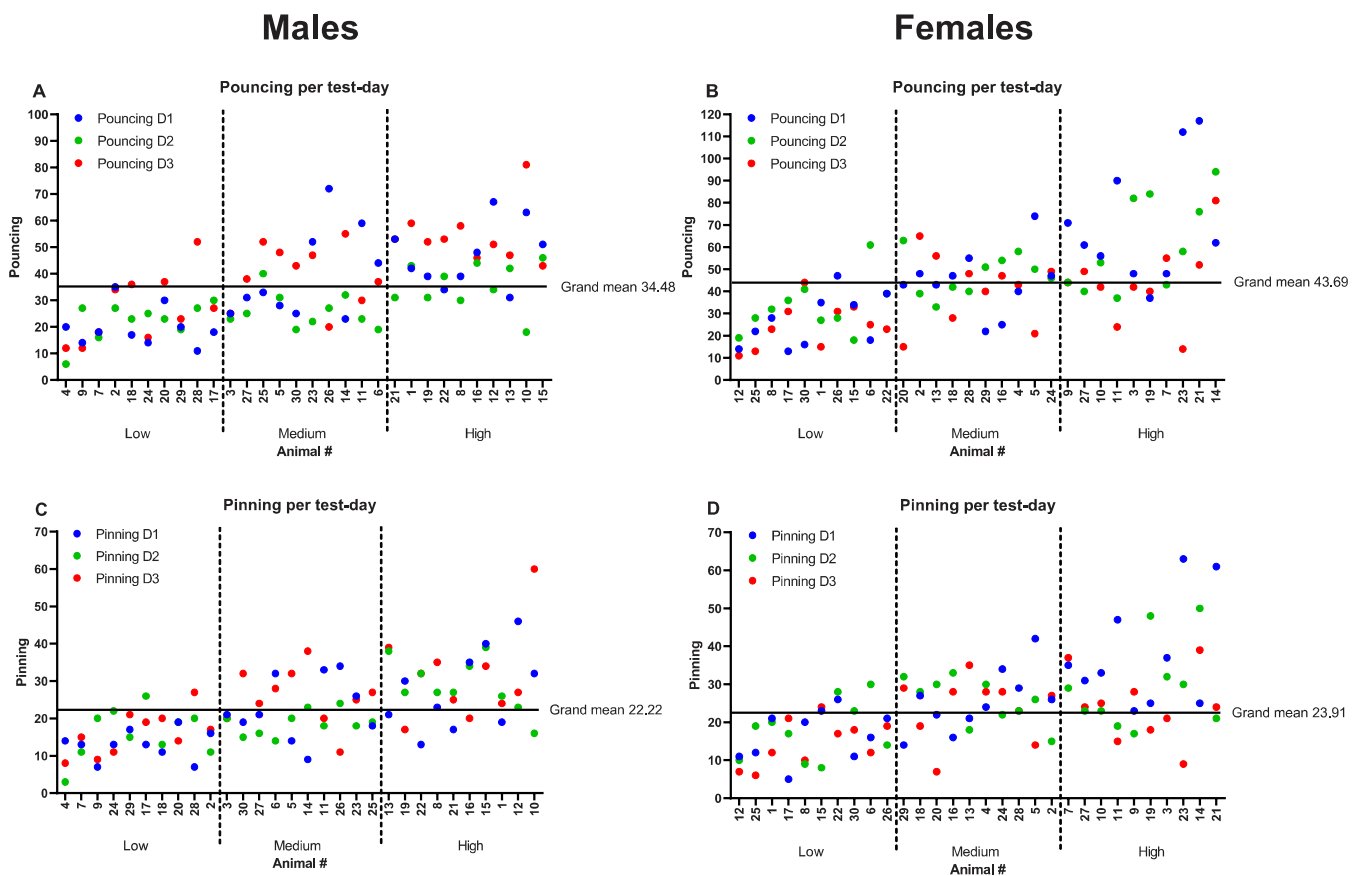


Fig. 3. Frequencies of pounces (A,B) and pins (C,D) per individual rat categorized in playertypes. Data is displayed over the three characterization days for both males and females. The horizontal line indicates the grand mean of the amount of pounces (A,B) or pins (C,D) displayed over three days. D1: test day 1 (blue dots), D2: test day 2 (green dots), D3: test day 3 (red dots). Four patterns can be discerned: 1) Low playing (LP) rats of both sexes have two or three days in which pounces and pins fell below the grand mean. 2) Medium playing (MP) rats of both sexes have one or two days in which the amount of pounces and pins were above the grand mean. 3) High playing (HP) rats of both sexes have two or three days in which the amount of pounces and pins are above the grand mean. 4) The day-to-day amounts of pounces and pins can differ substantially in both sexes.

Table 1

Consistency in play behaviors over the three days of characterization. Displayed are statistics per type of play behavior. D1: day 1, D2: day 2, D3: day 3 of characterization. Arrows indicate how the test days differ in behavior: < decrease, > increase. Statistical test used: Repeated measures ANOVA with day as within subject factor and player type as between subject factor. p * d: player type x day interaction. n = 10 rats per playertype per sex. Sexes were analyzed separately because they belonged to separate batches.

Consistency in play behaviors over three characterization days		
Behavior	Males	Females
Pouncing	Fday(2,54) = 8.43, p = 0.001	Fday(2,54) = 3.50, p = 0.04
	Fplayertype(2,27) = 41.72, p < 0.001	Fplayertype(2,27) = 32.95, p < 0.001
	Fp*d(4,54) = 1.80, p = 0.15	Fp*d(4,54) = 1.54, p = 0.20
	Post hoc days adjusted p = 0.0167	Post hoc days adjusted p = 0.0167
	D1 > D2 t(29) = 2.28, p = 0.03	D1 < D2 t(29) = 0.13, p = 0.99
	D1 < D3 t(29) = -1.51, p = 0.14	D1 < D3 t(29) = -10.27, p = 0.14
Pinning	D2 < D3 t(29) = -4.52, p < 0.001	D2 > D3 t(29) = 10.40, p = 0.02
	Fday(2,54) = 1.46, p = 0.24	Fday(2,54) = 2.62, p = 0.08
	Fplayertype(2,27) = 37.47, p < 0.001	Fplayertype(2,27) = 32.76, p < 0.001
	Fp*d(4,54) = 0.61, p = 0.66	Fp*d(4,54) = 1.37, p = 0.28
	Fday(2,54) = 6.66, p = 0.003	Fday(2,54) = 2.84, p = 0.07
	Fplayertype(2,27) = 1.52, p = 0.24	Fplayertype(2,27) = 4.41, p = 0.02
Being pounced	Fp*d(4,54) = 2.89, p = 0.03	
	Post hoc days adjusted p = 0.0167	
	D1 > D2 t(29) = 2.43, p = 0.02	
	D1 < D3 t(29) = -1.17, p = 0.25	
	D2 < D3 t(29) = -3.67, p = 0.001	Fp*d(4,54) = 0.94, p = 0.45
	Post hoc playertype per day	
D1: F(2,27) = 0.89, p = 0.42		
D2: F(2,27) = 1.55, p = 0.23		
D3: F(2,27) = 0.93, p = 0.41		
Fday(2,54) = 1.40, p = 0.27	Fday(2,54) = 2.69, p = 0.08	
Fplayertype(2,27) = 1.83, p = 0.18	Fplayertype(2,27) = 2.55, p = 0.10	
Fp*d(4,54) = 2.65, p = 0.04		
Post hoc days adjusted p = 0.0167		
Being pinned	D1 < D2 t(29) = -0.05, p = 0.96	
	D1 < D3 t(29) = -1.24, p = 0.23	
	D2 < D3 t(29) = -1.37, p = 0.18	Fp*d(4,54) = 0.96, p = 0.44
	Post hoc playertype per day	
	D1: F(2,27) = 0.34, p = 0.71	
	D2: F(2,27) = 1.26, p = 0.30	
D3: F(2,27) = 0.76, p = 0.48		

predicted that the amount of play initiated (i.e., measured as pounces) by one partner should increase the amount of play initiated by its partner. This was not what was found. Rather, for the three days, there was either no correlation, or the correlation was negative (Table 2). Comparing the degree of variation between pairs and individuals suggests a reason for why this prediction was not fulfilled. The total number of pounces varied significantly over the three characterization days for males (pairs: $F(2,28) = 8.01$, $p = 0.002$, individuals: $F(2,58) = 5.60$, $p = 0.006$) and for females (pairs: $F(2,28) = 4.90$, $p = 0.02$, individuals: $F(2,58) = 3.18$, $p = 0.05$). Moreover, the daily variation was larger for individuals than for pairs. The coefficient of variation (CV) was calculated for each day, with the average daily CV for pairs being 24.27% (males), 28.34% (females) and that for individuals being 40.35% (males), 46.84% (females). These findings suggest that individuals vary the number of pounces they initiate depending on the behavior of their partners, so either increasing or decreasing the number of pounces.

Given that the daily variance among individuals tends to be greater than the variance among pairs, to assess the variation in the contribution of one partner relative to the other, the absolute number of pounces by

one partner (animal A) was compared to the percentage of pounces contributed by the other (animal B). Not only did this reveal a strong correlation on each day of testing for both males and females, but it also showed that the association was negative, and in most cases, this remained significant across playertypes (Table 3).

The negative correlations indicate that, if one partner launched many pounces, its partner launched fewer. To explore this further, the full set of 126 males (i.e., combining batches 1, 3, 4, 5 and 6) was used as this provided sufficient pairings of different playertypes across days to be compared as shown in Fig. 5. What is evident is that, when paired with a low player, a rat launches more pounces, whereas, if paired with a high player, it launches fewer. That is, an individual rat, irrespective of playertype, modulates its play with that of its partner, and this modulation is bidirectional (full statistics can be found in Table 4).

Four patterns were discerned from the test against an equal contribution to the play interaction: 1) A low player always contributes less than 50% pounces to the play interaction except when paired with another low player. 2) A medium player contributes significantly more than 50% pounces when paired with a low player, equal amounts when paired with a medium player but significantly less than 50% when paired with a high player. 3) A high player always contributes more than 50% pounces to the interaction except when paired with another high player, then it fluctuates around equal. 4) The described patterns for low players are already present from test-day 1, whereas for medium and high players, this pattern emerges from day 2 onward (Table 4 and Fig. 5).

Next, we tested whether focal animals of a specific playertype in each of three pair compositions possible (LP can be paired with LP, MP or HP) contributed to the relative frequency of pounces differently over the characterization days. This was the case for all three playertypes on the three characterization days (Table 4). *Post hoc* analysis revealed that on day 1, LPs in a LP-HP pair contributed significantly fewer pounces compared to LPs in a LP-LP or LP-MP pair. On day 2 and 3, LPs in a LP-HP pair contributed less compared to LPs in a LP-LP pair. Over the 3 characterization days, MPs contributed relatively more pounces when coupled with a LP compared to when coupled with a HP. In addition, on the third characterization day, MPs coupled with LP contributed significantly more compared to when coupled with another MP. HP rats in HP-LP pairs contributed significantly more pounces in this pair compared to HP animals in HP-HP pairs. Only on day two did HPs in HP-LP pairs contribute more compared to HPs in HP-MP pairs. These data indicate that playertypes differ in their relative pounce contribution depending on the partner they are coupled with.

Interestingly, the absolute frequencies of pounces did not differ based on focal animal in a specific pair composition and this was similar for all three characterization days (*Low*: day 1: $F(2,37) = 1.76$, $p = 0.19$, day 2: $F(2,37) = 1.08$, $p = 0.35$; day 3: $F(2,37) = 0.18$, $p = 0.84$; *Medium*: day 1: $F(2,43) = 2.00$, $p = 0.15$; day 2: $F(2,43) = 0.04$, $p = 0.97$; day 3: $F(2,42) = 1.40$, $p = 0.26$; *High*: day 1: $F(2,37) = 3.03$, $p = 0.06$; day 2: $F(2,37) = 0.93$, $p = 0.41$; day 3: $F(2,38) = 1.57$, $p = 0.22$).

Together, these data suggest that, while adolescent rats have individual differences in the absolute amount of play they prefer, they modulate their relative contribution to a play trial depending on the behavior of their partner (as illustrated in Fig. 6).

4. Discussion

In the present study, we aimed to determine whether individual differences in social play of rats could be discerned from the partner's contribution in a dyadic encounter. Individuals paired successively with three, weight-matched, unfamiliar play partners and assessed for their playfulness were identified as high, medium or low players, in line with Lesscher et al. (2021). These differences in player types were relatively consistent over the three days of testing and was present in both sexes. Nonetheless, the amount of play initiated by rats was modified by the playfulness of the partner with which it was paired.

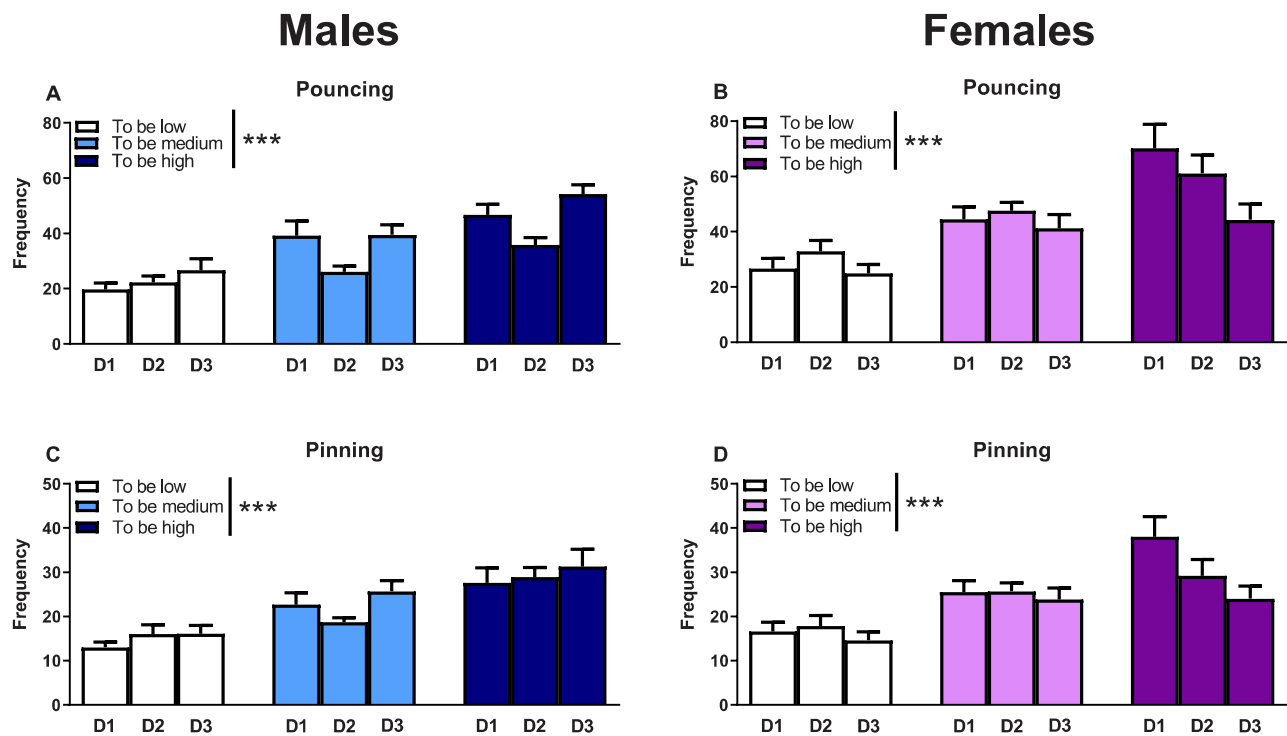


Fig. 4. Consistency in playtypes over the three characterization days in male (A,C) and female (B,D) rats. HP (males: dark blue bars, females: dark purple bars) display significantly more pounces (A,B) and pins (C,D) compared to medium (males: mid blue bars, females: mid purple bars) and low (white bars) players. The frequency of pounces fluctuated over days in both males (A) and females (B) but no interaction of playertype and day was found, indicating that the playtypes were consistent over days. The frequency of pins in both males and females was constant over the characterization days (C-D), no interaction of playertype and day was found. Full statistics can be found in [Table 1](#).

Table 2

Daily correlations between partners for pounces and pins. One animal from each play-pair was designated animal A and its behavior relative to its partner, animal B, is shown in each case. *Number of active pins of A, i.e., being on top in the pin configuration. **Pins are expressed as the percentage of pounces leading to a pin, with the amount of pounces initiated by that animal set to 100%. Significant correlations are indicated in bold. Sexes were analyzed separately because they belonged to separate batches.

Correlation (Pearson)	Day1		Day2		Day3	
	Males	Females	Males	Females	Males	Females
Number of pounces by A versus the number of times B is pinned*	$r = 0.93$ $p < 0.001$	$r = 0.93$ $p < 0.001$	$r = 0.97$ $p < 0.001$	$r = 0.84$ $p < 0.001$	$r = 0.61$ $p = 0.02$	$r = 0.90$ $p < 0.001$
Percentage of times A is pinned versus % of times B is pinned**	$r = 0.31$ $p = 0.26$	$r = -0.02$ $p = 0.95$	$r = -0.13$ $p = 0.64$	$r = 0.06$ $p = 0.83$	$r = 0.43$ $p = 0.11$	$r = -0.50$ $p = 0.06$
Number of pounces by A versus pounces by B	$r = 0.06$ $p = 0.83$	$r = -0.37$ $p = 0.04$	$r = 0.18$ $p = 0.52$	$r = -0.61$ $p < 0.001$	$r = -0.73$ $p = 0.002$	$r = 0.19$ $p = 0.33$

Two potential mechanisms by which the playfulness of one rat can modify the level of playfulness of the other rat, emotional contagion and homeostasis of preferred levels of play, were analyzed for their explanatory power to account for the daily variation we observed in the play of individuals. We found that while animals have an inclination for a particular baseline level of playfulness, they modulate the amount of play they initiate depending on the playfulness of their partner, seemingly to maintain their preferred level of play (see [Fig. 6](#)).

4.1. Individual differences in playfulness in male and female rats

Our data are consistent with those of [Lesscher et al. \(2021\)](#), [Melotti et al. \(2014\)](#) and [Lampe et al. \(2019\)](#), in showing that, over repeated trials, rats exhibit consistent individual differences in their degree of playfulness. Moreover, these individual differences are present whether rats are tested with familiar partners ([Lampe et al., 2019](#)) or strangers ([Lesscher et al., 2021](#); present study), and whether observed in the home cage or in staged dyadic encounters ([Melotti et al., 2014](#)). The present

study extends these findings by showing that such individual variation is present in both males and females, and that the variation across multiple trials can be accounted for by the influence of the playfulness of the partner.

Pharmacological approaches inducing discrepancies in playfulness have also been used to reveal individual differences in the play profiles of rats. Scopolamine hydrobromide passes the blood-brain barrier and when injected in juvenile rats, it selectively blocks social play but not exploration and social investigation ([Deak and Panksepp, 2006](#); [Pellis and McKenna, 1995](#); [Thor and Holloway, 1983a](#)). Consequently, when an untreated rat interacts with a scopolamine-treated rat, the treated rat's lack of playful response reveals the playfulness of the untreated attacker. In this type of dyadic interaction, some individuals consistently initiate more attacks on a scopolamine-treated target than others ([Pellis and McKenna, 1992, 1995](#)). The same is true for the stimulant and play-suppressant drug methylphenidate ([Vanderschuren et al., 2008](#)). These data suggest that, when one partner is not actively engaged in play, differences in initiating play indicate that there are intrinsic

Table 3

Correlations of the frequency of pounces of animal A with the percentage pounces of animals B over the three test days. One partner from each partner was designated animal A and its behavior relative to its partner, animal B, is shown in each case. All: all animals together. HP: high player, MP: medium player, LP: Low player. *Pearson correlation (r). r²: coefficient of determination. Sexes were analyzed separately because they belonged to separate batches.

Correlation* pounces animal A and % pounces animals B	Males	Females
Day 1: all	r = -0.74, p < 0.001, r ² = 0.54	r = -0.82, p < 0.001, r ² = 0.67
HP	r = -0.55, p = 0.10, r ² = 0.30	r = -0.90, p < 0.001, r ² = 0.81
MP	r = -0.70, p = 0.02, r ² = 0.49	r = -0.67, p = 0.03, r ² = 0.45
LP	r = -0.94, p < 0.001, r ² = 0.88	r = -0.34, p = 0.35, r ² = 0.12
Day 2: all	r = -0.65, p < 0.001, r ² = 0.42	r = -0.87, p < 0.001, r ² = 0.76
HP	r = -0.67, p = 0.04, r ² = 0.45	r = -0.87, p = 0.001, r ² = 0.76
MP	r = -0.50, p = 0.14, r ² = 0.25	r = -0.82, p = 0.004, r ² = 0.67
LP	r = -0.72, p = 0.02, r ² = 0.51	r = -0.89, p < 0.001, r ² = 0.79
Day 3: all	r = -0.90, p < 0.001, r ² = 0.81	r = -0.62, p < 0.001, r ² = 0.38
HP	r = -0.53, p = 0.12, r ² = 0.28	r = -0.17, p = 0.65, r ² = 0.03
MP	r = -0.93, p < 0.001, r ² = 0.86	r = -0.76, p = 0.01, r ² = 0.58
LP	r = -0.93, p < 0.001, r ² = 0.86	r = -0.80, p = 0.006, r ² = 0.64

differences in levels of playfulness in the other partner.

A potential confounding factor is that greater playfulness may be a by-product of some rats being generally more active or more motivated to engage in social behavior in general. This is an unlikely explanation because the period of social isolation preceding the dyadic test leads to an increase in social play, not an increase in social exploration (Panksepp, 1981) or general activity such as cage exploration. Given that our rats designated as different playertypes did not differ in either social or non-social exploration (Fig. 2), supports the view that individual differences in playfulness are not a by-product of differences in either sociability or overall activity. Some rats are indeed more playful than others.

Potential causes of individual differences in playertypes likely include genetic differences as some strains of rats are more playful than others (Himmler et al., 2014a; Reinhart et al., 2004, 2006; Siviy et al., 1997, 2003), perinatal influences, such as degree of exposure to testosterone (Meaney and McEwen, 1986; Ward and Stehm, 1991; Van Ryzin et al., 2020), postnatal rearing experiences, such as being exposed to maternal separation (Aguilar et al., 2009; Arnold and Siviy, 2002; Siviy and Harrison, 2008), and post-weaning experiences, such as being housed with rats from different strains (Himmler et al., 2014b; Schneider et al., 2016). It is also likely that these different influences may interact, producing subtle variation across individuals in their motivation to engage in play.

There may also be indirect influences that shape the degree of playfulness across individuals. For example, there are neural differences among individuals that lead to variation in boldness (Cools et al., 1990), and relative boldness could indirectly affect individual differences in playfulness (Pellis and McKenna, 1992). Also, some rats appear to attract more playful attacks than others (Ham and Pellis, 2023; Pellis and Pellis, 1990; Varlinskaya et al., 1999), which in turn, may stimulate them to initiate more play. That is, some rats may be indirectly more playful because they are more attractive play partners. We do not know if more playful rats are bolder, but we assessed whether rats that initiate more play also receive more play. Being pounced on and being pinned

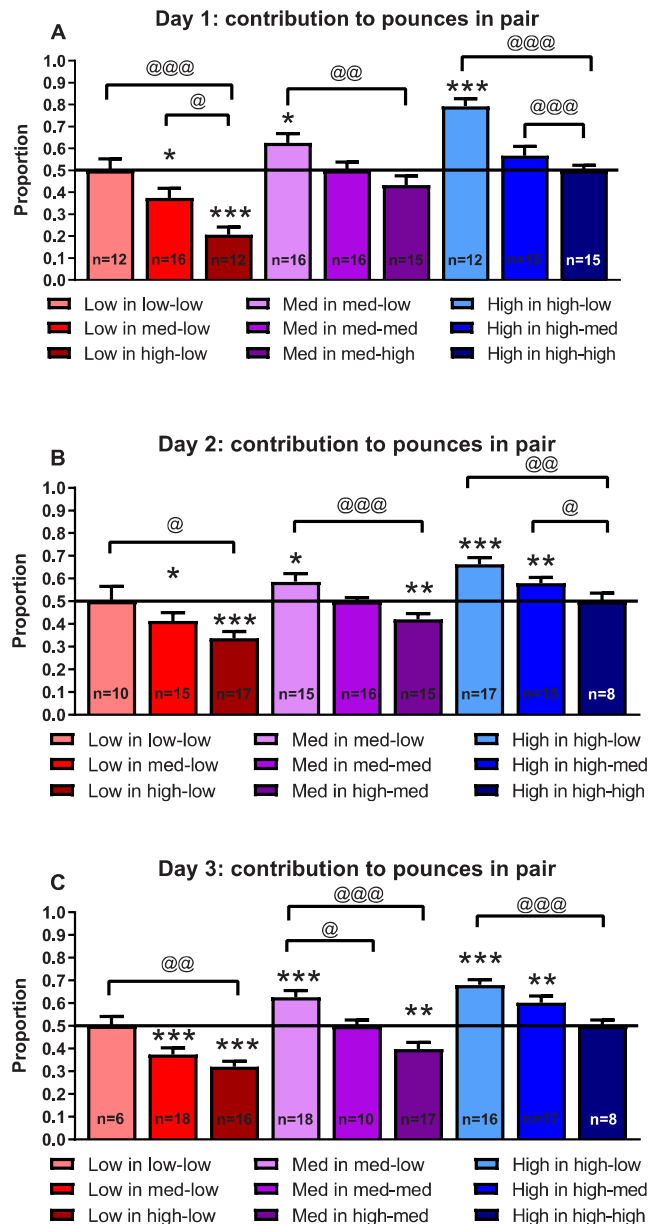


Fig. 5. Proportion contribution of pounces per animal in a pair for the three characterization days in a large sample of males. Low: low players (red bars), Med: medium player (purple bars), High: high player (blue bars). A. Day 1, B. Day 2 and C. Day 3. Four patterns can be discerned from the figures: 1) A low player always contributes significantly less pounces to the play interaction except when paired with a low player, then it is equal. 2) A medium player contributes significantly more pounces when paired with a low player, equal amounts when paired with a medium player but significantly less when paired with a high player. 3) A high player always contributes more pounces to the interaction except when paired with another high player, in which the contribution is equal. 4) The described patterns for low players are already present from test-day 1, whereas for medium and high players, this pattern emerges from day 2 onward. Data are represented as mean + SEM, *p < 0.05, **p < 0.01, ***p < 0.001, when tested against an equal contribution (proportion of 0.5). @p < 0.05, @@p < 0.01, @@@p < 0.001, within playertype differences depending on dyad composition. Indicated in the bars are the number of animals in that particular group.

did not differ across playertypes, suggesting that some rats did not play more because they were more attractive partners. An accounting of all the mechanisms by which rats come to differ in their degree of playfulness remains to be determined.

Table 4

Statistics of relative contribution of pounces per pair composition for three characterization days in male rats ($n = 126$). Low (L): low player, Med (M): medium player, High (H): high player. Low-Low: pair composed of both low players. Med in Med-High: contribution pounces of a medium player in a pair composed of a medium and a high player. The statistics used for the relative contribution in the first part of the graph are one-sample t-tests, proportions are tested against an equal proportion (0.5). t: t-test value, df: degrees of freedom. For the within playertype relative play contribution (second part of the graph) univariate ANOVAs followed by Bonferroni corrected t-tests were used when appropriate. Focal: focal rat in a certain pair composition. Significant differences are indicated in bold.

Relative contribution of play per pair composition										
Testing against 0.5	Day 1			Day 2			Day 3			
	t	df	p-value	t	df	p-value	t	df	p-value	
Low in Low-Low	0.00	11	1.00	0.02	7	0.99	0.04	5	0.97	
Low in Med-Low	-2.89	15	0.01	-2.41	14	0.03	-4.30	17	<0.001	
Low in High-Low	-8.61	11	<0.001	-5.55	16	<0.001	-7.61	15	<0.001	
Med in Med-Low	2.89	15	0.01	2.41	14	0.03	4.30	17	<0.001	
Med in Med-Med	0.00	15	1.00	0.00	15	1.00	0.00	9	1.00	
Med in Med-High	-1.57	13	0.14	-3.19	14	0.007	-3.48	16	0.003	
High in High-Low	8.61	11	<0.001	5.55	16	<0.001	7.61	15	<0.001	
High in High-Med	1.59	13	0.14	3.19	14	0.007	3.48	16	0.003	
High in High-High	0.03	13	0.98	0.00	7	1.00	0.00	7	1.00	

Within playertype relative play contribution			
Focal	Day 1	Day 2	Day 3
Low	F(2,37) = 9.98, p < 0.001 Post hoc: L-L vs L-M: p = 0.15 L-L vs L-H: p < 0.001 L-M vs L-H: p = 0.03 F(2,43) = 5.54, p = 0.007 Post hoc:	F(2,37) = 3.80, p = 0.03 Post hoc: L-L vs L-M: p = 0.50 L-L vs L-H: p = 0.03 L-M vs L-H: p = 0.40 F(2,43) = 9.61, p < 0.001 Post hoc:	F(2,37) = 5.99, p = 0.006 Post hoc: L-L vs L-M: p = 0.06 L-L vs L-H: p = 0.004 L-M vs L-H: p = 0.48 F(2,42) = 17.21, p < 0.001 Post hoc:
Med	M-L vs M-M: p = 0.99 M-L vs M-H: p = 0.007 M-M vs M-H: p = 0.80 F(2,37) = 18.98, p < 0.001 Post hoc:	M-L vs M-M: p = 0.08 M-L vs M-H: p < 0.001 M-M vs M-H: p = 0.12 F(2,37) = 6.46, p = 0.004 Post hoc:	M-L vs M-M: p = 0.03 M-L vs M-H: p < 0.001 M-M vs M-H: p = 0.09 F(2,38) = 8.22, p < 0.001 Post hoc:
High	H-L vs H-M: p < 0.001 H-L vs H-H: p < 0.001 H-M vs H-H: p = 0.51	H-L vs H-M: p = 0.04 H-L vs H-H: p = 0.001 H-M vs H-H: p = 0.11	H-L vs H-M: p = 0.11 H-L vs H-H: p < 0.001 H-M vs H-H: p = 0.08

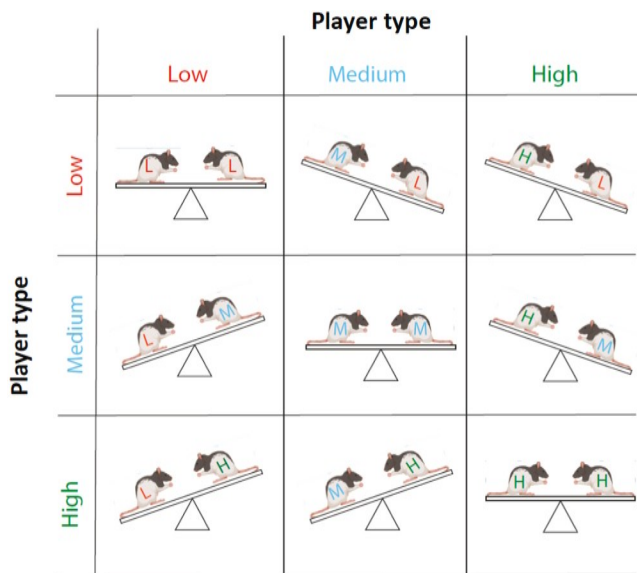


Fig. 6. A model for the homeostatic regulation of play. The schematic diagram illustrates how individuals of different player types adjust the amount of play they initiate (i.e., pounces) in response to partners of differing playfulness.

In the present study, in separate batches of male and female rats, although not statistically compared, similar patterns of individual differences in play were present, showing that female rats display discernable playertypes as well. Studies of sex differences in play suggest that not only contextual factors have a role in affecting how much play

rats engage in during a dyadic encounter, but that the upper limit of how much play they are motivated to engage in is intrinsically set. In whole litter setting males engage in more play than females (Meaney and Stewart, 1981), a finding sometimes replicated in the dyadic test paradigm (Pellis and Pellis, 1990; Smith et al., 1998). However, for the latter, the sex of the partner, rearing or test condition and length of test period can influence the presence and strength of that sex difference (Argue and McCarthy, 2015; Himmler et al., 2016a; Thor and Holloway, 1984; Bredewold et al., 2014). For example, male rats tend to initiate more play than female rats (Meaney, 1988) and when tested with scopolamine-treated target males, pounce more than do females (Thor and Holloway, 1983b). A contributing factor to the sex difference in the frequency of play per unit time arises from a neurobiological endogenous value (Meaney and McEwen, 1986; Meaney et al., 1981), established by perinatal exposure to testosterone (VanRyzin et al., 2020). That this set point is not at the maximum value is not only shown by early postnatal testosterone exposure being able to increase the play of females to the level typical of males (Smith et al., 1998; Thor and Holloway, 1983b; VanRyzin et al., 2020), but also by such exposure increasing the play of males above that typical of males (Pellis et al., 1992).

The sex difference in playfulness shows that individuals have intrinsically set preferred levels of play (Thor and Holloway, 1984), but it also shows that the identity of the partner can modify the expression of that preferred level (Argue and McCarthy, 2015). The latter is important, because pairing rats from strains with differences in preferred levels of play can also be induced to play either more or less depending on the playfulness of the partner (e.g., Reinhart et al., 2006; Schneider et al., 2016; Siviy et al., 1997). In the present study we show that in both sexes, in repeated trials, rats of a particular playertype can either increase or decrease the amount of play they initiate (see Figs. 3 and 4),

and a major factor inducing this variation is that a new partner is involved, and that partner has its own idiosyncratic level of playfulness (Lesscher et al., 2021; present study).

4.2. Preferred play level as a homeostatically controlled variable

Based on the extant literature we proposed two mechanisms (contagion vs homeostasis) by which an individual's degree of playfulness could be altered when encountering a novel partner. To distinguish between these two mechanisms, we tracked the playfulness of both partners in each of the three trials, allowing us to determine whether the variation in playfulness of rats from one trial to the next was influenced by that of its partner. If contagion was the main mechanism by which partners influenced one another, then LP rats should play more when matched with HP rats (Pellis and McKenna, 1992; Reinhart et al., 2006) either in absolute frequency or in relative contribution to the dyadic interaction. Whereas if homeostasis was the main mechanism, then LP rats should initiate more play with a LP rat and less play with a HP rat. The opposite pattern would be predicted for HP rats. Of note, the relative difference in playfulness between the rats matched could affect the strength of either mechanism, so that LP-MP and HP-MP pairs would show less contagion or regulation than LP-HP pairs. Critically, if contagion were the predominant mechanism, then HP-HP pairs should exhibit an increase in play, but if homeostasis were predominant, then such pairs should exhibit a decrease in play compared to other player-type combinations.

Contagion induced by the partner's playfulness was not obvious when investigating absolute amounts of play initiated. Indeed, the opposite pattern was evident: if one partner launched more playful attacks, the other one initiated fewer. In specific dyad combinations of playertypes, an LP rat consistently contributes significantly less to the play interaction whereas an HP rat predominantly contributes most to the play interaction, with a MP rat predominantly contributing more than a LP but less than a HP rat. The biggest differences in and most consistent contributions were found in LP-HP dyads. This pattern for LP rats is already present from day 1, whereas for MP and HP rats, the pattern emerges significantly from day 2 onward (Fig. 5).

Thus, when playing with a rat that initiates fewer playful attacks, the partner launches more, whereas, when playing with a partner that launches more, it will launch fewer. This bidirectional effect points to a homeostatic mechanism regulating dyadic playful encounters. That is, our data support the second hypothesis, that rats vary their degree of playfulness to adjust to that of the partner. This homeostatic view posits that animals have a level of play that they prefer to experience in a trial and will increase or decrease the amount of play they contribute to maintain that preferred level (Baldwin and Baldwin, 1976). Contagion may occur under some test conditions, such as when an individual from a low playing strain encounters a partner from a high playing strain (e.g., Reinhart et al., 2006), but often when encountering a low player, a high player increases the number of playful attacks it initiates (e.g., Pellis et al., 2017; Schneider et al., 2016), as was the case in the present study. Indeed, the low player, initiated less play (Fig. 5). This latter finding makes sense if the preferred level of play is being maintained in a homeostatic manner – the overall amount of play per trial remains relatively stable and to maintain this, partners modify their contribution based on the contribution of their partner (as illustrated in Fig. 6).

This homeostatic view also seems to be reflected in the vocal communication, the emission of ultrasonic calls, during play. Both when anticipating play and during play, rats emit 50-kHz calls (Burgdorf et al., 2008; Knutson et al., 1998), with some variants of these calls having been linked to signaling behavior that affects the ongoing interaction (Burke et al., 2018, 2020). When two devocalized rats are tested together, the amount of play is half as much as that of intact pairs (Kisko et al., 2015b), but when a devocalized rat is paired with an intact partner, the amount of play matches that of a pair of intact rats and the amount of calling by the intact rat doubles (Burke et al., 2018; Kisko

et al., 2015a). Similarly, hedonically depressed Kyoto Wistar rats initiate half as many pounces as do Wistar rats, and in the anticipation to play paradigm, plateau to emitting about half as many 50-kHz calls (Burke et al., 2021). Not only can play be depressed below typical levels and stimulated back to typical levels, as the contagion effect proposes (Pellis and McKenna, 1992) but also other aspects of the interaction - in this case, the associated calling - has a homeostatic component. That is, if one rat in a pair call less than expected its partner will call more, thus maintaining a 'preferred' level. We expect the same patterns to be found when pairing different playertypes together (work in progress).

That social behavior in general is maintained under homeostatic control has repeatedly been the subject of modelling and experimentation (for reviews see Matthews and Tye, 2019; Lee et al., 2021 and Bales et al., 2023). In the present paper we show that homeostasis may also explain some aspects of social play, particularly why an individual increases or decreases the amount of play it initiates when confronting a new partner.

5. Conclusion

There are individual differences in the preferred amount of play and the present paper provides evidence that these differences are consistent, present in both sexes and maintained in a homeostatic manner. However, it is also clear that value is flexible and capable of being manipulated experimentally both by altering the activity of specific neural mechanisms (e.g., Reppucci et al., 2020; Manduca et al., 2016; Achterberg et al., 2015; Trezza et al., 2008) and by changing the playful characteristics of the partner (e.g., Argue and McCarthy, 2015; Lampe et al., 2019; Reinhart et al., 2006; Schneider et al., 2016). As we come to understand the factors that can modify the play expressed by an individual at any given time better, the ability to develop paradigms to detect individual differences in preferred play activity reliably will be improved. In turn, being able to generate samples of rats that have a similar baseline in their play preference would refine current experimental protocols by reducing variability among studies conducted across laboratories that currently complicates comparison (Blake and McCoy, 2015; Pellis et al., 2022a, 2022b) and would also reduce the number of rats needed per study to be able to detect an experimental effect.

Declaration of Competing Interest

Apart from funding received from the mentioned grants, the authors declare no conflict of interest.

Data availability

Data will be made available on request.

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