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Pinning in the Play Fighting of Rats: A Comparative Perspective With Methodological Recommendations

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During play fighting, rats attack and defend the nape of the neck and during the course of this competitive interaction, they may adopt a configuration in which one animal stands over its supine partner (i.e., pin). Because the pin configuration is typically frequent and relatively easy to identify, it has been widely used as a marker to detect the effects of experimental treatments. In the present study, the frequency of pinning during standardized, 10-min trials in three strains of rats, Long Evans hooded (LE), Sprague-Dawley (SD) and wild (WWCPS), was compared. LE and SD had higher rates than WWCPS rats (#/min: 6.5, 5.5, 1.5, respectively). When adjusted for strain differences in the frequency of attacks, SD as well as WWCPS rats had lower rates of pinning compared to LE rats. Both SD and WWCPS rats were less likely to use tactics of defense that promote pinning. Moreover, while the majority of the pins achieved in LE rats arose from the defender actively rolling over onto its back, the majority of pins in WWCPS rats arose because one partner pushed the other onto its back. SD rats were intermediate in this regard. Finally, once they do adopt the pin configuration, SD rats are less likely to remain supine than LE and WWCPS rats. That is, both SD and WWCPS rats have significantly fewer pins than LE rats, but a different combination of factors account for this. These data highlight the need to use a battery of measures for ascertaining the effects of experimental manipulations on play. Some suggested guidelines are provided.

Play fighting in rats involves attack and defense of the nape of the neck, which if contacted is nuzzled with the snout (Pellis & Pellis, 1987; Siviy & Panksepp, 1987). The resulting grappling, wrestling and other maneuvering can lead to complex sequences of behavior (Pellis & Pellis, 1987; Poole & Fish, 1975, 1976). Indeed, because of this, many different schemes have been proposed to measure the frequency and content of such play (Blake & McCoy, 2015). Pinning, in which one rat is lying supine and its partner is standing on top, is a configuration that occurs frequently during play fighting, and given the high inter-observer reliability of this marker, it has been posited to be a highly reliable measure to use to score the frequency of play (Panksepp, 1981; Panksepp & Beatty, 1980), and has been widely used in neurobiological studies of play fighting in rats (e.g., Aguilar, Carames, & Espinet, 2009; Calcagnetti & Schechter, 1992; Flynn, Delclos, Newbold, & Ferguson, 2001; Panksepp, Normansell, Cox, & Siviy, 1994; Siviy, Crawford, Akopian, & Walsh, 2011; Thor & Holloway, 1983; Trezza & Vanderschuren, 2008; Varlinskaya & Spear, 2009).

Detailed analyses of play fighting sequences show that the pin configuration most often occurs when one partner maneuvers to block access to its nape and the partner presses the attack (Pellis & Pellis, 1987). That is, the pin configuration is almost invariably associated with attack and defense of the nape. Given that the pin configuration arises from the confluence of actions of both animals, there can be problems associated with interpreting an experimentally induced change in the frequency of pinning.

For example, pinning in the play of pairs of juvenile rats that have been decorticated at birth is reduced by about 50%, even though the frequency with which they initiate attacks to the nape remains about the same as pairs of intact littermates (Panksepp et al., 1994; Pellis, Pellis, & Whishaw, 1992a). In part, this decline in

the frequency of pinning can be explained by the decorticate rats preferentially using defensive tactics that make it less likely for them to be pinned (Pellis et al., 1992a). That is, a change in defense can lead to a change in the frequency of pinning. Conversely, in a comparative study of play in a strain of captive wild rats (Wild Warsaw Captive Pisula Stryjek or WWCPS) and in a commonly used strain of domesticated rats, Long Evans hooded (LE), the large discrepancy in the frequency of pinning (low in WWCPS, high in LE), could, to a large extent, be accounted for by the lower frequency of playful attack by the WWCPS rats (B. Himmler, Stryjek et al., 2013). That is, a change in attack can lead to a change in pinning. The interaction of attack and defense can become more complex in how they can change the frequency of pinning.

A study using the same housing and testing methodology as used in B. Himmler, Stryjek et al. (2013) on three other commonly used strains of domestic rats – the Brown Norway (Brown), Sprague-Dawley (SD) and Wistar - showed that the strain executing the most playful attacks (SD) had the least pins, while the other two strains had frequencies of pinning intermediate between WWCPS and LE, but with attack frequencies that were similar to LE (S. Himmler, Modlińska et al., 2014). That is, the frequency of pinning may not always be correlated with the frequency of attack. To overcome the problem that, as a play fight progresses, the actions of one animal are continually modified by the actions of its partner (Pellis & Pellis, 1987), a paradigm was developed to standardize the measure of how rats defend themselves when their nape is attacked. From the onset of an attack, the first two to three video frames are used to judge the type of defensive tactic the defender initiates, even though the continuing action of its partner may prevent the defender from completing the execution of that tactic (B. Himmler, Pellis, & Pellis, 2013).

Two main types of defensive tactics are used: evasion, in which the defender turns its head away from the attacker as it swerves, jumps or runs away, and facing defense, in which the attacker turns to interpose its face between its nape and the attacker's snout. Moreover, the facing defense can involve the rat rolling over to supine or remaining standing. In using this paradigm, it was found that the reduced frequency of pinning in decorticate rats resulted from them switching from mostly using rolling to supine, which promotes pinning, to standing defense, which reduces the likelihood of pinning (Pellis et al., 1992a). The comparisons across the five strains, however, show that this paradigm by which the type of defense is determined within the first few frames of an attack (B. Himmler, Pellis, & Pellis, 2013) does not adequately account for the differences in pinning (B. Himmler, Stryjek et al., 2013; S. Himmler, Modlińska et al., 2014).

Consideration of the three strains that were most different from one another illustrates the problem. On average, the frequency of pins in trials of 10 minutes in pairs of WWCPS is about 15, compared with about 65 for LE and about 55 for SD. Over the 10-min trials, pairs from the three strains differed in the frequency of playful attacks and since only those attacks that are defended against are likely to lead to a pin, the frequency of attacks has to be amended to the frequency of defended attacks (number of attacks x probability of defense) (Table 1). Dividing the average frequency of pins per pair by this corrected number shows that the percent of attacks leading to pins was highest in LE rats and equally low in SD and WWCPS rats (Table 1). The number of pins accounted for by rolling over to supine when rolling over to supine was the first tactic deployed, can then be calculated. This can be done by multiplying the probability of using rolling over as the first defense by the number of defended attacks, then dividing this number by the frequency of pins. These calculations show that rolling over when first attacked accounts for a majority of pins in LE rats and a very small percentage in SD rats (Table 1). Clearly, SD, and to a lesser degree, WWCPS, are being pinned less often than would be expected by the frequency of nape attacks, especially when compared to LE rats. Similarly, while the first used defensive tactic accounts for the majority of pins in LE rats, this accounts for fewer pins in the WWCPS and SD rats. Thus, neither the frequency of attack nor the defense used in the first instance is able to explain all the differences across strains with regard to their frequencies of pinning. As a reviewer pointed out for one of our recent papers (S. Himmler, Modlińska et al., 2014), perhaps we should assess what happens in the frames after

the beginning of the first defensive action. In this study, we take this suggestion to heart to identify what may explain these large differences, across strains, in pinning.

Table 1

The relative rates of pinning based on frequencies of attack, defense and types of defense used are shown for the three strains of rats.

Play measures*	LE rats	SD rats	WWCPS rats
Attacks/10min	85	140	50
Defended attacks/10min	80.8	140	40
Percentage of pins/defended attacks	80.5	39.3	37.5
Percentage of pins resulting from initial use of rolling over defense	57.2	15.3	42.7

*The logic of the transformations of the data is described in the text.

Videos of the male pairs of WWCPS, LE and SD rats from our previous studies (B. Himmler, Stryjek et al., 2013; S. Himmler, Modlińska et al., 2014) were re-analyzed to assess how rats ended up in the pin configuration. We chose these three strains and used males because subsequent studies confirmed that these strain differences were robust (B. Himmler et al., 2015; S. Himmler, Lewis, & Pellis, 2014). The findings of the present study suggest that not only should the measurement of play fighting be standardized across laboratories (Blake & McCoy, 2015), but that the currently used methodologies involving the measurement of nape contacts and pins (Panksepp, 1981; Panksepp & Beatty, 1980) and nape attacks and types of initial defense (B. Himmler, Pellis, & Pellis, 2013; Pellis et al., 1992a) need to be modified to make that standardized paradigm more widely applicable.

Method

Videotaped sequences of dyadic play trials in male LE, SD and WWCPS rats were obtained from our library of data that had been collected from two previous studies (B. Himmler, Stryjek et al., 2013; S. Himmler, Modlińska et al., 2014). The LE and SD rats were derived from commercially bought animals, whereas the wild rats (WWCPS) used were derived from five independent colonies of wild rats in Warsaw, Poland, where they were bred from two to five generations in the laboratory (Stryjek & Pisula, 2008). Six pairs of 30-35 day old rats were used for each strain, with each pair housed in a separate cage. All strains were tested in 50 cm x 50 cm x 50 cm Plexiglas boxes, lined with approximately 1-2 cm of bedding. Pairs were habituated to the testing enclosure for 30 min per day for three consecutive days, then, preceding testing, the pair mates were housed in isolation for 24 h. For testing, the pair mates were placed in the test enclosure and videotaped in the dark using cameras with night shot capability for 10 min (for further procedural details see B. Himmler, Pellis, & Pellis, 2013). The minor differences between housing and handling between the two laboratories (Lethbridge and Warsaw) were shown not to be sufficient to account for the strain differences (S. Himmler, Modlińska et al., 2014). Moreover, the same pattern of difference between LE, SD and WWCPS rats, especially in the males, has been replicated in subsequent studies (B. Himmler et al., 2015; S. Himmler, Lewis, & Pellis, 2014), and the pattern seen in these studies for LE rats is consistent with many previous studies using LE rats (e.g., Pellis & Pellis, 1990; Pellis, Pellis, & Dewsbury, 1989; Pellis et al., 1992a). Therefore, we are confident that the data from B. Himmler, Stryjek et al. (2013) and S. Himmler, Modlińska et al. (2014) are representative of these strains.

It is the differences in pinning reported in those two studies that led to the present analysis of how pins arise in these strains. It should also be noted that weight differences between pair mates was around 5% and there were no systematic biases in weight asymmetry in any particular strain, making it unlikely that the strain differences in pinning was an artifact of size differences in pair mates. Nonetheless, individual differences between pair mates may contribute to differences in patterns of pinning (Panksepp, Siviy, & Normansell, 1984; Pellis & McKenna, 1992), and how they may do so in relation to strain differences should be examined in future studies.

The studies from which the current data were obtained involved six pairs each of LE and SD rats, but more than six pairs of males of WWCPS rats (B. Himmler, Stryjek et al., 2013; S. Himmler, Modlińska et al., 2014). Therefore, to equalize the sample sizes, only six pairs of WWCPS rats were used. Given that all pairs of LE and SD rats met the criterion of having the minimum number of 10 pins per 10-min sample and the wild rats have the lowest absolute frequency of pins, to avoid a selection bias, the first six male pairs of WWCPS in the experimental series that met this criterion were used. To make the selection of pins random in LE and SD pairs, both of which had higher absolute levels of pinning, the first ten pins from each pair of each strain were used. For each sequence, the point in the video clip in which a pin (as defined by Panksepp, 1981) was clearly present was identified. The video was then rewound to the point at which the attack by the partner had begun (as defined in B. Himmler, Pellis, & Pellis, 2013). Frame-by-frame analysis was used to identify and characterize how the pin ensured. In addition, several temporal measures were scored related to the onset and duration of each pin.

Behavioral Measures

Tactics of attack and defense leading to pins. Pins can result from one animal rolling over to supine by rotating around its longitudinal axis cephalocaudally (i.e., head to rump), so as to avoid an attacker contacting its nape. Conversely, the defender may remain standing on all four of its feet or only on its hind feet, facing the attacker head on or laterally. From these standing positions, the defender may end up on its back as it is pushed over, done either quickly or incrementally, by the attacker (Pellis & Pellis, 1987). Therefore, pins were scored for whether they involved the partner ending up on its back by rotating to supine (i.e., roll over pins) or being pushed over (i.e., pushed pins). All 10 pins from each pair in all pairs from each strain were scored for whether the pins were roll over or push pins. To compare across strains, the proportion of roll over pins relative to the total number of pins was calculated.

Latency measures associated with pinning. Some of the differences in pinning across strains may arise from what the different rats do following their initial defensive response to a nape contact. For example, in all rats, pinning may arise mostly from rolling over, but with differences in how quickly after a nape attack they do so. Our standardized scoring scheme only takes into account the first 2-3 video frames (B. Himmler, Pellis, & Pellis, 2013), which would not capture a roll over that occurred on the fourth frame or later. That is, all strains may use the rolling over defense at comparable rates, but this would be missed in our standardized scoring scheme if the time of onset of the tactic differed among the strains. Similarly, analysis of age-related changes in rolling over to supine in LE rats show that when they first start using this tactic it can be quite slow, taking six or more frames to complete, with the mature form taking no more than three frames (Pellis & Pellis, 1997). Our standardized scoring scheme would count a quickly achieved supine position as a roll over, but not a slow one, so potentially underestimating the use of roll over defenses across different strains. Differences in the speed of rolling over may contribute to strain differences in how pins are achieved. Finally, differences across strains may arise from differences in how long they remain in the supine position. For example, rolling over to supine as a defensive tactic in aggressive encounters in mice was underestimated because, unlike rats, once a mouse blocks a bite to its rump by rolling to supine, it immediately rolls back to prone and flees. The transitory roll over to supine may be too short a time to warrant being scored as a supine defense (Pellis, Pellis, Manning, & Dewsbury, 1992b). Differences across strains of rats in how long they remain in the supine position may similarly have influenced the likelihood that they are scored as pins. Therefore, three measurements were taken for those cases in which pinning arose from the defender rolling over to supine: (1) the latency to begin to rotate following the onset of an attack, (2) the latency to be fully supine once rotation began, and (3) the duration of remaining supine in the pin configuration. These were measured as the number of frames from beginning to end and these values were then converted to seconds (s) or milliseconds (ms), as appropriate, for graphical representation.

(i) Latency to begin to rotate to supine following the onset of an attack. The time between the start of a nape attack to the start of a roll over was measured. The frame at which the attacking animal makes its final lunge to attack the defending animal's nape determines the start of the attack. This is also the frame preceding the one in which the first defensive movement by the defending animal occurs. Once this frame is identified, the number of frames until the frame in which the defending animal makes its first rotatory movement was recorded.

(ii) Latency to be fully supine once rotation began. This begins with the frame in which the defending animal makes its first rotatory movement and ends with the frame in which the animal ceases rotating. In some cases, animals will roll fully onto their backs, while others may remain partially tilted to one side. Therefore, the frame marking the last rotational movement to the supine position was utilized as the end of this measurement as it signals that the animal has rotated as far as it will go. A rat that is tilted to one side may rotate further so as to be fully supine, but this is only so if its partner pushes or reaches further to access its nape.

(iii) Duration of the pin configuration. The number of frames a defending animal spends on its back once it has fully rotated was measured. This measure begins with the frame in which the defending animal ceases rotating (see above) and ends when the defending animal makes its first movement that leads to it resuming the standing position.

Statistical analyses

One-way analyses of variance were used to compare across the strains with Scheffe's post hoc tests for pairwise comparisons in cases in which an ANOVA was significant. Significance was set at $p \leq .05$. To compare within strain differences, matched sample t-tests were used and to compare the variability in measures, the C-statistic was used to compare differences in the coefficient of variation ($CV = SD/M$ x 100; Lehner, 1996).

To ensure that the measurements across strains were consistent, an assessment of inter-observer reliability was conducted. One observer (S. M. H.) scored all the video sequences. A subset of those sequences, two pairs from each strain, were rescored by another observer (B. T. H.). For the proportion of pins arising from being pushed was compared using the Spearman's rank correlation and for the measures of duration Pearson's correlation was used. The correlation coefficients were significant ($r_s = .92$; $r > 0.90$; $p < .05$) indicating a high level of reliability. Therefore, the data presented are those collected by the original observer.

Results

There were significant strain differences in the percentage of roll over pins, $F(2, 17) = 21.36$, $p < .01$, with follow-up pairwise comparisons showing that all strains significantly differed from one another: LE > SD > WWCPS (Figure 1). That is, while the overwhelming majority of pins in LE rats arose by the performer rolling over to supine to defend its nape, the majority of pins in the WWCPS rats arose from one pair mate being pushed over.
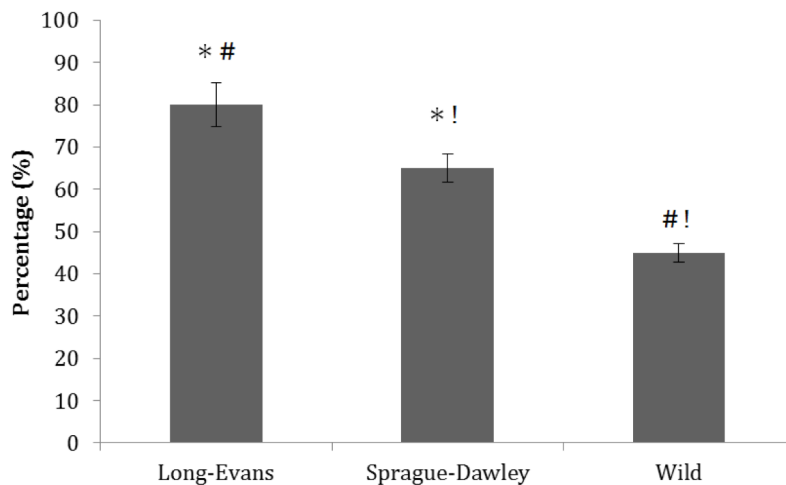

Figure 1. The mean and standard error are shown for the percentage of pins, among the three strains (Long-Evans (LE), Sprague-Dawley (SD) and wild (WWCPS), that arise from the defender actively rotating to the supine position. A one-way ANOVA revealed a significant group difference with follow-up pairwise comparisons using the Scheffe test showing significant differences between all strains ($p < .05$). The symbols at the top of the graphs, *, # and !, designate the significant paired comparisons.

However, over the course of a trial, there is a gradual decline in the likelihood of pinning (Manduca, Campolongo et al., 2014), and given the marked strain differences in pinning rates, the temporal distribution of the pins over the course of the 10-min samples could bias the likelihood of which type of pin occurred. For example, in strains in which pins are frequent, such as in LE rats, most of the sample of 10 pins may have been

obtained early in the trial, when play motivation was high, whereas, in WWCPS rats in which pinning is rare, more of the pins could have been obtained later in the trial when play motivation is lower. To test for this possibility, the frequency of pins arising from being pushed in the first five pins sampled was compared to that in the last five pins. For all strains, the frequency of being pushed over into the pin configuration did not differ significantly over the course of the sample (Table 2). These findings suggest that the strain differences in how pins arise (Figure 1) are unlikely to be fully explained as an artifact of how pins were sampled, although future studies should test this possibility more vigorously.

Table 2

The frequency of pins arising from being pushed over is compared for the first and second half of the pin samples for the three strains.

| Strain/part of sample	First 5 pins	Last 5 pins	Matched pairs *t*-tests
LE rats	1.00 + 0.45	1.00 + 0.26	0.00 (ns)*
SD rats	2.17 + 0.31	1.50 + 0.56	0.83 (ns)
WWCPS rats	2.67 + 0.21	2.83 + 0.40	-0.28 (ns)

*The *t*-value is given with *ns* indicating no significant difference.

Given the differences in agility in the three strains (B. Himmler, Stryjek et al., 2013; S. Himmler, Modlińska et al., 2014), how pushing led to pinning varied. The most common situation in the SD rats was for one rat to push laterally against the flank of its partner while maneuvering its head and neck so as to access its partner's nape. In contrast, in the WWCPS rats, the most common situation followed the defender evading by jumping forward and rapidly turning to face its attacker. The original attacker typically pounced on the evading defender, but if the defender turned around in time it was positioned to catch the attacker in mid-air and knock it to the ground. Both of these actions were present in LE rats, even though pinning from being pushed was infrequent in this strain.

Even though how the majority of pins arose differed across strains, they all used cephalocaudal rotation to get into the supine position for at least some pins, and when it occurred, all three strains could have used this tactic in the same manner. Because out of the pins that were sampled, there were such large differences in the frequency of rotation, all pins could not be compared across strains. The smallest number of roll over pins for WWCPS rats was four for a particular pair. Therefore, for comparative purposes, the first four rotation-derived pins in all pairs from all strains were used. As within-strain comparisons revealed no significant differences, for the cross-strain comparisons, all four roll over pins per pair were used to ensure that as much of the variability as possible could be included.

The latency to begin to rotate following the onset of an attack did not differ significantly, $F(2, 71) = 2.62$, $p = .08$, although there was a trend with SD rats being the outliers (Figure 2A). Indeed, the highest values for the LE and WWCPS rats were 0.77 s and 0.83 s, respectively, whereas the highest for SD rats was 3.5 s. Comparison of the CV values between strains showed that the SD rats (174.26%) were significantly more variable than the LE rats (104.67%; $C = 2.37$, $p < .05$) and WWCPS rats (94.08%; $C = 2.81$, $p < .05$). Thus, in at least some cases, the SD rats seemed more resistant to begin to rotate. However, once rotation began, there were no significant differences among the strains ($M \pm SE$ ms duration: LE = 190.0 0 ± 35.32; SD = 260.50 $\pm$ 13.73; WWCPS = 210.00 $\pm$ 17.10; $p > .05$). In contrast, there was a significant strain difference in the length of time spent in the pin configuration, $F(2, 71) = 4.14$, $p = .02$), with pairwise comparisons showing that LE > SD, but the WWCPS rats did not differ significantly from either the LE or SD (Figure 2B). Overall, LE rats were likely to remain in the pin configuration for longer. Comparison of the CV values between strains revealed no significant differences among the strains for either the duration of rotating to supine or for the duration of pins ($p > .05$).

A. Latency to begin rolling to supine
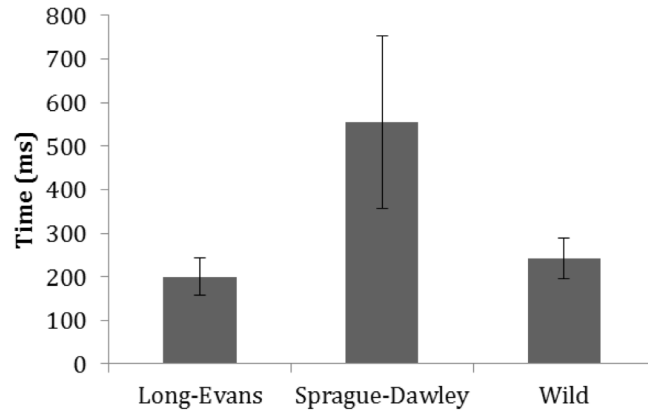
B. Duration of pinning
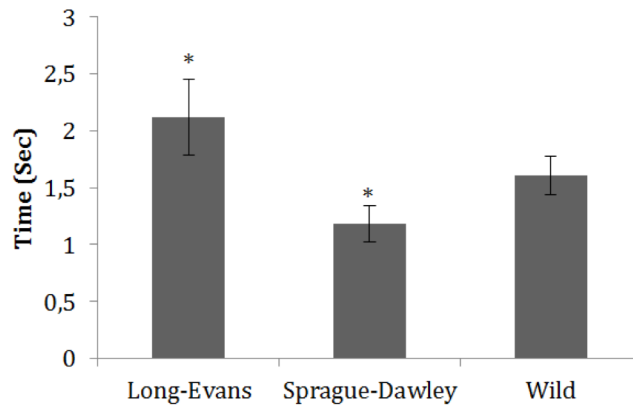
Figure 2. **The means and standard errors are shown for two of the measures of latency among the three strains.** (A) The latency to begin rotating from the onset of attack (ms) and (B) the duration of time spent in the pin configuration (s). An ANOVA did not reveal a significant group difference for the latency to begin rotation, but did reveal a significant group difference for the duration of pinning once pinned. For the latter, follow-up pairwise comparisons using the Scheffe test showed that LE > SD ($p < .05$), but WWCPS did not differ from either strain ($p > .05$). The symbol * designates the significant paired comparison.

It is possible that rats that end up on their backs due to they themselves initiating a rotation to gain that position behave differently once on their backs compared to when they are pushed over onto their backs. To test this possibility, the duration of push pins were compared to roll over pins. As one pair of LE rats did not have any instances of pins resulting from pushing only five pairs of this strain contributed to this analysis. Also, LE rats had the fewest push pins (see Figure 1), with several pairs only having two such pins, thus setting the maximum number that could be compared across pairs and strains. The first two push pins and the first two roll over pins arising were sampled from each pair in all three strains (Table 3).

Table 3

Means and standard errors are shown for the duration of pins (in s) arising from being pushed over compared to comparably sampled pins that arise from the defender rotating to supine.

Type of pin	LE rats	SD rats	WWCPS rats
Pushed pin	2.30 + 0.53	1.48 + 0.28	0.77 + 0.22
Rotatory pin	2.17 + 0.58	1.13 + 0.20	1.55 + 0.25
Matched pairs *t*-tests (2-tailed)	$t(9) = 0.16, p > .05$	$t(11) = 0.88, p > .05$	$t(11) = -2.49, p = .03$

Given that in Table 3 data across strains is independent, but within strain the data for the two types of pins is matched, a 2 x 2 ANOVA was not an appropriate statistical comparison. At a qualitative level, the values of the truncated sample of roll over pins in Table 3 are similar to those in Figure 2B, with LE having the longest duration in both. Two analyses were used to compare the durations of push pins. An ANOVA revealed a significant between group difference, $F(2, 31) = 4.87$, $p = .02$, with pairwise comparisons showing that the LE rats differed significantly from both the SD and WWCPS rats ($p < .05$), but the SD and WWCPS rats did not differ significantly from each other ($p > .05$), as was found for the duration of roll over pins (see above and Figure 2B). A within strain comparison of the duration of push pins and roll over pins using two-tailed matched sample *t*-tests showed a significant difference for WWCPS rats, but not for LE and SD rats (Table 3). WWCPS rats remained in the pin configuration longer when the animal being pinned gained that position by rotating to supine.

Discussion

For pairs of WWCPS and SD rats, only about 37% and 39% of their playful attacks, respectively, led to pinning. However, the SD rats had a higher absolute number of pins than the WWCPS rats (55 versus 15), which can be mostly accounted for by the much higher number of playful attacks launched by SD rats (Table 1), especially when that number is corrected for those attacks that led to defense. Thus, although WWCPS rats pinned less often, they pinned more often than expected for the number of attacks as compared to the SD rats. LE rats initiated more attacks than the WWCPS rats, but fewer than the SD, but had more pins than either, yielding a much greater percentage of pins than either of the other strains (about 80%; B. Himmler, Stryjek et al., 2013; S. Himmler, Modlińska et al., 2014). Therefore, these cross-strain comparisons are consistent with experimental studies showing that, in some cases, pins and nape attacks are not mutually supportive measures to estimate the frequency of play (e.g., Panksepp et al., 1994; Pellis et al., 1992a; Webber et al., 2012). This suggests these measures may reflect different underlying mechanisms. However, if all pins arise from the same combination of actions by the attacker and defender, the absolute of frequency of pinning could still reflect an experimental effect on that underlying mechanism, whatever that may be. The present paper specifically tested this latter possibility.

The most likely defensive action taken by LE rats when they are first attacked is for them to roll over, whereas both SD and WWCPS rats are more likely to evade, but if they do face their attacker, they are more likely to remain standing than to roll over (B. Himmler, Stryjek et al., 2013, 2015; S. Himmler, Lewis, & Pellis, 2014; S. Himmler, Modlińska et al., 2014). Consequently, while the first defensive action can account for nearly 60% of pins in LE rats, it accounts for about 43% in WWCPS rats and about 15% in SD (Table 1). The limitation of this method of assessing defense is that only the first defensive action in a bout of play fighting is scored (B. Himmler, Pellis, & Pellis, 2013). It is possible that rolling over to supine occurs later in the sequence in WWCPS and SD rats, so that when they do get pinned, it is because of this action by the defender. The present data do not support this possibility. Rotating to supine, wherever it occurred in a bout of

attack and defense, accounted for 80% of pins in LE rats, but 65% in SD rats and only 45% in WWCPS rats (see Figure 1). That is, for the WWCPS rats, 55% of pins, and for the SD rats 35%, arose not by the defender rolling over to supine, but from the defender being pushed over by its partner. In contrast, the vast majority of pins by the LE rats arose from an active rotation by the defender to supine. Thus, a change in the frequency of pinning could be achieved by changes in the likelihood of the defender rolling over to supine, the attacker being more forceful in pushing the defender to supine, or some combination of both. That is, a change in the frequency of pinning may not involve the same combination of mechanisms in different experimental treatments. The present results thus have implications for both the experiences gained by different strains of rats when they engage in play fighting and the methodologies that are most sensitive to detect experimental effects on play.

The Biology of Play

The pin configuration is thought to provide the opportunity to maximize body-on-body contact during play fighting - contact that has been hypothesized to be critical for the activation of the brain's pleasure circuits (e.g., Niesink & van Ree, 1989; Panksepp & Burgdorf, 1999). Moreover, it has also been hypothesized that the pin configuration provides a platform by which rats can maximize their experience of uncertainty during play. This experience has been thought to promote the beneficial effects of play on the development of the prefrontal cortex (Pellis, Pellis, & Bell, 2010) - changes that have been hypothesized to account for at least some of skills that are improved by juvenile play experience (e.g., Baarendse, Counotte, O'Donnell, & Vanderschuren, 2013; van den Berg et al., 1999; van Kerkhof, Damsteegt, Trezza, Voorn, & Vanderschuren, 2013; von Frijtag, Schot, van den Bos, & Spruijt, 2002). The data on the SD and WWCPS rats would appear not to support either hypothesis.

Not only did a large percentage of pins in WWCPS and SD rats arise from being pushed over, rather than the defender rolling over to supine, but also, when compared to LE rats, they remained in the pin configuration for a significantly shorter duration (Figure 2B). SD rats remained in the pin configuration for the least amount of time, which is consistent with their reluctance to adopt the supine position by rotating to supine. Also, revealing is that, when the duration of pins arising from being pushed onto their backs was compared, the LE rats still had the longest durations. Moreover, within strain comparisons showed that while LE and SD rats had similar durations for the two types of pins that of the WWCPS rats differed significantly (Table 3). The duration of remaining in the pin configuration was halved in rats in which pins arising from being pushed over onto their backs were compared to those which arose from rats rolling over to supine. This suggests that, at least for some strains, the quality of the pin configuration depends on how the pin is achieved.

Although the group means did not differ, for cases in which rats rolling over to supine did lead to them being pinned, the duration from the initiation of the attack to the defender beginning to rotate was more variable in SD rats (Figure 2A). Some values in SD rats exceeded 3.5 s, whereas for both the WWCPS and the LE rats, the largest values did not exceed 0.83 s. However, once rotation to supine began, the duration for them to rotate fully onto their backs was the same across strains. It appeared that SD rats only rotate to supine after they attempted all other tactics to defend their napes, and once on their backs, they regain their footing as quickly as possible. In this regard, the WWCPS rats seem intermediate to SD and LE. Thus, while the LE rats are likely, as their first tactic, to roll over to supine and then stay in this configuration for longer, the SD and WWCPS rats are not. Consequently, the experience of ventral-ventral contact would be quite different between the LE rats and the SD and WWCPS rats.

It would be of interest to evaluate the neurochemical changes in the brain's reward systems (Siviy & Panksepp, 2011; Vanderschuren, 2010) during these encounters across these strains to assess what aspects of

the interaction are the most rewarding and if these differ among strains. Certainly, selected lines of rats with diminished dopaminergic activity in the reward circuits are less playful (Siviy et al., 2011). Yet, even though SD rats gain proportionately less pins, they initiate the most attacks. Perhaps there is some threshold level of ventral-ventral contact that suffices and this threshold can be reached by increasing the frequency of attack, rather than by increasing the likelihood of rolling over to supine. But this does not account for the low values of both attack and rolling over to supine in the WWCPS rats.

During play fighting, rats emit large numbers of 50 kHz calls, which reflect positively affective states (e.g., Brudzynski, 2007; Burgdorf, Kroes, Beinfeld, Panksepp, & Moskal, 2010; Burgdorf et al., 2008; Wright, Gourdon, & Clarke, 2010). However, it should be noted that 50 kHz calls and playful actions are dissociable and there are strain differences in the frequency of emission of the vocalizations (Manduca, Campolongo et al., 2014; Manduca, Servidio et al., 2014), making the use of one measure to directly assess changes in the other, difficult. Nonetheless, by assessing when, during play fights, these are emitted most frequently (B. Himmler, Kisko, Euston, Kolb, & Pellis, 2014; Kisko, Himmler, Himmler, Euston, & Pellis, 2015), it may be possible to identify whether different facets of the encounters are perceived as most rewarding by the different strains. Thus, while a partner that actively defends itself is more rewarding to attack than one that does not (Pellis & McKenna, 1995), the differences across strains in the experiences derived from play fighting offers a venue by which to pinpoint exactly what is most rewarding during such interactions.

For playful fights to remain playful there has to be some degree of reciprocity, so that one partner does not overwhelmingly achieve the advantage (Altmann, 1962; Dugatkin & Bekoff, 2003). In rats, and some other animals, reciprocity is facilitated by the partners self-handicapping themselves when attacking and defending (Pellis, Pellis, & Reinhart, 2010). That is, once one partner gains the advantage, it restrains itself in its ability to block its partner's counterattacks, thus making it more likely that the counterattack is successful and the roles of the two animals are reversed (Pellis, Pellis, & Foroud, 2005). In LE rats, while this self-handicapping was most clearly characterized when the rats are in the pin configuration (Foroud & Pellis, 2003), the likelihood of counterattacks is just as great in other configurations – such as that following an evasion or from a standing position (Pellis et al., 1989). Indeed, the frequency of role reversals is the same for LE, SD and WWCPS rats (B. Himmler et al., 2015; S. Himmler, Himmler, Pellis, & Pellis, 2016), suggesting that, despite strain differences in the preferred tactics of defense, they play in a way that still creates the experiences of unpredictability and loss of control that have been hypothesized to be crucial for the training of emotional resilience (Špinka, Newberry, & Bekoff, 2001). Such resilience may depend on the play-induced changes to the prefrontal cortex (Vanderschuren & Trezza, 2014; Pellis, Pellis, & Bell, 2010). Again, the differences across strains in preferred tactics may provide a tool by which to identify the crucial play-generated experiences that lead to these changes in emotional regulation.

Measuring Play

From a methodological perspective, the present analysis of strain differences in how the pin configuration is achieved suggests that simply counting the frequency of pins per trial (Panksepp, 1981) does not provide sufficient information. A decrease or an increase in pins, or indeed, no change in pins, may underestimate or overestimate an experimental effect. Similarly, measuring the first defensive tactic (B. Himmler, Pellis, & Pellis, 2013) when an attack is initiated and using the frequency of rolling over to supine as a correlate of pinning may also underestimate the frequency by which encounters eventually culminate in the pin configuration. When measures of outcome (pins) and of process (rolling to supine) both converge on a similar value, then using both measures can be redundant (Siviy, Baliko, & Bowers, 1997; Siviy, Love, DeCicco, Giordano, & Seifert, 2003). However, it cannot be assumed a priori that these measures yield a comparable picture. For instance, for SD rats, given the frequency of playful attack, pins are less frequent than

expected, but more frequent than expected given the low likelihood of them using the rolling over to supine tactic (S. Himmler, Modlińska et al., 2014). Moreover, in other studies, it is the shift in the tactics of defense (B. Himmler, Pellis, & Pellis, 2013) that is the most revealing of the experimental effects (e.g., Kisko et al., 2015; Mychasiuk, Hehar, Farran, & Esser, 2014). The method used in the present paper bridges this gap.

By sampling pins for their occurrence and then moving backwards so as to identify how the rats came to be in the pin configuration, it is possible to characterize whether all experimental conditions lead to pins in the same manner. As seen in the present study, the most frequent ways in which the pin configuration is achieved by different strains differs, so it is important to know that if the frequency of pinning is reduced by a particular experimental manipulation, whether that arises because all processes by which pinning can arise are compromised or because a particular process is compromised. The effect of such compromises may be strain-specific. For example, the decrease in pinning that is present in LE rats that have been decorticated occurs because the preferred tactic of defense is changed from rolling over to supine to standing defense (Pellis et al., 1992a). It remains to be determined if the decrease in pinning in other strains that have been decorticated (Panksepp et al., 1994) arises for the same reason. Even if the same tactic were affected by decortication, if wild rats were used, given their low frequency of attacks and that only 45% of their pins arise from the defender rolling over to supine, a significant decrease in pinning may not be detected. The baseline for preferred tactics and the means by which pinning arises need to be established for the strain to be used before the effects of a particular treatment can be assessed.

To evaluate the effects of experimental treatments on play fighting in rats effectively, we recommend using a multi-layered sequence of measures. At the first level of analysis, the frequency of launching nape attacks and the frequency of pins per trial should be measured (B. Himmler, Pellis, & Pellis, 2013; Panksepp, 1981, 1998). A quick assessment of discordance can be obtained by calculating the percentage of pins given the number of attacks (e.g., Raza et al., 2015; Webber et al., 2012). Changes in this percentage relative to the control condition can alert one that something has changed – such as the lower than expected percentage of pins compared to the inflated frequency of attack in SD rats (S. Himmler, Modlińska et al., 2014). This should then lead to a second level of analysis. The preferred tactics of defense are scored by assessing what the tactic of 'choice' is when an attack first begins (B. Himmler, Pellis, & Pellis, 2013). If this accounts for the changed frequency of pins, as is the case for LE rats that have been decorticated (Pellis et al., 1992a), then this would suffice. However, if this is not the case, in that given the preferred tactics, there are still too few or too many pins, then a third level of analysis should be conducted. In this case, as done in the present paper, a random selection of pins should be chosen and the process by which they are achieved should be identified and quantified.

Finally, what animals do once they are in the pin configuration is also important and a simple clue can be derived from scoring the duration of the pin configuration. A short pin may reflect this position as a transitory action that is used to defend the play target, but quickly abandoned once this is achieved, whereas a long pin may reflect active prolongation of close body contact – as shown in the present study by the differences between the LE and SD rats. Moreover, when scoring pin durations, the observer will likely notice whether the animals are passive or squirming; this may suggest whether making finer measurements of what the animals are doing in the pin configuration is necessary. Indeed, the reduced duration of pins following being pushed to supine compared to rolling over pins in WWCPS rats (Table 3), would suggest that being pushed over makes them behave more like SD rats - that is, they are reluctant to remain pinned. Finer-grain analyses of what the pinned partner does when pinned may provide additional insight into whether pins are comparable across experimental manipulations.

For example, in the juvenile period, LE rats that are on their backs in the pin configuration are highly likely to counterattack (Pellis & Pellis, 1990), which accounts for the active wrestling that is seen when they

are in this position (Pellis & Pellis, 1987). However, as they approach sexual maturity, such counterattacks become less frequent, and the rats will remain unmoving for a larger portion of the time when in the pin configuration (Pellis & Pellis, 1990).

The proposed approach obviates the need to measure 'everything' up front only to find that, in a particular case, the added measures were uninformative (Siviy et al., 1997, 2003). Rather, each level of analysis provides clues as to whether another, finer-grained level of analysis is likely needed. For example, scoring the frequency of attacks, pins and initial tactics of defense were insufficient to explain the discrepancies in pinning rates across strains (B. Himmler, Stryjek et al., 2013; S. Himmler, Modlińska et al., 2014). As demonstrated in the present paper, another level of analysis provided a fuller account of these strain differences.

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