

## Utilization of same- vs. mixed-sex dyads impacts the observation of sex differences in juvenile social play behavior.

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### Abstract

The study of juvenile social play behavior has gained popularity due to the disruption of social behaviors in several psychiatric illnesses. In contrast to many tests currently utilized in animal models of psychiatric illness, juvenile social play behavior is part of the normal behavioral repertoire in the laboratory rat and can be observed in a controlled setting but without evocation by the experimenter. Understanding sources of naturally occurring differences in the juvenile social play behavior of the rat is a fundamental first step to guide future research on identifying factors that disrupt this behavior. One of the most commonly found variations is a sex difference, with male rats displaying higher levels of rough-and-tumble play behavior relative to females. This sex difference is also observed in human play. In our recent paper published in *Biology of Sex Differences*, we investigated how the sex and familiarity of the play partner can impact different components of rough-and-tumble play behavior (pouncing, pinning, boxing, and chasing) and the observation of sex differences within each of these components. Our findings suggest that juvenile male rough-and-tumble play behavior is impacted by the sex of their play partner, while females are more sensitive to the familiarity of their play partner. In this review, we discuss our recent findings and provide a comprehensive comparison of methodology and the reporting of sex differences in the literature on this topic.

**Keywords:** juvenile; play partner; sex differences; social play behavior

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Juvenile play behavior is a necessary component for normal social and cognitive development [1–4]. Animals that have been experimentally deprived of the opportunity for social play during the juvenile period display behavioral and neurodevelopmental deficits [3,5]. In humans, social behaviors, including play, are often atypical in individuals with psychiatric illnesses [6–10]. Children diagnosed with attention deficit hyperactivity disorder play with less intensity and skill relative to age-matched children without this diagnosis [6]. Autistic children are delayed in their development of social play relative to their peers and their solitary play does not attract the attention of other children so that it may develop into social play, as it frequently does in non-autistic children [discussed in 7]. Moreover, social withdrawal is a characteristic symptom of schizophrenia and socially withdrawn children may be at a higher risk for affective disorders [8–10]. Interestingly, the same illnesses that manifest with disrupted social behaviors also display sex differences in their prevalence [11]. Specifically, women have a higher lifetime prevalence of anxiety and depression relative to men, while boys have a higher incidence of autism, attention deficit hyperactivity

disorder, and antisocial personality disorder relative to girls [12–14]. Play fighting, or rough-and-tumble play, is the most common form of social play behavior exhibited by many species, including many popular laboratory animals [15–18]. Both humans and several species of laboratory animals display sex differences in their play behavior with males exhibiting a higher frequency and spending more time engaged in rough-and-tumble play behavior relative to females [19–28]. In laboratory rats, one of the most popular species for studies of sex differences and behavioral models of psychiatric illnesses, there is disagreement about the magnitude of the sex difference in juvenile play. These discrepancies may be largely due to variation in the experimental design [19,24]. Clarifying variations in play behavior under normal conditions will facilitate appropriate design of experiments exploring changes in this behavior in response to experimental manipulations.

The medial amygdala is a critical brain region for sexual differentiation of play behavior since implantation of testosterone capsules into this region in neonatal rats is sufficient to masculinize juvenile female rough-and-tumble

play [29]. This finding not only establishes the importance of the medial amygdala, but also provides supporting evidence that sex differences in juvenile social play are organized by the actions of steroid hormones during the neonatal period [30]. These, and several other studies of sex differences were completed in the Long Evans strain, which raises the question of whether the observed sex differences in rat play are strain specific. A recent study by Himmler and colleagues addressed this question through comparisons of sex differences in different aspects of juvenile play in commonly used strains of laboratory rats [31]. They found no sex differences in any of the strains for the number of playful attacks or in the number of pins, however there was a sex difference in Sprague Dawley rats with males demonstrating a higher probability of evading an attack relative to females and females demonstrating a higher probability of using a complete rotation defense tactic relative to males [31]. These results were different from what we had observed and in many cases were in opposition to other reports in the literature [for example 21,32]. Potential sources of variation include use of social isolation to evoke play behavior or mixed-sex groups versus same- sex pairs. Although isolation is useful to increase components of play to higher levels so that they may be analyzed in detail, some studies have suggested that group differences are not evident with an isolation paradigm because it increases play to ceiling levels [33]. Additionally, with a mixed-sex paradigm, there is concern about the confound of the contagiousness of play, meaning that animals that are naturally less playful might be spurred to increase their play by being paired with an extremely playful partner [34–39].

In our recent study published in *Biology of Sex Differences*, we investigated how sex and familiarity of the play partner can impact juvenile rough-and-tumble play in Sprague Dawley rats [40]. For this study, all animals were paired with a same- and an opposite-sex partner, the order of which was alternated daily over the 12-day observation period. One caveat to this design is that the experience with the opposite-sex partner may have carried over to the behavior observed with the same-sex partner and

*vice versa*. However, the design was chosen based on the observation that there is a great deal of individual variability in relative playfulness, making it necessary to assess play of one individual with same- and opposite-sex partners using a repeated measures analysis [40]. We divided play behavior into its constitutive components of pouncing (initiation of play behavior), pinning (a defensive strategy in which the animal that has been pounced upon rotates to a supine position with the attacker standing on top), boxing (two rats stand on their hind legs and batting at each other with their front paws), and chasing (one rat chases after the fleeing opponent). An important distinction is that defensive behaviors were scored for the initiator/attacker of the play bout even though pinning (which facilitates continuation of the play bout) and chasing (which acts to terminate the play bout), require action by both members of the dyad (the opponent must rotate to supine for pinning to occur and the opponent must run away to be chased). We also combined all components of play together to get a measure of total frequency and timed the total amount of time engaged in play behavior, which is considered reflective of play quality.

Our results are summarized in Table 1 and show that males pounce more when paired with female play partners relative to other males, but achieve more pins when paired with other males compared to when paired with females. Chasing increased for both males and females when paired with an opposite- relative to a same-sex play partner. The total time spent engaged in play behavior was greatest for male-male dyads and least for female-female dyads, with mixed-sex pairs falling in the middle. However, neither males nor females displayed a change in total play frequency as a result of being paired with a same- or opposite-sex play partner. These sex of play partner effects resulted in males displaying higher initiation of play (pouncing) relative to females only in mixed-sex dyads. Males displayed higher levels of pinning, boxing, and total play relative to females in both same- and mixed-sex pairs, and no sex differences were observed for chasing behavior. Body weight was not a confounding factor in the observed sex differences. To investigate the effect of

**Table 1: The use of same- vs. opposite-sex play partners can alter the various parameters of social play behavior and impact the observation of sex differences in rough-and-tumble play behavior.** > indicates instances in which play in the first group was higher than play in the second group, < indicates instances in which play in the first group was lower than play in the second group, and = indicates instances in which play in the two groups was not significantly different. Significant differences are in blue text.

Play Component	Partner Effects		Sex Differences	
	Males	Females	Same-Sex Dyad	Mixed-Sex Dyad
<b>Total Play Behavior</b>	Same = Opposite	Same = Opposite	<b>Males &gt; Females</b>	<b>Males &gt; Females</b>
<b>Pouncing</b>	<b>Same &lt; Opposite</b>	Same = Opposite	Males = Females	<b>Males &gt; Females</b>
<b>Pinning</b>	<b>Same &gt; Opposite</b>	Same = Opposite	<b>Males &gt; Females</b>	<b>Males &gt; Females</b>
<b>Chasing</b>	<b>Same &lt; Opposite</b>	<b>Same &lt; Opposite</b>	Males = Females	Males = Females
<b>Boxing</b>	Same = Opposite	Same = Opposite	<b>Males &gt; Females</b>	<b>Males &gt; Females</b>
<b>Time</b>	<b>Same &gt; Opposite</b>	<b>Same &lt; Opposite</b>		

partner familiarity, novel play partners were introduced on day seven of the twelve-day paradigm. The animals were re-partnered with their familiar partners on day eight. This resulted in a decrease in total play observed on day seven with the introduction of the novel partner in female-female dyads only [40].

These results may explain why sex differences do not always carry across different studies. This is particularly apparent when the focus is on initiation of play (pouncing), or pinning rather than analyzing a variety of components of play. Moreover, in studies where a new play-partner is introduced daily, females will be more affected by the

novelty than males. Our results also confirm that rough-and-tumble play behavior is contagious since mixed-sex pairs showed an intermediate time engaged in play behavior between male-male dyads, which played for the most time, and female-female dyads, which played for the least time [40].

To summarize how differences in methodology can impact whether sex differences in juvenile rough-and-tumble play behavior are observed, we have compiled a list of multiple studies in which sex differences, or the absence thereof, have been reported (Table 2). This list is not comprehensive for all studies on the topic, but does provide a wide range

**Table 2: Comparison of experimental design and the observation of sex differences in juvenile rough-and-tumble play behavior.** All mentioned differences were reported to be statistically significant in the original manuscript.

Publication	Species	Ages Studied	Isolation Protocol	Partner	Cage	Findings
Argue and McCarthy. 2015 [40]	Sprague Dawley	PN27-38	none	Same-Sex, Non-Littermate, Non-Cagemate Pairs	Novel	Males had higher total play, pinning, and boxing frequencies than females. Male pairs spent more time engaged in play than female pairs.
				Opposite-Sex, Non-Littermate, Non-Cagemate Pairs		Males had higher total play, pouncing, pinning, and boxing frequencies than females.
Beatty and Costello. 1983. [41]	Albino	PN27-41	none	Mixed-Sex, Cagemate Groups	Home	Males initiated more play than females and trended towards higher play fighting frequency.
Beatty et al. 1981. [42]	Albino	PN31-40	none	Mixed-Sex, Cagemate Groups	Home	Males initiated more play and had higher total play frequency than females.
Birke and Sadler. 1983. [43]	Wistar-derived	PN22-43	none	Same-Sex, Littermate, and Cagemate Groups	Home	Males played more than females.
Bredewold et al. 2015. [44]	Wistar	PN31-32	2 days	Same-Sex, Unfamiliar Partner	Not Stated	No sex differences
Edelmann et al. 2013. [45]	Sprague Dawley	PN26-29	none	Mixed-Sex, Cagemate Groups	Home	Males played more than females.
Götz et al. 1991. [46]	Wistar	PN26-44	none	Mixed-Sex, Cagemate Groups	Home	Males played more than females.
Himmler et al. 2014. [31]	Brown Norway	PN35	24 hours	Same-Sex, Cagemate Pairs	Novel	No differences in total play or play initiation.
	Sprague Dawley					No differences in total play or play initiation. Males were more likely to evade than females. Females were more likely to use a rotation to supine defense tactic than males.
	Wistar					No differences in total play or play initiation. Males were more likely to evade than females.
Jessen et al. 2010. [47], Kurian et al. 2008. [48], and Olesen et al. 2005. [49]	Sprague Dawley	PN25-29	none	Mixed-Sex, Cagemate Groups	Home	Males played more than females.
Lukas and Wöhr. 2015. [50]	Wistar	PN40	none	Same-Sex Pairs	Novel	Males spent more time playing than females.
Meaney and McEwen. 1986. [29]	Long Evans	PN26-40	none	Mixed-Sex, Cagemate Groups	Home	Males played more and initiated more play than females.

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Meaney and Stewart. 1981. [51]	Long Evans	PN21-55	none	Mixed-Sex, Cagemate Groups	Home	Males initiated more play than females PN26-55, but not PN21-26.
Meaney and Stewart. 1981. [30] and Meaney et al. 1983. [52]	Long Evans	PN26-40	none	Mixed-Sex, Cagemate Groups	Home	Males played more and initiated more play [29] than females.
Meaney et al. 1981. [53] and Meaney et al. 1982. [54]	Holtzman Albino	PN26-40	none	Mixed-Sex, Cagemate Groups	Home	Males played more and initiated more play than females.
Northcutt and Nguyen. 2014. [55]	Long Evans	PN30-33	none	Same-Sex, Cagemate Partner	Home	No sex differences
Oloff and Stewart. 1978. [21]	Sprague Dawley	PN22, 26, 30, 40	none	Same-Sex, Non-Cagemate Pairs	Novel	Males spent more time playing than females
Panksepp and Beatty. 1980. [56]	Holtzman Albino	PN25-30	none or 24 hours	Same-Sex Unfamiliar Pairs	Novel	No sex differences
Parent and Meaney. 2008. [23]	Long Evans	PN37-51	none	Mixed-Sex Pairs	Home	Males pounced and pinned more than females.
Paul et al. 2014. [57]	Wistar	PN35-36	none	Same-Sex Partner	Novel	No sex differences
Pellis and Pellis. 1990. [58]	Long Evans	PN31-35	24 hours	Same- or Mixed-Sex Pairs	Novel	Male pairs had more play fights than mixed-sex pairs or female pairs, while mixed-sex pairs had more play fights than female pairs. Males initiated more play than females. Males and females were more likely to defend against a male attacker than a female attacker. Males were more likely to evade than females. Females were more likely to use a supine defense than males.
Poole and Fish. 1976. [59]	PVG/C	PN25-35, 35-49	none	Mixed-Sex, Cagemate Groups	Home	Males were more playful than females.
Takahashi and Lore. 1983. [60]	Long Evans	PN24-90	none	Same- or Mixed-Sex, Littermate Pairs	Novel	Male pairs were more playful than female and mixed-sex pairs at younger ages, however male pairs were less playful after PN54.
Thor and Holloway. 1982. [61]	Long Evans	PN32-38	several days	Stimulus Male	Novel	Males engaged in more pinning and wrestling than females.
Thor and Holloway. 1984. [33]	Long Evans	PN36	30min	Stimulus Group-House, Non-Littermate Female	Home	No sex differences in pinning frequency or duration.
			24 hours			No sex differences in pinning frequency or duration.
			15 days			No sex differences in pinning frequency or duration.
			24 hours			No sex differences in pinning frequency or duration.
PN30-35	24 hours	Stimulus Group-Housed, Non-Littermate Male or Female	No sex differences in pinning frequency or duration.			
PN35-37	6 days	Males pinned more and had longer pinning duration than females.				
Veenema et al. 2013. [62]	Wistar	PN35	2 days	Unfamiliar	Home	No sex differences

of rat strains, ages, play partner paradigms, and isolation protocols for comparison. Although all of the reported differences are for the control animals from that study, it is important to note that many of the studies involved experiential, surgical, or pharmacological manipulations resulting in control animals that received different levels of handling, sham surgeries, vehicle drug administration, or been housed with or paired for play with an animal that received some sort of experimental manipulation. As is evident from Table 2, and as previous research reports have specifically investigated, it is possible to alter play initiation without impacting play defense and *vice versa* [39,40]. Analyzing as many components of play behavior as possible is the best practice to detect variations. For other aspects of experimental design, it is impossible for all researchers to adhere to the same guidelines. Certain studies may require that a specific rat strain is used, that animals be individually housed prior to play to recover from a surgery, that animals be paired with a like-treated partner so that ultrasonic vocalizations can be recorded without needing to decipher which animal is vocalizing, etc. What is important for this field to move forward is for researchers to understand how these variations impact play so that they can most accurately interpret their findings. Observations regarding natural variations in play under different circumstances are a necessary first step to determine manipulations that produce aberrant social play behavior.

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