USING TINBERGEN'S APPROACH TO UNDERSTAND PLAY

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ABSTRACT

Play fighting has been proposed to provide animals with experiences that promote behavioural flexibility, which helps them to successfully navigate their physical and social environments later on in life. However, there is much variation in how closely related species play, indeed, even among different strains of rats aspects of their play differ. Is all this variation adaptive in serving play's critical developmental role? The integrative approach advocated by Tinbergen, suggests not. Among rat strains, the differences in play are simply byproducts of subtle sensorimotor differences. Irrespective of the form of the play, rats of all strains converge on the same key experiences during play that are useful for promoting the development of the prefrontal cortex. That is, the experiences derived from play, despite variations, leads to the same outcome. The lessons learnt from rats serve as a guide for cross-species comparisons.

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LIST OF ABBREVIATIONS

SD	Sprague-Dawley rats
LE	Long-Evans
WWCPS	Wild Warsaw Captive Pisula Stryjek rats
BN	Brown Norway rats
W	Wistar rats
ANOVA	Analysis of variance

Chapter 1: The Structure and Function of Play Fighting in Rats

1.1 Abstract

Studies on rats have revealed that experience with social play in the juvenile period is important for the refinement of the functioning of the prefrontal cortex, whose role in executive function is critical for attending to important stimuli, impulse control and decision making. But there is much variation in how closely related species play, indeed, even among different strains of rats play can differ markedly. Is all this variation adaptive in serving play's critical developmental role? The integrative approach advocated by Tinbergen, suggests not. Among rat strains, the differences in play are noise, byproducts of subtle sensorimotor differences. Irrespective of the form of the play, rats of all strains converge on the same key experiences during play that are useful for promoting the development of the prefrontal cortex. The lessons learnt from rats serve as a guide for cross-species comparisons.

1.2 Introduction

Navigating complex social networks requires animals to be attentive to the identity of their partners (e.g., dominance, sexual status, etc.), to the actions that those partners are performing (e.g., invitation to groom, threat, etc.), as well as having a repertoire of appropriate behaviours to deal with any given encounter (e.g., submission, genital inspection, etc.). The complexity of social systems can vary with regard to the nuances in identity, salient actions and the range of allowable responses. Macaques provide an illustrative model. These Old World monkeys comprise about 20 species that have a wide distribution, from the temperate mountains of Northern Africa, the deserts and forests of Central Asia, the rainforests of Southeast Asia, to the cold mountainous forests of Japan (Thierry & Kaumanns, 2004). They all share a common social system, comprising multi-male, multi-female troops. The females are philopatric and are organized in matrilines - that is, networks of related females: grandmother, mothers and aunts and their dependent young. The males leave the natal troop in late adolescence and join new troops. The females form an age-based dominance hierarchy within matrilines, and among matrilines, there is also a dominance hierarchy. The males form their own dominance hierarchy that is independent of that formed by the females (Chapais, 2004). In this complex social network, dominance relationships form the organizing structure for inter-animal interactions, but there is a telling variation across species.

The different species of macaques sit on a gradient of tolerance, where, at one extreme, the dominance relationships are maintained very rigidly, and at the other, they are only loosely applied (Thierry, 2007). For example, among Japanese macaques, at the intolerant extreme, a dominant can take food from or strike a subordinate with impunity, but in Tonkean macaques, at the other extreme, while dominants can act this way some of the time, any given case could lead to the subordinate retaliating (Thierry, 1985). This uncertainty as to how much abuse a subordinate is willing to take from a dominant monkey creates a more complex style of social living. To be effective in such a tolerant social system, individuals have to be able to attend to more subtle cues about a partner's likely course of action and have a more nuanced repertoire for dealing with partners. Rough-and-tumble play, or play fighting, provides an avenue for negotiating social nuances.

In social systems in which relationships are not rigidly structured, adults can use play fighting with one another as a means of assessing and manipulating their relationships (Brueggeman, 1978; Pellis & Iwaniuk, 1999b, 2000), and this can be done both within and between troops (e.g., Palagi 2006, 2008; Antonacci, Norscia, & Palagi 2010; Norscia & Palagi, 2011). Not surprisingly, adult-adult play is frequent in Tonkean macaques, but infrequent in Japanese macaques (Ciani, Dall'Olio, Stanyon, & Palagi, 2012). That is, in social systems in which there is uncertainty about the ability to exercise power, animals can use play fighting as a non-aggressive way to test and probe partners, and so ascertain how far the other animal can be pushed before it may retaliate (Pellis, 2002a). The advantage of using play in this manner is that, if the partner begins to retaliate, there is a greater chance of diffusing the situation by signaling that, "after all, it was only play" (Pellis & Pellis, 2009). In this way, play can be used to navigate complex social relationships without needing to resort to strategies of avoidance or all out fighting – play affords animals a more peaceful route via which to pursue their social goals (Pellis, 2002a; Pellis & Iwaniuk, 2004). But play is not only used in such utilitarian ways among older animals, it is also used in the juvenile period to train one with the skills needed to deal with complex social systems that require attention to more subtle exchanges (Pellis & Pellis, 2009, 2011).

Play fighting is inherently ambiguous, in which a strike can be forcefully delivered, but still be playful. A partner needs to monitor the force of the blow, the immediate context in which it occurs and the history of prior actions by the same partner – one strong blow too many may lead to aggressive retaliation, but such escalation is rare (Pellis & Pellis, 1998a). Animals follow rules of engagement during play that allow for sufficient reciprocity so that the interaction can continue to be perceived as playful by both partners (Altmann, 1962; Dugatkin & Bekoff, 2003; Pellis, Pellis, & Reinhart, 2010b). In the event that it is unclear whether a particular action is playful or not, there is always the option, for those species that possess them, to use play signals to de-escalate an encounter (Aldis, 1975; Bekoff, 1995; Pellis & Pellis, 1996; Demuru, Ferrari, & Palagi, 2014). Nonetheless, in any given play fight, each animal has to monitor the actions of their partner, evaluate patterns over time and match these against their own actions – this is the kind of monitoring and decision making that taxes and trains the executive functions of the prefrontal cortex (Diamond, 2013; Diamond, Barnett, Thomas, & Munro, 2007). The unpredictability that occurs during play and the need to adjust rapidly to fast-paced events may be crucial in training animals to be resilient and adaptable in the face of uncertainty (Špinka, Newberry, & Bekoff, 2001). In this context, it may not be surprising that juvenile Tonkean macaques more frequently engage in play fighting that is more unpredictable than do Japanese macaques (Reinhart, Pellis, Thierry, Gauthier, VanderLaan, Vasey, & Pellis, 2010).

1.3 Tinbergen and the 4-whys

The seminal paper by Tinbergen (1963) made the case that to understand behaviour fully, four different questions need to be answered: what is its adaptive function, what mechanisms produce it, how did it evolve and how does an individual acquire it? Tinbergen not only stressed the need to answer each question, but also the need to integrate these into a cohesive framework – what are the mechanisms that can be co-opted to achieve particular functions and how can those mechanisms be modified over development and evolutionary time to refine those functions? In the past few decades, our understanding of the play fighting of rats has expanded (e.g., Panksepp, 1998; Panksepp & Biven, 2012; Panksepp, Siviy, & Normansell, 1984; Pellis & Pellis, 1998b, 2009;

Siviy, 2010; Siviy & Panksepp, 2011; Vanderschuren, 2010; Vanderschuren, Niesink, & van Ree, 1997; Vanderschuren & Trezza, 2014), providing insight into all of Tinbergen's whys.

During the juvenile period, the experience of play and other social interactions, refine a variety of social, emotional and cognitive skills (e.g., Arakawa, 2002, 2003, 2007a, b; Einon & Morgan, 1977; Einon, Morgan, & Kibbler, 1978; Hall, 1998; van den Berg, Hol, van Ree, Pruijt, Everts, & Koolhaas, 1999; Von Frijtag, Schot, van den Bos, & Spruijt, 2002). Thus, at a functional level, play fighting in juvenile rats has been implicated in improving a range of skills crucial for navigating complex social environments. With regards to the mechanisms by which such refinement occurs, a critical one appears to be by improving the executive functions of the prefrontal cortex (Baarendse, Counotte, O'Donnell, & Vanderschuren, 2013), and this is achieved, in part, by modifying the neural circuits involved (Bell, Pellis, & Kolb, 2010; B. T. Himmler, Pellis, & Kolb, 2013b). Studying play fighting in rats also provides a clue as to how the experience of play induces these changes in the brain.

Play fighting in rats involves the attack and defense of the nape, which is nuzzled with the snout if contacted (Pellis & Pellis, 1987; Siviy & Panksepp, 1987). To protect the nape from playful attack, rats can use one of two types of tactics. The defender can turn away from the oncoming attacker by taking evasive action, such as swerving or running away, or, alternatively, the defender can turn to face and block the attack by wrestling with the attacker. When turning to face their attacker, the defender can either rotate onto its back (supine defense) or use a variety of tactics that involve remaining standing on one or both of its hind paws (standing defense) (Pellis & Pellis, 1987).

Facing defenses are considered to be more complex because rats must be able to coordinate their movements to a greater degree with those of their partner (Bell, McCaffrey, Forgie, Kolb, & Pellis, 2009; Pellis, Pellis, & Dewsbury, 1989).

Supine defense, compared to standing defense, involves relinquishing some of the control the defender has over its partner. When standing upright, the defender has more options for further blocking the attacker's maneuvers, such as kicking with its ipsilateral hind foot, delivering a hip-slam or rearing up and pushing with its forepaws - all actions that put the partner on the defensive and so increases the performer's chance for successfully counterattacking with a lunge to the nape of its own. While the supine position is effective in blocking further attempts to contact the nape by the attacker that is standing on top, the ability to launch successful counterattacks from this position are more limited (Pellis & Pellis, 1987).

The likelihood of using the supine defense to protect the nape is increased in the juvenile period (Pellis & Pellis, 1990, 1997). This may be a way for a defender to increase its experience of loss of control, and so unpredictability. This possibility is supported by the finding that, also at this age, the rat standing on top of its supine partner will engage in actions that, in turn, reduces its ability to control the movements of its partner (Foroud & Pellis, 2002, 2003), and so increase the likelihood that the supine partner can launch successful counterattacks (Pellis, Pellis, & Foroud, 2005). That is, both the actions of the defender and the attacker increase their respective loss of control over their subsequent actions and those of their partner, creating the unpredictable experiences that seem to be important for training the executive functions of the brain (Pellis, Pellis, & Bell, 2010a)

During adulthood, rats, especially males, use play fighting to negotiate dominance relationships. Rat colonies consist of multiple males and females with one male becoming dominant (Barnett, 1963; Calhoun, 1962; Flannelly & Lore, 1977). However, all subordinates will preferentially engage the dominant male in affinitive interactions, including allogrooming and play (Adams & Boice, 1989; Pellis & Pellis, 1991; Pellis, Pellis, & McKenna, 1993). Subordinate males will respond in a more juvenile-typical manner when playfully attacked by a dominant, so that they will be more likely to roll over to supine; whereas when a subordinate attacks a dominant, the dominant will be more likely to defend in a more adult-typical manner by remaining standing (Pellis & Pellis, 1991; 1992). Within a colony, subordinates may increase how roughly they play (i.e., by using standing defense more) to test the dominant for weakness, and so potentially reverse the relationship (Pellis et al., 1993). Similarly, when confronting one another in a neutral enclosure, unfamiliar males from different colonies will initially play roughly, then one will either assume the subordinate status and begin to roll over, or they will escalate the encounter into serious fighting until one becomes dominant (Smith, Fantella, & Pellis, 1999). That is, by increasing or decreasing the roughness of play, rats can use the encounter either to reinforce existing dominance relationships or seek to reverse them (Pellis, 2002a).

These assessment and manipulative functions of play fighting involve the same neural circuits of the prefrontal cortex that are modified by play and associated social experiences in the juvenile period. Rats that have been reared with peers and are then subjected to lesions of the prefrontal cortex will exhibit deficits in modulating playful actions appropriate to the dominance status of their partner (Pellis, Hastings, Shimizu, Kamitakahara, Komorowska, Forgie, & Kolb, 2006) and will have difficulty in coordinating complex actions with another rat (Bell et al., 2009; B. T. Himmler, Bell, Horwood, Harker, Kolb, & Pellis, 2014). That is, rats with such brain damage show similar social deficits to those seen in rats that have been deprived of social partners and so play, in the juvenile period (Moore, 1985; Pellis, Field, & Whishaw, 1999; van den Berg et al., 1999).

The example of play fighting in rats, then, provides insight into how two of Tinbergen's questions – function and mechanism – are integrated (Pellis et al., 2010a; Pellis & Pellis, 2011). But not all species of rodents that engage in play fighting as juveniles have nuanced social interactions as adults (Pellis & Iwaniuk, 2004) and nor are cognitive functions improved in all cases (Einon, Humphreys, Chivers, Field, & Naylor, 1981; Einon & Morgan, 1977). This leads us to Tinbergen's third question - the one that asks about the evolutionary history of a trait – why is play in the functional forms seen in rats present in this lineage, but not in others?

It is now clear that play, of any sort, is relatively rare in the Animal Kingdom – so far it has only been identified in some species from 5 of the 30 or so Phyla (Burghardt, 2005). Even within one limited clade, the Order Rodentia, within which rats reside, not all species engage in play fighting, and some in no form of play at all (Fagen, 1981). Of those that do, the complexity of that play fighting can vary dramatically (Pellis & Pellis, 1998b; Pellis & Iwaniuk, 1999a). Some engage in simple approach and withdrawal, in which the defense only involves evasion; in others, the play can involve wrestling, in which the defense involves turning to face. Of those that engage in play fighting involving wrestling, this can vary both qualitatively and quantitatively – that is, if present, play fighting can range from simple to increasing levels of complexity (Pellis & Pellis, 2009). To date, the training and the social navigation functions of play have only been demonstrated for rats (Pellis & Pellis, 2009, 2011). No doubt there are other rodents that will likely be found for which play has comparable functions, but what is clear is that there are rodents that do not have these functions (Pellis & Iwaniuk, 2004).

More interesting with regard to Tinbergen's why question of phylogenetic history is that at least some members of other mammalian orders appear to have functions of play fighting comparable to rats. First, about 50% of primate species use play fighting as adults for social assessment and manipulation (Pellis & Iwaniuk, 1999b, 2000, Palagi, 2011). Second, in humans and at least in some Old World monkeys and apes, play seems to function during the immature phase as it does in rats – to facilitate and refine the development of social skills and associated executive functions (e.g., Kalcher-Sommersguster, Preuschoft, Crailsheim, & Franz, 2011; Kempes, Gulickx, van Daalen, Louwerese, & Sterk, 2008; Pellegrini, 1995). That is, in several lineages, play has been transformed in a manner that its functions have converged. Thus, even though rats and macaques have play fighting that originated from different sources - the former from sex, the latter from aggression - it has been transformed in a way so as to serve the function of refining executive function. This pattern of transformation, revealed by cross-species comparisons, provides a means of integrating mechanism and function with phylogenetic history (Pellis, Pellis, & Himmler, 2014). But what about the fourth why – how is development integrated with the other whys?

From the material already reviewed, it is clear that experiences gained from play in the juvenile period refine the brain mechanisms needed to use play effectively in adulthood as a tool for social assessment and manipulation. Given that in terms of function, play in the juvenile period provides fitness enhancing experiences, how is this juvenile-typical, mature form of play developed from precursors earlier in infancy? However, before being able to answer that question, a digression is necessary.

1.4 Diversity in play fighting: what do strain differences among rats tell us?

While there are some reports of play in wild rats (e.g., Ewer, 1971; Robitaille & Bovet, 1976), most of what we know about the play of rats is derived from studies of domesticated laboratory strains (e.g., Panksepp et al., 1984; Pellis & Pellis, 1998b; Siviy & Panksepp, 2011; Thor & Holloway, 1984; Vanderschuren et al., 1997). However, laboratory rats have been domesticated for over a century and domestication involves animals being selectively bred so that they can adapt to the conditions of captivity. Domesticated animals are typically bred to be more docile, making them easier to handle and better able to be housed with conspecifics in smaller living spaces (Blanchard & Blanchard, 1994; Castle, 1947; Lockard, 1968; Trut, 1999). While the affinitive, aggressive and sexual behaviour of domesticated rats is similar to that of wild rats (Blanchard, Flannelly, & Blanchard, 1988; Price, 1980; Whishaw & Whishaw, 1996), domesticated laboratory rats have a shorter flight distance (Blanchard, Flannelly, & Blanchard, 1986), are less motorically competent (Whishaw, Gorny, Foroud, & Kleim, 2003) and their senses are less acute (Prusky, Harker, Douglas, & Whishaw, 2002) than wild rats. That is, the changes in brain and behaviour produced by the domestication process may mean that the mechanisms and functions characterized for play fighting in domesticated laboratory rats may be a byproduct of domestication, not inherent characteristics of play.

In addition, there are multiple strains of domesticated laboratory rats with diverse origins (Castle, 1947) that differ in a variety of behavioural and physiological traits (e.g. Castle, 1947; Lockard, 1968; Pisula, Turlejski, Stryjek, Nałęcz-Tolak, Grabiec, & Djavadian, 2012; Takahashi & Blanchard, 1982). Indeed, strains from different selection regimes differ in how playful they are and in how they play (e.g. Reinhart, Pellis, & McIntyre, 2004, Reinhart, McIntyre, Metz, & Pellis, 2006; Siviy, Baliko, & Bowers, 1997; Siviy, Crawford, Akopian, & Walsh, 2011; Siviy, Love, DeCicco, Giordano, & Seifert, 2003). Such variation among strains raises the issue of whether the findings from one strain can be generalized to other strains.

Using a standardized testing and scoring paradigm (B. T. Himmler, Pellis, & Pellis, 2013a), we compared the play fighting of four laboratory strains (Brown Norway, Long Evans hooded, Sprague Dawley and Wistar) to wild rats (B. T. Himmler, Stryjek, Modlinska, Derksen, Pisula, & Pellis, 2013c; S. M. Himmler, Modlinska, Stryjek, Himmler, Pisula, & Pellis, 2014b). In all strains, over 90% of play fights start with a nape attack. The frequency of nape attacks differs significantly with wild rats initiating fewer playful attacks than all the domesticated strains and of the domesticated strains, Sprague Dawley rats initiating the most. However, the likelihood of defending against a nape attack does not differ among strains. With regard to the tactics of defense, all strains use all types, but there is a significant difference in the likelihood of their use. Wild and Sprague Dawley rats use evasive tactics more often than do the other strains. Of the turning to face tactics, the complete rotation tactic (i.e., turning to supine) is used most often by Long Evans rats and least often by Sprague Dawley rats.

The picture that emerges is that domestication has had a uniform effect in increasing playfulness. However, with regard to the tactics of defense, the direction of change from the wild type varies markedly across domesticated strains. There are well established neural circuits associated with the motivation to play (Siviy & Panksepp, 2011; Vanderschuren, 2010), and some of the variation in playfulness across domesticated strains arises from differences in the functioning of this circuitry (Siviy et al., 2011). The same may apply to strain differences in the use of different defensive tactics.

Long-Evans rats, with lesions to the motor cortex, attack and defend the nape at similar frequencies as do intact rats, but display a large decrease in the use of supine defense and an increase in the use of evasive tactics (Kamitakihara, Monfils, Forgie, Kolb, & Pellis, 2007). That is, damage to the motor cortex changes Long Evans to make their defense more like that of Sprague-Dawley rats. Moreover, our preliminary data show that the dendritic arbor of the basilar field of the neurons of the motor cortex are significantly longer in Sprague-Dawley (SD) than in Long Evans (LE) rats (mean $\pm$ standard deviation: 81.19 ± 15.61 for SD versus 60.85 ± 8.94 for LE; $t(10) = 2.770$, $p = 0.020$) (see Bell et al., 2010 and B. T. Himmler et al., 2013a for methods of analysis). It is possible that, with domestication, the motor cortex has been changed in differing ways relative to that present in wild rats, yielding strain differences in their preferences for using different tactics of defense. However, before jumping to this conclusion, a simpler possibility needs to be considered: even though all strains attack the nape, different strains may differ in the speed and orientation of their attack, creating postural differences that make different tactics more effective in some strains relative to others.

Postural and contextual factors may be sufficient to produce strain or species differences without modifying brain mechanisms (Barrett, 2011; Blumberg, 2005; Pellis, 1997).

To test this possibility, juvenile Long Evans and Sprague Dawley rats were tested with unfamiliar juvenile Long Evans and unfamiliar juvenile Sprague Dawley partners. Irrespective of whether attacked by a partner of the same strain or of a different strain, Long Evans rats maintained their preference for supine defense and Sprague Dawley rats maintained their preference for evasive defense (S. M. Himmler, Lewis, & Pellis, 2014a). That is, strain typical preferences for particular tactics are not byproducts of strain differences in body morphology and style of playful attacks. The preference for particular tactics appears to arise from strain differences in a neural bias for those tactics. A surprising finding supports this conclusion. In a variant of the experiment described above, quads of rats, which included two Long Evans and two Sprague Dawley rats, were housed together, from shortly after weaning at 24 days to the juvenile period when the frequency of play is at its peak (30-40 days: Thor & Holloway, 1984). Instead of maintaining their strain-typical pattern as predicted, they changed their preference so that both Long Evans and Sprague Dawley rats had a frequency of evasion and supine defense that was intermediate between that typical of the two strains (S. M. Himmler et al., 2014a). That is, strain-typical preferences in defense tactics involve brain-based biases, but those biases can be changed by social experiences.

Irrespective of these variations in the frequency and content of play across strains, the experience of play in the juvenile period has been shown to influence the development of the prefrontal cortex in several strains (e.g., Long Evans, Lister hooded, Wistar) (e.g., Baarendse et al., 2013; Bell et al., 2010; B.T. Himmler et al., 2013b). Such common effects on brain development would suggest that the key experiences derived from play should be similar across strains, irrespective of the strain differences in the frequency of play and in the defensive tactics used.

As explained above, during the juvenile period, both the attacker and the defender use maneuvers that facilitate losing control over the situation, which, in turn, facilitates the ability of the partner to gain the upper hand, albeit transiently. These maneuvers facilitate role reversals, in which the defender successfully attacks the original attacker (Pellis et al., 2005). Since for play to remain playful interactions need to be reciprocal (Pellis et al. 2010b), the role reversals can be used to assess reciprocity (B.T. Himmler, Himmler, Stryjek, Modlinska, Pisula, & Pellis, 2015; Kisko, Himmler, Himmler, Euston, & Pellis, 2015). In rats, play fights occur in bouts lasting between 3-5 seconds, before the partners stop, or, more usually, move apart, and then resume with a new attack and defense (Pellis & Pellis, 1987; Poole & Fish, 1976). Some bouts involve a role reversal (Pellis et al., 2005). Therefore, two measures can be used to evaluate role reversals: (1) the probability that a bout will include a role reversal and (2) the absolute frequency of occurrence of role reversals in a test trial. If play fighting in the different strains has a similar function, then, irrespective of the differences in their use of defense tactics, they should all have a similar likelihood of experiencing role reversals.

The ten min play trials from B. T. Himmler et al. (2013c) and S. M. Himmler et al. (2014b) were re-analyzed to calculate role reversals. There were no significant differences in the probability of role reversals across strains ($F(4,35) = 2.308$, $p = 0.77$) (Figure 1.1A), but there was a significant group difference for the total number of role reversals ($F(4,35) = 2.759$, $p = 0.043$) (Figure 1.1B). Pairwise comparisons show that this difference arises because Sprague Dawley rats have significantly more role reversals than wild rats ($p < 0.05$), but neither differed significantly from the other strains ($p > 0.05$). This difference is due to the high frequencies of playful attack by the Sprague Dawley rats and low frequencies by the wild rats.

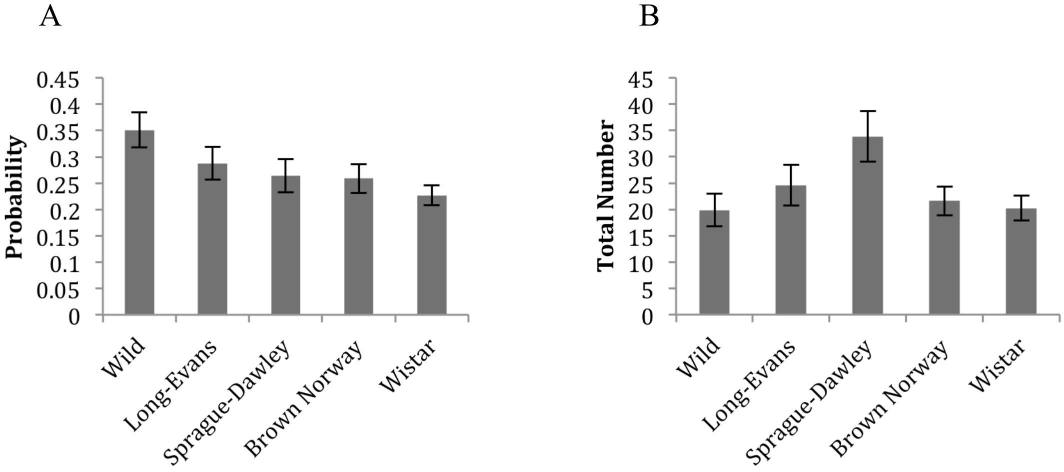

Figure 1.1 Measures of role reversals during play fighting show (A) the probability that a play fight will include a role reversal and (B) the total number of role reversals that occurred in the ten min play trials for the five strains of rats. Data are derived from B. T. Himmler et al., 2013c and S. M. Himmler et al., 2014b and are for males. As females showed the same pattern, in this paper, for simplicity's sake, the data on males are used to illustrate the patterns.

Given that all strains experience levels of role reversals that are at the same level, or exceed, that of Long Evans and Wistar rats, two strains that show a play-induced change in the anatomy and functioning of the prefrontal cortex, it would seem likely that the play of all of them would be capable of providing the experiences necessary to influence the development of the prefrontal cortex and associated executive functions. (Pellis et al., 2010a, 2014). Therefore, while the differences among strains may not diminish the functional uses of play fighting, they do highlight the possibility that experiences preceding the juvenile period may be important in shaping some aspects of the play present in juveniles.

1.5 Development: Pre-juvenile influences

Play fighting achieves its mature form, capable of influencing the development of the prefrontal cortex, in the juvenile period (Bell et al., 2010; B. T. Himmler, 2013b). The juvenile period is defined as the age at which young animals are nutritionally independent, which in mammals occurs at weaning, to when they become sexually mature (Pagel & Harvey, 1993). Although standard laboratory practice is to wean rats between 21-23 days after birth, when left together with their mothers, weaning is a gradual process beginning in the third week after birth, when the young open their eyes and ears and start ingesting solid food. Then, over the following week, the frequency of lactation declines, until the young are fully independent of maternal milk at around 34 days (Cramer, Thiels, & Alberts, 1990; Thiels, Alberts, & Cramer, 1990). Play between peers begins between 15-18 days of age (Baenninger, 1967; Bolles & Woods, 1964; Thiels et al., 1990; Pellis & Pellis, 1997), but does not achieve its completely mature juvenile form until 28-30 days (Pellis & Pellis, 1997). With regard to the development of play, infancy can be divided into two phases: the first two weeks (early infancy: birth-14 days) when no play occurs, and the following two weeks (late infancy: 15-30 days) when play matures.

1.5.1 Early infancy

For the first two weeks after birth, rats are completely dependent on their mother for food, warmth and for the evacuation of waste (i.e., urine, feces) (Bolles & Woods, 1964; Cramer et al., 1990; Stern, 1996). Mothers do not play with their infants, but they do intensely interact with them; they pick pups up in their mouths to carry them to new locations, they feed them by standing over them in an arched posture allowing the young to suckle from beneath and they lick and groom them, especially around the anogenital area (Stern, 1996). Licking and grooming of the young by the mother, as well as handling of the infants by experimenters can affect the frequency of play fighting exhibited by juveniles (e.g., Aguilar, Carames, & Espinet, 2009; Edelmann, Demers, & Auger, 2013; Moore & Power, 1992; Parent & Meany, 2008; Siviy & Harrison, 2008) These effects on play may be indirect, as maternal licking and grooming also affect the development of the stress-response system (Meaney, 2001) and neurochemical systems that are involved in regulating social behaviour, such as oxytocin and vasopressin (Veenema, 2012). Indeed, maternal licking and grooming has been show to influence the development of various aspects of social, sexual and maternal behaviour (e.g., Cameron, Sharhrokh, Del Corpo, Champagne, & Meaney, 2008; Parent, Del Corpo, Cameron, & Meaney, 2013). Where such early maternal influences on play have been demonstrated, it has been on its frequency, not in how the juveniles play.

1.5.2 Late infancy

Not only do peer-peer interactions involving play begin and mature in this period, but interactions with the mother also continue (Bolles & Woods, 1964; Cramer et al., 1990; Stern, 1996; Thiels et al., 1990), so that both could influence the development of the mature juvenile pattern of play. Manipulating the age of weaning or the amount of pre-weaning exposure to the mother can affect the frequency of play in the juvenile period (e.g., Arnold & Siviy, 2002; Birke & Sadler, 1987; Ikemoto & Panksepp, 1992; Janus, 1987; Karkow & Lucion, 2013; Moore & Power, 1992; Shimozuru, Kodama, Iwasa, Kikusui, Takeuchi, & Mori, 2007; Veenema & Neumann, 2008). As is the case for maternal influences on play arising from interactions in early infancy, maternal influences in late infancy seem to affect how playful rats are in the juvenile period, not how they play.

From its first appearance, peer-directed play involves targeting the nape. However, the tactics used to defend against nape contact are not used in their juvenile typical frequencies until about 30 days of age. Moreover, when they first appear, the form of the tactics are also immature – for example, supine defense initially takes over 60 msec to execute, but does not reach its fluid form, taking only 30 msec, until between 28-30 days (Pellis & Pellis, 1997). Similarly, the quality of play experienced in the later stages of this period can alter some aspects of the form of juvenile play fighting, such as the strain-typical preferences for the defense tactics (S. M. Himmler et al., 2014a), and social skills more generally (Green, Barnes, & McCormick, 2013). These experience-based changes in how play is organized would suggest that, in late infancy, the development of the juvenile-typical form of play should be highly dependent on the experiences derived from playing with peers. This does not appear to be the case.

Rats that are reared without peers during late infancy, and so are devoid of peer-peer play experience, develop their juvenile-typical pattern of play fighting by the time they are 30 days or so (Foroud, Whishaw, & Pellis, 2004; B. T. Himmler et al., 2015).

This is the case whether rats are reared in complete isolation for only a limited portion of this period (23-28 days) - the same age in which our earlier study found that cross-strain rearing changes the strain-typical, juvenile-typical preferences for defense tactics (S.M. Himmler et al., 2014a) - or when reared alone, only with their mother or with peers and no mother over a longer period (15-28 days). Comparison of the effects of these non-play and mismatched play rearing conditions for aspects of both attack and defense are revealing (Table 1.1). The motivation to play in rats is increased when they are reared as isolates or when reared with peers and without a mother, but not when reared alone with a mother. Lack of experience with peer play does not diminish the role of the nape as the target of attack. However, the accuracy with which the nape is contacted is diminished. But this is true whether rats are reared with siblings alone, with play occurring, or with the mother alone, where no practice occurs. This suggests that the social influences on play at this age are more likely to affect motivational factors that impinge on execution, rather than directly on improving execution by practicing attacks. This conclusion is supported by the finding that the vigor of launching attacks is greater in both the juveniles reared only with mothers and those reared only with peers (B. T. Himmler et al., 2015). Most strikingly, in the absence of practice, the strain-typical preferences of juveniles for using particular defense tactics emerge unscathed (Table 1.1). That is, the rats do not need to experience the execution of immature versions of defense tactics for them to mature into the juvenile-typical patterns.

Table 1.1 The effects of the absence of peers and/or mothers during the late infancy period and the effects of neonatal decortication on the development of juvenile-typical play fighting are shown for various measurements of playful attack and defense.

	Total Playful Attacks	**Probability of Defense**	**Probability of Supine Defense**	**Probability of Evasion**	**Aim**	**Role Reversals**
Mix-housed*	–	–	⬇	⬆	–	–
Social Isolates**	⬆	–	–	–	–	–
Mother-only reared**	–	–	–	–	↓	–
Sibling-only reared**	⬆	–	–	–	↓	–
Decorticates***	–	–	⬇	⬆	–	–

*From S. M. Himmler et al., 2014b, **from B. T. Himmler et al., 2015, ***from Pellis et al., 1992b
Symbols: Arrows show the strength (➔= greater effect; → = smaller effect) and direction of difference to the respective control group, and the minus sign shows

1.6 All Roads Lead to Rome

Exposure to a rearing environment involving atypical peers – members of a strain that has different preferences for using particular tactics of defense – has the effect of altering the juvenile-typical use of those tactics (S. M. Himmler et al., 2014a). Given that the absence of play partners has no effect on the development of juvenile-typical patterns of playful defense (Table 1.1), the alteration in the pattern of preference by playing with both same and different strain peers over this age seems puzzling.

Behaviour that emerges in the absence of the need for functional practice has been termed ‘prefunctional’ (Hogan, 2001). Dust bathing, which is used by birds to clean their feathers by passing grit through them, illustrates this concept. For example, in chickens, it involves six distinct behaviour patterns that are executed in a specific sequence. Over a two-week period following hatching, these different patterns emerge with the order of appearance being the same as the order in which they are executed in the mature sequence. Whether the chicks are raised on a wire mesh floor or on a substrate containing particles (e.g., sand, soot, wood shavings), the order of appearance and the sequence in which they are used when all are present is the same (Vestergaard, Hogan, & Kruijt, 1990). That is, chicks do not need the functional feedback of having actual particles pass through their feathers. However, even though there is no functional feedback, chicks do experience repeated performances of the dust bathing actions.

In mammals, there are five to six distinct types of righting that are triggered by particular sensory stimuli, such as vestibular, tactile and visual ones. In adults, these have an order of priority as to which is executed when more than one releasing stimulus is present (Magnus, 1926; Pellis, 1996). Rats can successfully right themselves from the day of birth and over the next 3 weeks, the different types of righting emerge in an overlapping order, so that, as the next one becomes available, it can be co-activated with the preceding one if the releasing stimuli for both are present. Eventually, as all types of righting become fully matured, the adult-typical priorities are established (V. C. Pellis, Pellis, & Teitelbaum, 1991). Marsupials are born at an immature state and at birth they make their way to the mother's pouch where they attach themselves to a teat. The attachment involves a growth of skin around the mouth, securing an unbreakable hold on the teat that is not released until the infant is mature (Nelson & Gemmell, 2004). In the marsupial cat, righting reflexes do not begin to emerge until about 20 days after attachment and do not develop the adult-typical pattern until over 40 days later while the infant is still attached to the teat. The order of appearance of the types of righting and the establishment of which ones have priority when multiple releasing stimuli are present follow the same order of emergence as in rats (Pellis, Pellis, & Nelson, 1992a). In rats, a reverse pattern of righting is used to move to a supine position under the lactating mother, and then use the righting patterns available to return to prone when they have finished suckling (Eilam, Goffman, & Smotherman, 1999; Eilam & Smotherman, 1998). That is, from birth, rats use the immature forms of righting in a functional manner. In the case of the marsupial, not only is the functional experience of righting not present, but the opportunity to perform the righting movements is also absent; clearly, righting in the marsupial develops prefunctionally.

In the pre-juvenile period, the absence of the need for functional feedback from playing with peers to develop the juvenile-typical pattern of play (Table 1.1) suggests that the development of play fighting, like dust bathing and righting, is prefunctional. So why the altered pattern of playful defense in rats housed with different strain peers (S. M. Himmler et al., 2014a)? One possibility is that the development of play proceeds in the same manner with or without actually having play experience, but the system is malleable when confronted with play experiences that are atypical. That is, the system wires itself up in the same way if there is no feedback or if the feedback confirms the pattern, but if the feedback is at odds with the expected pattern, then the system is wired up differently. There is precedent for prefunctional and functional patterns of development working together.

Chicks begin to peck at small objects that are contrasted against the background from the day they hatch and then, over days, their accuracy in striking the object improves. Just as one's accuracy in shooting a gun or a bow at a target improves with practice, the feedback from the failed pecks may similarly be the basis for the improved striking accuracy. In an experiment, chicks were outfitted with a leather helmet and goggles, but the goggles had refracting lenses, so that the object being looked at was displaced in the visual field. The chicks were placed on a large tray with a substrate of plasticine on which nails with flat heads were embedded. The nail heads provided for the chicks contrasting targets at which to peck and the tip of their beaks left impressions in the plasticine. Chicks without the helmets, or with helmets without the refracting lenses, showed that, day-by-day, the pecks were clustered more tightly around the nail head – their accuracy improved. The chicks wearing helmets with refracting lenses showed the same pattern of tighter clustering over time, but because of the visual displacement produced by the lenses, the cluster was several centimeters away from the nail head. That is, the improvement did not come from feedback from the strikes, but rather, arose from sensorimotor motor maturation that progressed independently of the pecking (Hess, 1956). This experiment shows that the development of more accurate pecking is prefunctional.

In another experiment, chicks were hatched in an incubator without light. Then, every day, some were taken out and tested for their accuracy in pecking at wheat grains on the floor by counting the number of misses. The chicks that were tested for the first time on day one after hatching made more errors than those tested for the first time on day 2 and so on, so that it was clear that, over the five day period, there was an improved accuracy independent of previous experience with visually guided pecking, confirming the prefunctional emergence of pecking. However, irrespective of the day of first testing, each chick was tested daily over the duration of the experiment. This revealed that the rate of improvement was faster for the chicks that were tested more often. That is, experiential feedback enhanced the age-related improvement in accuracy (Cruze, 1935). Thus, pecking accuracy develops prefunctionally, but performance can be improved further by functional feedback.

For the pre-juvenile development of play fighting in rats something similar may apply, with the rats' developmental system following the rule of "develop according to plan unless confronted with styles of play that do not conform, then adjust the pattern of play to match the prevailing social conditions". This makes sense from the perspective of the adaptive value of juvenile play. The experiences derived from play are critical for refining the development of brain mechanisms that are needed for executive functions (Pellis et al., 2010a; Vanderschuren & Trezza, 2014). Ensuring that the pattern of play providing those experiences adapts to the vagaries of the social partners available provides insurance that the essential experiences are forthcoming. The sensitivity to the social environment to modify play is highlighted by the development of sex differences.

Generally, male rats engage in play fighting more frequently than do females, and this sex difference is contingent on exposure to male gonadal hormones in the perinatal period (Meaney, 1988; Pellis, 2002b). However, the presence and the strength of this sex difference differs across studies, with the strongest sex difference emerging when rats are reared in mixed sex groups from weaning onwards and the least when housed alone or only with same sex partners (e.g. Beatty, Dodge, Traylor, & Meaney, 1981; Meaney, Dodge, & Beatty, 1981; Meaney & Stewart, 1981b; Olioff & Stewart, 1978; Panksepp, 1981; Pellis & Pellis, 1990; Poole & Fish, 1976; Thor & Holloway, 1983). While some of this variation across studies may be due to strain differences (Reinhart et al., 2004, 2006) and the testing paradigms used (Thor & Holloway, 1984), testing and scoring in the standard way that we have developed in our laboratory shows that multi-animal, mixed sex groupings enhance the development of sex differences (Pellis & Pellis, 1990), while housing with a single, same sex partner diminishes them (B. T. Himmler et al., 2013c; S. M. Himmler et al., 2014b). The sensitivity to these social settings may be adaptive, ensuring that the amount of play experienced over the juvenile period remains the same irrespective of the available play partners. Similarly, the changes in the use of defense tactics when housed in mixed strain groups (S. M. Himmler et al., 2014a) may adjust the form of the play to ensure that the important experiences to be derived from play are present.

Recall that the most critical experiences derived from play fighting appear to be those that create unpredictability (Špinka et al., 2001; Pellis et al., 2010a), and that this unpredictability is enhanced in the juvenile period by interjecting movements that make it easier for the opponent to gain the advantage (Pellis et al., 2005). This is reflected in the occurrence of role reversals (B.T. Himmler et al., 2015; Kisko et al., 2015). As shown for the wild and domesticated strains of rats, irrespective of differences in preferred defensive tactics, they experience comparable levels of role reversals (Figure 1.1). If the adjustments in the preferences for defensive tactics is adaptive, then it should be the case that the rats housed in mixed strain groups should have rates of role reversals comparable to those of rats housed with partners of the same strain. Analyzing the videotaped material of those rats (S. M. Himmler et al., 2014a) revealed exactly that – that the probability of role reversals was the same (t(10) = -1.352, p = 0.206) (Figure 1.2A), as was the absolute frequency of their occurrence (t(10) = 1.127, p = 0.286) (Figure 1.2B). In fact, none of the rats that were reared atypically (i.e., isolated, alone with their mother, with peers and no mother) showed any differences in the number of role reversals as compared to their control groups (Table 1.1). This suggests that experience with play in late infancy is not needed to develop the mechanisms that make role reversals possible. Given that in the juvenile and post-juvenile periods play fighting is modified contextually

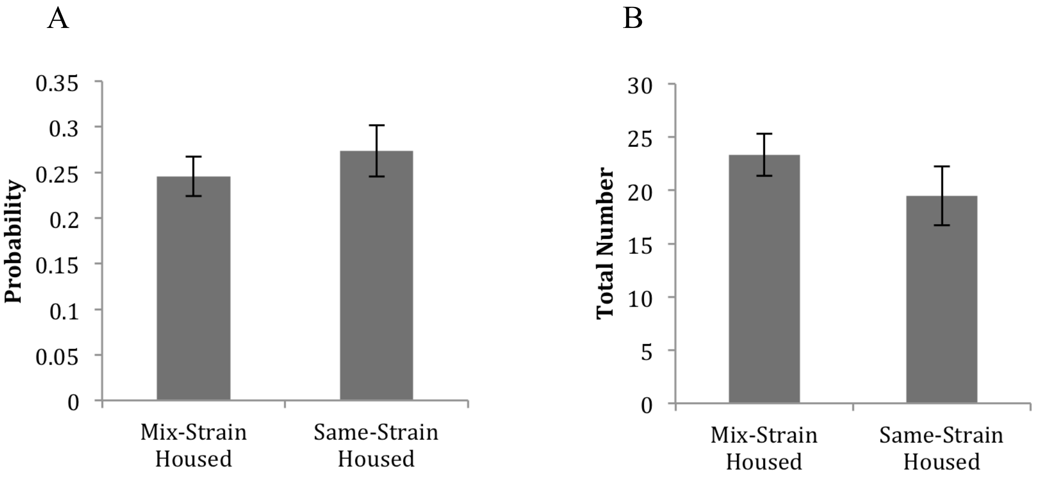

Figure 1.2 The two measures of role reversals, (A) their probability and (B) their total frequency (see caption for Figure 1), are shown for Long Evans rats that were housed in same strain quads or mixed strain quads (with two Sprague Dawley cage mates). Data are derived from S. M. Himmler et al. (2014b).

with regard to the identity of the partner and the actions performed by the partner and this is achieved with the aid of prefrontal cortical systems (Bell et al., 2009; B. T. Himmler et al., 2014; Pellis et al., 2006), it would seem likely that the ability to move so as to foster role reversals would also require cortical control.

Complete decortication at birth (Pellis, Pellis, & Whishaw, 1992b) or ablation restricted to the motor cortex in the days proceeding birth (Kamitakahara et al., 2007) changes the juvenile-typical preferences in use of defensive tactics (Table 1.1). If cortical systems are needed to adjust role reversal enhancing movements to meet changing contexts, then these rats should be deficient in role reversals. Analysis of the videotaped material of the decorticated rats (Pellis et al., 1992b) revealed that their probability of experiencing a role reversal in a given play fight was the same as that of the intact rats ($t(10) = -1.322$, $p = 0.216$) (Figure 1.3A) and that the experience of the absolute number of role reversals over the time of the testing did not differ between the decorticates and intact rats ($t(10) = 1.225$, $p = 0.249$) (Figure 1.3B). That is, all the facets of play fighting, even the most seemingly sophisticated ones, like being able to ensure the occurrence of role reversals, are organized subcortically and these do not require functional feedback during the pre-juvenile period to come on-line in the fully mature form in the juvenile period. Some of the modifications in the defensive patterns used likely require cortical control (Foroud et al., 2004; Kamitakahara et al., 2007), but these appear to be minor changes that do not affect the emergence of a form of play fighting in the juvenile period that is capable of generating the experiences needed to enhance the development of executive functions.

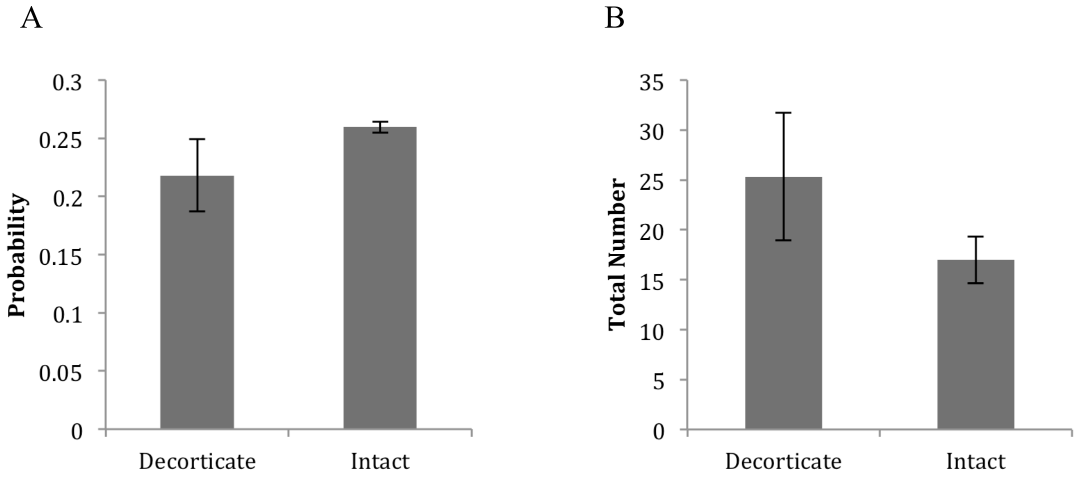

Figure 1.3. The two measures of role reversals, (A) their probability and (B) their total frequency (see caption for Figure 1.1), are shown for pairs of Long Evans rats that were decorticated at birth and for pairs of intact siblings. Data are derived from Pellis et al. (1992b).

That being able to modulate the tactics of playful attack and defense develops without cortical control and independently of prior social experience with play is a surprising finding. However, even more extreme forms of social isolation, involving the absence of contact with mother and siblings from birth to adulthood, has been reported not to affect some forms of learning, such as appetitive food conditioning that requires the ability to engage in goal-directed behaviour (Lomanowska & Kraemer, 2014). Such extreme forms of isolation rearing do affect impulse control and the ability to inhibit superfluous behavioural acts (Lomanowska & Kraemer, 2014; Lovic, Kee, Fletcher, & Flemming, 2011), which is consistent with the motivational and impulse control disruptions arising from more moderate reductions in maternal and sibling contact during infancy and the juvenile period (B.T. Himmler et al., 2015; Meaney, 2001; Vanderschuren & Trezza, 2014). These deficiencies may alter how species-typical and learned behaviours are executed, but do not effect how to perform them effectively. Thus, it would appear that play, along with other important cognitive and behavioural capabilities, develop relatively normally even when reared in atypical social environments.

1.7 Conclusion: Tying Tinbergen's whys together

Play likely originated as a byproduct of a confluence of factors (e.g., extended developmental period, surplus resources, protected environment, capacity to experience boredom, etc.), and in its first, rudimentary forms, was likely non-adaptive. Then, with new contextual opportunities, play was further transformed for novel adaptive functions (Burghardt, 2005). Play fighting in extant rodents shows a pattern of variation that is consistent with this theoretical framework (Pellis & Pellis, 2009). For some species, play has no discernible functions, but in some, the experience of play facilitates general motor development (Byers & Walker, 1995) and the refinement of the adult behaviour being simulated by play (Moore, 1985). For some species, such as the rat, play has a more general influence on the development of cognitive abilities (Einon et al., 1978) and social skills (van den Berg et al., 1999; von Frijtag et al., 2002). That is, play has been transformed in the lineage leading to modern rats (i.e., Tinbergen's evolutionary history why) so that it has novel functions (i.e., Tinbergen's adaptive function why) (Pellis et al., 2014). These novel cognitive and social functions involve particular neural systems, incorporating the prefrontal cortex (Bell, et al. 2010; B. T. Himmler et al., 2014), that are involved in the executive functions of the brain (Baarandse et al., 2013). That is, the executive functions that are influenced by the experience of play in the juvenile period can be attributed to specific causal processes, involving particular neural networks and behavioural feedback (i.e., Tinbergen's mechanism why) (Pellis et al., 2010a).

In the present paper, we attempt to integrate these whys with the final one in Tinbergen's pantheon – the developmental why. As we have shown, the emergence of juvenile play – the mature form that is critical for refining executive function – is influenced by non-play experiences with the mother during infancy. Surprisingly, peer-peer play in the immature phase of development of play is not needed for the juvenile-typical form to mature (B. T. Himmler et al., 2015). Why, then, does this prefunctional development of play exist? The likely answer is that, in rats, the experiences derived from play in the juvenile period have become so critical for the development, not only of motor systems (Byers & Walker, 1995) and mating (Moore, 1985), but also for the refinement of the prefrontal cortex and its role in executive function (Pellis et al., 2010a), that it cannot be left up to chance for minor experiences in earlier stages of development to change the content to the degree that those critical experiences may be compromised. Minor deviations are allowable, but none of these minor changes affect the development of play in a way that the critical experiences that are derived from juvenile play are diminished (Table 1.1). In turn, the executive training functions of play in the juvenile period lay the foundations for the ability of adult rats to use play as a tool for the assessment and manipulation of social partners – as is clearly suggested by the social incompetence demonstrated by adult rats with damage to those areas of the prefrontal cortex that are influenced by play and other social experiences in the juvenile period (Bell et al., 2009; B. T. Himmler et al., 2014; Pellis et al., 2006).

With regard to the role of play in peacemaking, in rats, functional, mechanistic, historical and developmental processes all converge on making adult rats more versatile in their social life, able to navigate relationships with more nuance, making it more likely for individuals to find non-aggressive ways to solve their problems. Such processes must similarly be present in other species that are able to use play in adulthood as a tool for social assessment and manipulation (Palagi, 2011; Pellis, 2002b; Pellis & Pellis, 2011). An important quest for future research is to identify how and why this has arisen in particular lineages of animals and not others (Pellis et al., 2014).

Chapter 2: The Development of Strain Differences in the Play Fighting in Rats*

2.1 Abstract

Laboratory strains of rats are a commonly used subject to study play behaviour. Recent research has shown that play in one laboratory strain of rat (e.g., Long-Evans hooded) differs in a number of ways from its wild counterparts. These findings suggest that domestication affects some aspects of play behaviour. However, there are multiple strains of laboratory rats, which have been domesticated through different lineages all derived from wild rats and it cannot be assumed that all domestic strains are identical in their play. Therefore, the aim of this study was to compare the play behaviour of three other strains of laboratory rats (e.g., Wistar, Sprague-Dawley, and Brown Norway). All strains were similar to each other as they all engaged in high frequencies of play, tolerated similar interanimal distances before initiating playful defense and displayed similar acrobatic capacities, suggesting domestication produces some common changes in play and other factors that influence play. However, strains differed significantly from one another in the use of tactics that promote bodily contact during play. Indeed, in this regard, some strains were more similar to wild rats than others, suggesting that some domestication-induced changes are either unique or more prominent in some laboratory strains than others. Such a mosaic pattern of transformation not only offers the possibility of using strain differences to characterize the genetic factors contributing to different

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facets of play, but also cautions researchers from making rat-general conclusions from studies on any one strain.

2.2 Introduction

Rough-and-tumble play is one of the most commonly studied forms of play (Pellis & Pellis, 1998a) with domesticated laboratory rats being the subjects most often used in experimental studies (e.g., Bolles & Woods, 1964; Meaney & Stewart, 1981a; Panksepp, et al., 1984; Pellis, 2002a; Thor & Holloway, 1984; Vanderschuren et al., 1997). However, domestication affects a variety of traits, including bodily composition, physiology, neural mechanisms, and behaviour (e.g., Albiach-Serrano, Brauer, Cacchione, Zickert & Amici, 2012; Castle, 1947; Coppinger & Coppinger, 2001; Lockard, 1968; Pisula et al., 2012; Takahashi & Blanchard, 1982). Many of these domestication-induced changes in psychological characteristics are likely to affect how animals play (Pisula, Gonzalez-Szwacka, & Rojek, 2003). Therefore, it cannot be assumed that the play of domesticated rats represents the situation across all mammals, domesticated and nondomesticated. Indeed, there are differences in the behavioural organization of play in wild and domesticated rats (B.T. Himmler et al., 2013c).

The B.T. Himmler et al. (2013c) study compared play behaviour in the Long-Evans hood strain of domesticated rats with the Wild Warsaw Captive Pisula-Stryjek (WWCPS). The WWCPS strain was derived from five independent colonies of wild rats in Warsaw, Poland, and was handled in a way that minimized human contact (Stryjek & Pisula, 2008). While play fighting in both WWCPS and Long-Evans rats involves attack and defense of the nape, there are differences in the frequency with which different behaviour patterns are used (B.T. Himmler et al., 2013b). Long-Evans rats initiate playful attacks more frequently and are more likely to defend when attacked. In addition, Long-Evans rats are more likely to engage in playful defenses that promote bodily contact (B.T. Himmler et al., 2013c). Although this earlier study is useful in identifying the ways in which play behaviour may be modified by domestication, it is limited by only comparing one domesticated strain of rat with a wild type. Domesticated strains of rats have been found to differ in a variety of behavioural traits (Pisula, Stryjek, & Nałęcz-Tolak, 2006; Pisula et al., 2012; Stryjek, Modlinska, & Pisula, 2012). Therefore, it cannot be assumed that the play of all domesticated strains differs from that of the wild in the same ways. Indeed, differences among selected lines of domestic strains of rats have been reported (e.g., Reinhart et al., 2004; Reinhart et al., 2006; Siviy et al., 1997; Siviy et al., 2003).

Based on a search of "play behaviour" and "rats" in Google Scholar encompassing the past 20 years (1990 –2012), 40% of the studies used the Long-Evans (LE) strain, 35% used the Wistar (W) strain, and 25% used the Sprague Dawley strain (SD), making these strains three of the most commonly used ones. Although all domesticated strains share the same common ancestor, the wild Norway rat (*Rattus norvegicus*), different strains have different origins. The first domesticated lineage led to the W strain from which the LE was derived by a backcross with a wild rat and SD was derived by further selective breeding of a line of W rats (Castle, 1947; Lockard, 1968). Some other domestic strains, like the Brown Norway (BN), were derived independently from wild rats (Castle, 1947; Krinke, 2000). Given the diverse origins of the currently used strains of rats, the possibility exists that some of the experimental differences detected across different laboratories may arise from the use of different strains as experimental subjects.

Domestication can affect play behaviour in at least three ways. First, domestication has selected for animals to reach sexual maturity more quickly (e.g., Coppinger & Coppinger, 2001; Morey, 1994; Trut, 1999), and so be more playful as a result of the retention of juvenile traits (Budiansky, 1999; Burghardt, 1984, 2005). Second, domestic animals demonstrate a significant reduction in defensive aggression (e.g., Blanchard & Blanchard, 1994; Trut, 1999). This leads them to be less hostile and more tolerant of closer interpersonal distances (Blanchard, Blanchard, Takahashi, & Kelley, 1977; Blanchard, Flannelly, & Blanchard, 1986; Takahashi & Blanchard, 1982), and therefore more likely to have opportunities to engage in play compared to their wild counterparts (B.T. Himmler et al., 2013b). Third, domesticated animals become much fatter, have less lean muscle, and a weaker bone structure (Price, 1999; Ritcher, 1959) and are therefore less likely to engage in energetically and physically taxing behaviours. In play, by being more sedentary, the animals are more likely to remain in close proximity, and so more likely to engage in behaviour promoting bodily contact (B.T. Himmler et al., 2013c). All three factors have been shown to contribute to the differences in the play of LE rats compared with WWCPS rats (B.T. Himmler et al., 2013c). It is unknown, however, whether these factors have changed the play of other domesticated strains of rats in the same way as in the LE strain.

There are two possibilities. First, all domesticated strains have been influenced in the same way and, consequently, the differences in play reported for several selected lines of rats (e.g., Reinhart et al., 2004; Siviy et al., 2003) are smaller than their differences to wild rats. Second, different traits that can influence play may have undergone a mosaic pattern of change, with different traits changing to differing degrees across different strains. If so, then some strains may, for some features of play, be more similar to wild rats than to other domestic strains. Indeed, studies on other behavioural features of rats (e.g., exploration, circadian rhythms) have shown that traits do not have a uniform change from the wild type (Pisula et al., 2006; Stryjek et al., 2012; Stryjek, Modlinska, Turlejski, & Pisula, 2013). Therefore, the present study builds on and extends the findings of the earlier study (B.T. Himmler et al., 2013c) by providing a detailed analysis of the play of an additional three strains of domestic rats.

In the previous study, the LE strain was used, and in the present study, two of the strains used include the W and SD strains, which along with the LE strain are the strains most often for laboratory studies of play (see above). However, given that in differing ways LE and SD were both derived from W rats, it is possible that any similarities in their play may arise from a shared common genetic heritage, rather than domestication per se. Therefore, as an independent check on the effect of domestication on the play of rats, we also analyzed the play of the BN strain, a strain for which play has not been previously described, but also one that was derived from wild rats independently of the W lineage (Castle, 1947; Krinke, 2000). Common differences in play from the WWCPS strain among LE, SD, W, and BN would indicate that the mechanisms regulating play have been transformed in the same way repeatedly by domestication.

2.3 Method

2.3.1 Subjects

In total, 108 subjects were used for this study. Of these, 32 were W rats (16 males, 16 females, average weights being 124.6 g and 102.2 g, respectively) and 32 were BN rats (16 males, 16 females, average weights being 79.7 g and 68.9 g, respectively). These were bred and obtained from the Mossakowski Medical Research Centre at the Polish Academy of Sciences and housed at the vivarium at the Department of Psychology, Helena Chodkowska University of Management and Law, Warsaw, Poland. All animals were weaned at 22 days old and were then randomly paired with a sex- and age-matched partner for the entire experiment. All animals were housed in Eurostandard Type IV cages (61 X 43.5 X 21.5 cm), with dust-free softwood granules Tierwohl Super as bedding and were given ad libitum access to water and standard laboratory fodder. Animals were on a 12:12 hour light:dark cycle and maintained at a constant 21–23 °C. All animals were cared for in accordance with the Regulation of the Polish Minister of Agriculture and Rural Development of March 10, 2006, on laboratory animal care.

The remaining 44 rats included 32 SD rats (16 males, 16 females, average weights being 133.6 g and 124.2 g, respectively) and 12 W rats (6 males and 6 females) obtained at around 23 days of age from Charles River Laboratories were housed at the Canadian Centre for Behavioural Neuroscience. Animals were randomly paired with a sex- and age-matched partner and remained with the same partner for the entire experiment. Animals were kept in 46 X 25 X 20 cm polyethylene tubs, with corncob bedding; food and water provided ad libitum. Animals were maintained at a constant 21–23 °C on a 12:12 hour light:dark cycle. All animals were handled and cared for in accordance with the Canadian Council for Animal Care regulation.

2.3.2 Procedure

All pairs were tested between 30 and 35 days, which is the peak period for playful interactions in rats (Thor & Holloway, 1984). Animals were habituated to the play apparatus for 30 min each day for 3 days before testing. To enhance playfulness for testing, each subject was socially isolated 24-hr before testing. Each pair was tested twice with each testing session lasting 10 min after which play partners were rehoused together for a 24-hr period before being isolated for a second test day (Pellis & Pellis, 1990, 1997). All testing and habituation occurred in complete darkness as it has been shown that play behaviour increases in frequency when in darkness compared with low light conditions or red light (Foroud & Pellis, 2002; Pellis & Pellis, 1987, 1990, 1997; Smith, Forgie, & Pellis, 1998).

2.3.3 Equipment

Play testing occurred in a 50 X 50 X 50 cm Plexiglas box, which was lined with 1–2 cm of corncob bedding for SD and W rats tested in Lethbridge and Tierwohl Super bedding for BN and W rats tested in Warsaw. After each testing session for all rats, the boxes were emptied of bedding and cleaned with Virkon to reduce any odors left from previous rats.

Play trials were recorded from two different angles, at 45 degrees obliquely from the front and 90 degrees directly from above. The rats in Lethbridge were recorded using a DVD103 Sony Handycam for the 45-degree angle and an HDR-XR500V Sony Handycam for the 90-degree angle. For the rats tested in Warsaw, they were recorded using a BCS 0804LE-A DVR system, with an LC-471 camera for filming from above and an LC-308D from the 45-degree angle. All cameras used the night-shot option to film in the dark.

2.3.4 Behavioural Analysis

The 45-degree video orientation was used for the scoring of play behaviour. Trials were viewed at full speed, in slow motion, and frame-by-frame. Because of the fact that SD, W, and BN rats all have mono-colored coats, pair mates were not tracked individually during play bouts. Therefore, for each strain, the behaviour of each pair was recorded as a unit, summing the scores of attack and defense behaviour of both pair mates. This also made the results of this study comparable with those from B.T. Himmler et al. (2013c), which used the same procedure.

Play in rats involves attack and defense of the nape, which is nuzzled if contacted (Pellis & Pellis, 1987; Siviy & Panksepp, 1987). In contrast, serious fighting involves bites directed at the face or rump (Blanchard et al., 1977; Pellis & Pellis, 1987). Thus, playful attacks can be scored as the snout of one rat approaches or makes contact with the nape of the other rat (B.T. Himmler et al., 2013a). Occasionally, however, as the attacking rat approaches from the rear, the recipient may begin to defend itself when contact is made on the rump or dorsum, thus, before the nape is reached. If such lower body contact is made without ensuing defense, or even if the recipient initiates a defensive action, the attacker shifts that contact toward the nape (Pellis & Pellis, 1987). Nonetheless, it is possible that given defensive actions, it may not be operationally possible to measure attacks as nape attacks. Thus, one measure of the role of the nape in organizing play fighting is the percentage of attacks that are coupled with defense that actually involve the snout moving toward or contacting the nape (B.T. Himmler et al., 2013c). As not all attacks lead to defense by the recipient, a measure of the playfulness of rats is to score the total frequency of nape directed attacks that occur in the 10 min trials, irrespective of the movements performed by the recipient. The likelihood of response to an attack is another measure of playfulness, with the probability of attacks leading to defense providing a numerical value (B.T. Himmler, Pellis, & Pellis, 2013a).

When rats defend themselves against a playful attack, there are several distinctive tactics that they can use. The two major forms of playful defenses include: (a) evasion, in which the defender moves its nape away from its attacker and does so by running, leaping or swerving away and thus faces away from its partner, and (b) facing defense, in which the defender moves its nape away by turning to face its partner, putting its teeth between its partner and its own nape. Facing defense can take one of three forms: (a) complete rotation, in which the defender rolls completely over onto its back, (b) partial rotation, in which the defender rotates its forequarters, but maintains contact with the ground with one or both of its hind feet, and (c) other, in which defensive actions involve rotations or other movements in other dimensions. Defensive tactics used were determined by movements within the first 2–3 video frames after an attack to ensure that the first defensive tactic attempted was recorded rather than the eventual outcome of protracted attack and defense (B.T. Himmler et al., 2013a).

Another measurement used to assess potential strain differences in play behaviour was pinning, a configuration in which one partner lies on its back and the other stands on top (Panksepp, 1981). As pins are commonly used to measure play in rats (see Discussion section), it is important to know how variable this behaviour is across strains. Following past practice (Panksepp, 1981; Panksepp & Beatty, 1980), whenever the rats achieved the on-top/on-bottom configuration, this was scored as a pin and these events were recorded as the absolute frequency of pins occurring over the 10-min play trials.

The 90-degree video orientation was used to score sensorimotor differences between strains. Video clips were digitized and scored using the Vicon Motus motion capture software (e.g., Bell & Pellis, 2011; Pellis & Pellis, 1994; Sacrey, Alaverdashvili, & Whishaw, 2009). To measure the interanimal distance, clips of one animal attacking from the side from at least one rat-length away were used. Distances were measured between the tip of the attacking rat's nose and the middle of the nape of the defender once the defender made its first movement to swerve laterally away from the approaching partner. The distances jumped were measured when the performer leapt directly forward rather than upward, as leaping forward is more likely a defensive action than a play solicitation action (Pellis & Pellis, 1983). Because the head could change position during a jump, the location of the snout was not a good marker for tracking the distance jumped. Therefore, the center of the nape was tracked to measure the distance of the jump. The velocity of jumps was also measured on the frame at which all four of the rat's feet lost contact with the ground (B.T. Himmler et al., 2013c).

2.3.5 Statistical Analyses

Data were derived from the 16 pairs BN and 16 pairs W rats from Warsaw, Poland and the 16 pairs SD rats from Lethbridge, Canada. The 6 pairs of W rats from Lethbridge were not included in the strain comparisons, but rather, they were used to evaluate interinstitution reliability in data collected across the two institutions (see below the Validation of Testing Protocols and Behavioural Analyses, section). That is, the W rats from the two institutions were analyzed and compared with one another (see below the Validation of Testing Protocols and Behavioural Analyses, section). In addition, given that all pairs were tested twice, the data used for cross-strain comparisons represent an average score of both play sessions. The data were analyzed using a two-way analysis of variance (ANOVA), with strain (SD, BN, or W) and sex as independent variables; for pairwise comparisons, the least significant difference test was used for post hoc tests. For significant main effects of strain or sex, effect sizes were calculated using Cohen's d, with values of 0.8 or greater representing large effects. Differences were considered significant for p values of < 0.05. For graphical representation of the data, values are given for group means and 95% confidence intervals.

2.3.6 Validation of Testing Protocols and Behavioural Analyses

Two issues regarding the reliability of the data collected and scored were dealt with. First, this article, as well as the preceding one (B.T. Himmler et al., 2013c), involved comparing different strains that were tested at two different laboratories. Moreover, although all the strains were tested in the same sized testing arena, from weaning until completion of testing, the animals maintained in Poland were housed in home cages that were 2.3 times larger and were tested on different bedding. Therefore, to test whether being housed in different sized cages and being tested in different bedding could affect subsequent play behaviour the 6 pairs of W rats that were tested in Lethbridge were compared with the videotaped trials of the 16 pairs of W rats tested in Warsaw. As shown in the (Results section) there were no significant sex differences, so the data from each sample from each laboratory were combined across the sexes. The 6 Lethbridge pairs were compared with the 16 Warsaw pairs on five measures of play (frequency of attack, probability of defense, probability of evasion, probability of complete rotation, and frequency of pinning) using independent *t*-tests. The two samples did not differ significantly on any measure ($p > 0.05$). Therefore, differences in cage size and bedding type are unlikely to account for strain differences for animals tested in the two laboratories.

Second, to ensure interobserver reliability, the 6 W pairs sampled in Lethbridge were rescored by another experimenter (Brett T. Himmler), who was experienced with the scoring scheme used but had not previously applied it to W rats. Comparison using Pearson's correlation of these two scores for playful attacks, probability of defense, total pins, evasive defense, and complete rotation defense showed a high level of interobserver reliability (*r* range: 0.772 to 0.993, with four of five comparisons being significant, < 0.05). Given the consistency in the data collected across laboratories and strains, as well as the consistency in the scoring by different raters, it can be concluded that any significant differences across the strains was because of strain differences rather than any confounding factors. To ensure maximum consistency across all the strains, the data presented in the (Results section) are all based on scores by the same rater (Stephanie M. Himmler). Finally, to facilitate comparisons with the earlier study, each graph shows the average value of the trait as a separate horizontal line for LE and WWCPS (B.T. Himmler et al., 2013c). This provides easy determination as to whether each trait in W, SD, and BN rats more closely resembles the wild state or that of another domesticated strain.

2.4 Results

2.4.1 Targets of Attack

A 3 X 2 ANOVA for the proportion of playful interactions that began with the defense of the nape rather than contact with some other area of the body showed a significant strain difference, ($F(2,42) = 10.071$, $p = 0.0001$). Both BN and W rats targeted the nape more frequently than did SD rats ($p < 0.05$), but there were no significant differences between BN and W rats ($p > 0.05$) (see Figure 2.1). There was no effect of sex ($p > 0.05$), nor a significant strain by sex interaction ($p > 0.05$). Despite some statistically significant strain differences, it should be noted that, in all strains, the nape was the target of attack in well over 90% of attacks in all strains.

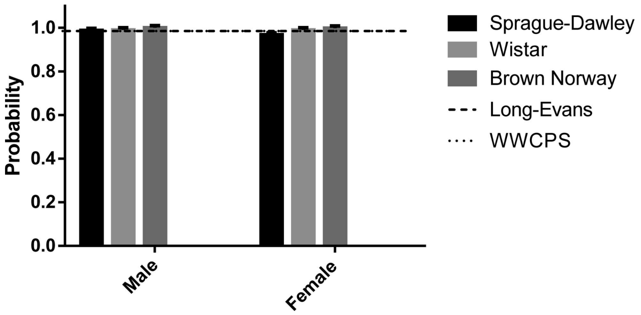

Figure 2.1. The probability of a playful attack being directed at the nape. The error bars represent 95% confidence intervals. For all figures, dark dashed lines (▬ ▬) represent the average score for the LE strain and light dashed lines (. . . . .) represent the average score for the WWCPS strain as given in B.T. Himmler et al. (2013c).

2.4.2 Frequency of Play

A 3 X 2 ANOVA for the total number of playful attacks revealed a main effect for strain, ($F(2, 42) = 53.883$, $p = 0.0001$) but no effect of sex ($p > 0.05$), nor a significant strain by sex interaction ($p > 0.05$). Post hoc analysis revealed that SD rats attacked more than W rats ($p < 0.05$, $d = 3.06$) and BN ($p < 0.05$, $d = 3.56$) (Figure 2.2A).

There was no significant effect between W and BN rats ($p > 0.05$).

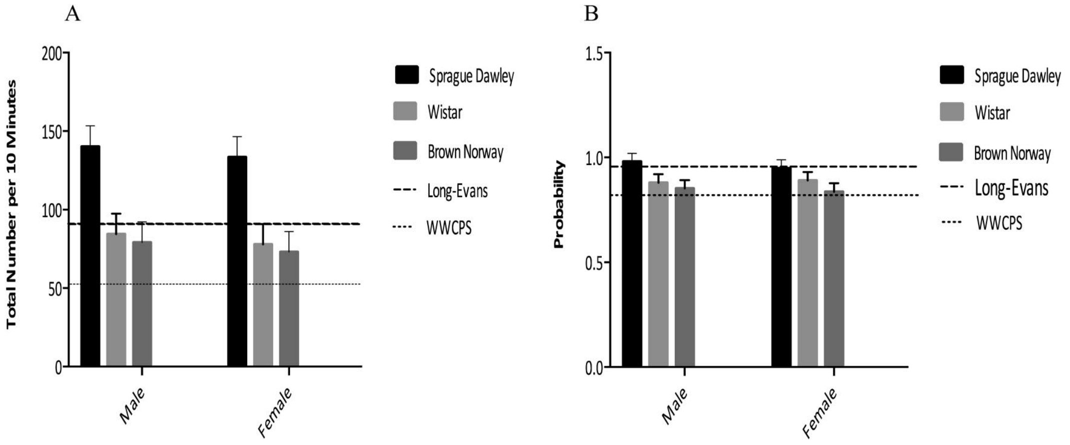

Figure 2.2. (A) The mean number of playful attacks per 10 min. (B) The mean probability of defending against a playful attack.

There was a main effect for strain for the probability of defending against a nape attack, ($F(2, 42) = 18.602$, $p = 0.0001$) but no significant effect of sex ($p > 0.05$), nor a significant strain by sex interaction ($p > 0.05$). Post hoc analysis revealed SD rats were more likely to defend against an attack than both W ($p < 0.05$, $d = 1.93$) and BN ($p < 0.05$, $d = 2.13$) rats, while W rats were more likely to defend than BN ($p < 0.05$, $d = 0.66$) rats (Figure 2.2B).

A 3 X 2 ANOVA for the frequency of pins revealed a main effect for strain, ($F(2, 42) = 23.446$, $p = 0.0001$) but no significant effect of sex ($p > 0.05$), nor a significant strain by sex interaction ($p > 0.05$). Post hoc analysis revealed that playful interactions in SD rats ended in the pin configuration more frequently than both W ($p < 0.05$, $d = 2.18$) and BN ($p < 0.05$, $d = 2.09$) rats (see Figure 2.3).

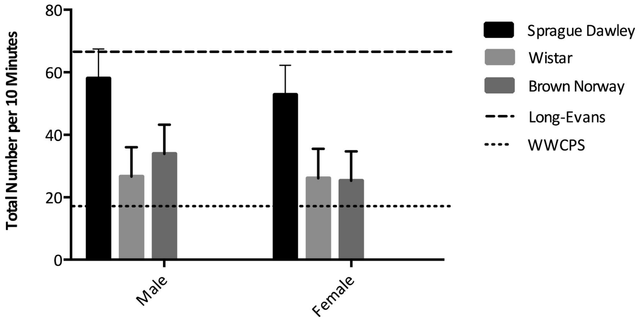

Figure 2.3. The mean number of pins in the 10 min trials

2.4.3 Tactics of Playful Defense

A 3 X 2 ANOVA revealed a main effect for strain, ($F(2, 42) = 51.876$, $p = 0.0001$) for the probability that animals would evade an attack. Post hoc analysis revealed that SD rats were more likely to evade than both W ($p < 0.05$, $d = 1.72$) and BN ($p < 0.05$, $d = 3.16$) rats and that W rats were more likely to evade than BN ($p < 0.05$, $d = 2.01$) rats (Figure 2.4A).

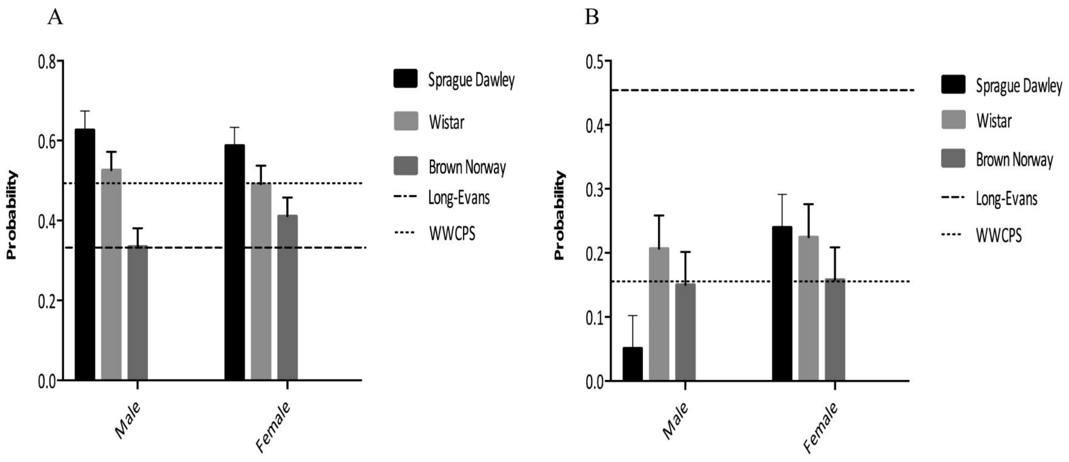

Figure 2.4. (A) The mean probability of engaging in evasion when defending against a playful attack. (B) The mean probability of engaging in a full rotation defense, as a proportion of all facing defense, when defending against a playful attack.

There was no significant main effect of sex ($p > 0.05$), but there was a significant strain by sex interaction, ($F(2, 42) = 4.043$, $p = 0.025$) with female SD rats being more likely to evade an attack than both W ($p < 0.05$, $d = 2.12$) and BN ($p < 0.05$, $d = 2.48$) females. Male SD rats were also more likely to evade than W ($p < 0.05$, $d = 1.63$) and BN ($p < 0.05$, $d = 4.56$) males. Male SD rats were also more likely to evade than female SD rats ($p < 0.05$, $d = 0.64$) and male W rats were more likely to evade than female W rats ($p < 0.05$, $d = 0.77$).

For facing defense, the partial rotation and "other" tactics were sometimes difficult to distinguish across the strains consistently, whereas complete rotation to supine could be. Therefore, because in both the partial rotation and other tactics the defender remains standing on at least one hind foot (see B.T. Himmler et al., 2013a), these two types of defenses were combined into "standing defense." In this way, the proportion of facing defense (combing standing and supine defense tactics) that involved the complete rotation tactic could be calculated.

A 3 X 2 ANOVA revealed main effects for complete rotation for both strain, ($F(2, 42) = 4.557$, $p = 0.016$) and sex, ($F(1, 42) = 11.807$, $p = 0.001$). Post hoc analysis revealed that W rats were more likely to use the complete rotation tactic than SD ($p < 0.05$, $d = 0.70$) and BN ($p < 0.05$, $d = 1.02$) rats (Figure 2.4B), and females were more likely to use the complete rotation tactic than were males ($p < 0.05$, $d = 0.39$). There was also a significant strain by sex interaction, ($F(2, 42) = 7.925$, $p = 0.001$)

with SD females being more likely to use this tactic than BN females ($p < 0.05$, $d = 0.91$) and W males more than both SD ($p < 0.05$, $d = 3.17$) and BN ($p < 0.05$, $d = 1.11$) males and BN males than SD males ($p < 0.05$, $d = 4.01$). Female SD rats were more likely to use the complete rotation tactic than SD males ($p < 0.05$, $d = 2.35$).

2.4.4 Defense Distance and Acrobatic Capability

For each pair (SD = 8 males, 8 females; W = 8 males, 8 females; BN = 8 males, 8 females), interanimal distance was measured when defense was initiated during a playful attack. A 3 X 2 ANOVA revealed no significant main effects for strain and sex or for strain by sex interaction ($p > 0.05$) (see Figure 2.5).

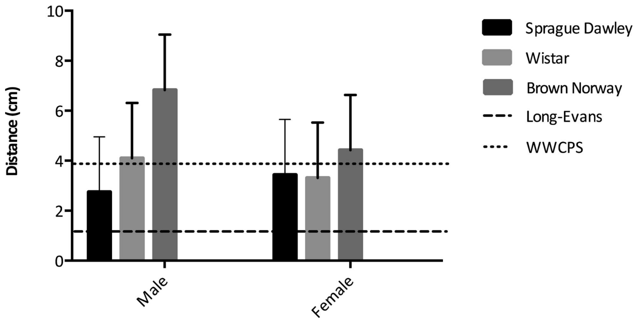

Figure 2.5. Distance (centimeters) between the nose of the attacker and the nape of the defender on the frame of the first defensive movement against an attack.

Jump distances away were measured for each pair. A 3 X 2 ANOVA revealed a significant strain effect, ($F(2, 42) = 8.804$, $p = 0.001$) with SD rats jumping shorter distances than both W ($p < 0.05$, $d = 1.21$) and BN ($p < 0.05$, $d = 1.44$) rats (Figure 2.6A). A 3 X 2 ANOVA revealed no significant main effects or interactions for jump velocities ($p > 0.05$) (Figure 2.6B).

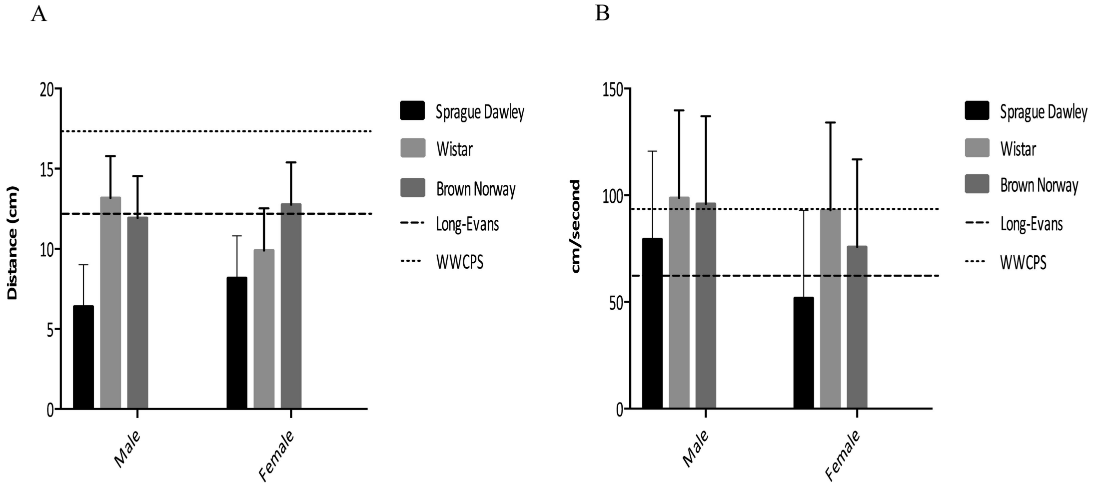

Figure 2.6. (A) The mean distance (centimeters) of jumps away from partners. (B) The mean velocity (centimeters per second) of jumps away from partners.

2.5 Discussion

The main aim of this study was to determine whether or not the effects of domestication on play are similar across different strains of laboratory rats. To determine this, three strains of laboratory rats were compared with another domesticated strain (LE) and a wild strain (WWCPS) of rat from a previous study (B.T. Himmler et al., 2013c). The present study, however, does not include a statistical comparison among all five strains, but rather, uses the average values of the WWCPS and LE as reference points to highlight graphically whether the three strains evaluated here are more like the other domesticated strain than the wild strain. Detailed comparisons of how similar or how different they are from the LE and WWCPS rats are thus not possible from the current study.

Like both the wild (WWCPS) rats and the domesticated LE rats (B.T. Himmler et al., 2013c), the W, SD, and BN strains almost exclusively attacked and defended the nape of the neck during play fighting. Thus, the four domesticated strains all retain the wild-type pattern of competing for access to the nape, which is nuzzled if contacted (Pellis, 1988; Siviy & Panksepp, 1987). Given that the nape is a body target contacted during adult courtship encounters, it suggests that play after domestication across several strains retains the ancestral pattern of being a simulation of precopulatory behaviour (Pellis, 1993; Pellis & Pellis, 1998a).

The review of the literature shows that there are at least three ways in which domestication can affect the expression of play. First, domestication increases playfulness (Budiansky, 1999; Burghardt, 1984, 2005). On most behavioural traits that affect the frequency of play, the three strains in this study are similar and are more like LE rats than WWCPS rats. W, SD, and BN rats have a frequency of playfully attacking the nape and have a likelihood of responding to a nape attack that is more similar to that of LE than WWCPS rats, suggesting that all of the domesticated strains are more playful than WWCPS rats (B.T. Himmler et al., 2013c).

Second, domestication reduces defensive aggression and by doing so increases the tolerance of greater proximity of conspecifics (Blanchard & Blanchard, 1994; Trut, 1999). This decreased interanimal distance would increase the likelihood of contact and so promote the occurrence of play, especially play involving close-quarter wrestling. The measure of the distance at the moment when the recipient of an attack began its defensive movement did not differ among strains and the average group distances were closer to the larger values seen in in WWCPS rats (B.T. Himmler et al., 2013c). However, the variation was extremely large and it is interesting that the lower values of all the domestic strains were well outside the low end of the distribution seen in WWCPS rats

(B.T. Himmler et al., 2013c). This indicates that all these domesticated strains can tolerate closer proximity by the partner than is the case for wild rats.

Third, domesticated animals have reduced acrobatic capacity because of changes in muscle composition and body proportions (Price, 1999; Ritcher, 1959), and this would again promote close-quarter contact and so increase the likelihood of play, especially playful wrestling. Jump distances were significantly lower in SD rats than the other two strains. In part, SD rats having higher body weights than both BN and W rats may account this for. In all cases, the values were closer to those of LE rats than WWCPS rats (B.T. Himmler et al., 2013c). The velocity of jumps did not differ among the strains and the average values were, in some cases, closer to WWCPS than LE rats. As the case for response distance, however, the variation on this measure was very large and the lowest values were well lower than any seen in the WWCPS rats (B.T. Himmler et al., 2013c), suggesting that acrobatic performance has been eroded by domestication.

Taken together, these findings show that after domestication, play retains the basic organization present in wild rats, that of attacking and defending the nape, which if contacted is nuzzled, not bitten (Pellis & Pellis, 1998b). In addition, the findings show that all strains of domesticated rats increase their frequency of playing by domestication-induced changes that either directly affect the occurrence of play (i.e., increased motivation to play) or indirectly do so (i.e., reduced interanimal distance and reduced capacity to leap away from the partner). However, closer comparison of how the rats play, especially with regard to the defensive tactics used, indicate that not all the changes from wild state to domesticated state are similar; this suggests that some features of play may have changed in a mosaic manner across different strains.

2.5.1 Strain Differences in Playful Defense

There were three major disparities in patterns of defense. First, in LE rats, only 20 –30% of defenses involve evasion (Pellis & Pellis, 1990, 1997) compared with the 50% or more in WWCPS rats (B.T. Himmler et al., 2013c), and the values for evasive defense for BN, SD, and W rats were more like those of WWCPS rats; although BN males appear to have values for evasive tactics closer to that of LE rats. Indeed, SD rats had the highest likelihood of using evasion, with over 60% of all defenses involving evasion. This finding was unexpected because the reaction distances and jumps for the SD rats were among the lowest recorded for the three strains, leading them to remain at close quarters for most of the play bouts. Thus, even though they remained close to one another, the SD rats were more likely to evade attacks than to face their partners. This is counter to our previous conclusion that the higher evasion values in WWCPS compared with LE rats was because of the WWCPS rats having superior acrobatic capacities and defensive reactions that begin at substantially greater interanimal distances (B.T. Himmler et al., 2013c). A preference for evasion compared with facing defense may not be a byproduct of strain-specific patterns of interactions that lead to divergent opportunities to use evasion, but rather, may reflect brain-based biases in the selection of different defensive tactics (Pellis & Iwaniuk, 2004; Pellis & Pellis, 1998b); a conclusion supported by the other disparities in defense across strains.

Second, when engaged in facing defense, the most likely tactic in LE rats is for them to rotate around their longitudinal axis (50 – 60%), leading them to achieve a supine position. Once supine, the defender wards off further attacks by the attacker

(Pellis & Pellis, 1987, 1990). This is substantially more than the 20% in WWCPS rats (B.T. Himmler et al., 2013c). Surprisingly, the values for complete rotation in the BN, SD, and W rats were low, more like those of WWCPS rats than LE rats. Indeed, SD rats had the lowest, and significantly so, rate of using the complete rotation tactic, with males barely using this tactic 10% of the time. Again, this is surprising given that the SD rats were the strain in which the pairs remained in close proximity most of the time, affording them plenty of opportunity to use this tactic. In this regard, the LE rats stand out as being different to the rest, as they exaggerate the use of the complete rotation tactic not only compared with the WWCPS rats (B.T. Himmler et al., 2013c), but also to the other domesticated strains (this article). That the difference may be at a neural level of tactic selection is suggested by findings in LE rats. Rats that have been decorticated at birth go on to develop as juveniles that initiate play at the same frequency that intact rats do (Panksepp, Normansell, Cox, & Siviy, 1994; Pellis & Pellis, 1992). Even though decorticate LE rats launch playful nape attacks and defend against such attacks at the same frequency that intact matched controls do, they only use the complete rotation tactic around 20% of the time (Pellis & Pellis, 1992). Indeed, limiting the damage to the removal of the motor cortex leads to the same outcome (Kamitakahara et al., 2007). Comparative evidence suggests that species-typical preferences for using different tactics of defense during play involve neural control at the subcortical level, likely involving the basal ganglia (Pellis & Iwaniuk, 2004; Pellis & Pellis, 1998a, 2009). Thus, the selective effect of cortical damage on the use of the complete rotation tactic suggests that in LE rats, a second level of control over defense tactics is present. The cortex, likely involving the motor cortex, modifies the selection preferences in the basal ganglia leading to an increased use of the complete rotation tactic compared with other tactics of facing defense (Kamitakahara et al., 2007). In this way, the extreme differences between LE rats (the highest rate) and the SD rats (the lowest rate) in using the complete rotation tactic may reflect differences in neural control mechanisms. Of course, it is possible that other subtle differences in the attack patterns of these strains differentially affect the opportunity to use the complete rotation tactic. This alternative needs to be evaluated before it is concluded the strain differences have resulted from differential changes in neural mechanisms of defense.

Third, play fighting in a pair of LE rats is more likely to end in the pinning configuration (one rat lying supine and the other standing on top; Panksepp, 1981) than in WWCPS rats. Given that LE rats are more likely to use the complete rotation tactic than WWCPS rats, our assumption was that pinning is more common in LE rats than WWCPS rats because the LE rats more frequently use a defensive tactic that facilitates pinning as an outcome of the actions taken (B.T. Himmler et al., 2013c). The present findings require this conclusion to be reevaluated. Surprisingly, even though it was the SD rats that had the lowest use of the complete rotation tactic, it was this strain that had the highest frequency of pinning, while BN and W rats appeared to display frequencies of pinning more similar to that of WWCPS rat. Given that previous studies on LE rats have shown that the majority of pins arise from the defender adopting the complete rotation tactic (Pellis & Pellis, 1987, 1990, 1992, 1997), the present findings, especially for SD rats, suggests that there are multiple ways in which the pinning configuration may be achieved.

This is an important finding because pinning is not only easy to observe and has a high interobserver reliability (Panksepp, 1981; Panksepp & Beatty, 1980), but it is also a common marker used for the neurobiological study of play in rats (Aguilar et al., 2009; Calcagnetti & Schechter, 1992; Panksepp et al., 1994; Thor & Holloway, 1983; Trezza & Vandershuren, 2008). In SD rats, most pins do not arise from the defender rolling to supine in a smooth, continuous movement lasting 2–3 video frames, as is the case in LE rats (B.T. Himmler et al., 2013a). Rather, the impression gained from scoring the behaviour was that the pins, in most cases, arose from incremental turning to supine lasting 5– 6 frames because of the persistent attempts to gain access to the nape by the attacker. Such pinning arising from continued attack and defense is occasionally seen in LE rats (Pellis & Pellis, 1987, 1990), but in the SD rats, this appears to be the predominant mode of action leading to pinning. The BN and W rats are intermediate in this regard. These strain differences in how the pin configuration is achieved need to be empirically evaluated further. However, the current findings suffice to alert researchers that an experimentally induced increase or decrease in pinning cannot be assumed to involve the same mechanisms in all strains. The overall cautionary lesson from the present findings is that studies of play should use multiple measurements, rather than relying on only one or two.

With regard to domestication, these findings on the use of different defensive tactics and in the variation in the likelihood of pinning suggest that different strains have not uniformly diverged from wild rats. A 30% reduction in brain size has been found to accompany domestication in a variety of species, including rats (Kruska, 1988). However, there are differences in brain size across strains (Yanai, 1979) as well as functional differences in neurochemical systems (Siviy et al., 2011). Moreover, as already noted above, there is a link between regulatory mechanisms in the motor cortex and the expression of one particular kind of facing defense (Kamitakahara et al., 2007). Taken together, these findings suggest that there are strain-specific idiosyncrasies in how different brain mechanisms may have been modified during domestication from the ancestral wild type. In turn, these brain differences may account for differences in patterns of play across strains (e.g., Siviy et al., 2011). An implication of these strain differences in brain and behaviour is that caution must be taken when the findings of one study using one particular strain of rats are compared with those from studies using different strains. Added power may be gained if researchers routinely repeated experiments with multiple strains of rats to determine if findings are consistent across strains.

2.6 Conclusion

All four domesticated rat strains, BN, LE, SD, and W, organize their play fighting as do wild rats—they attack and defend the nape of the neck (B.T. Himmler et al., 2013c; present study). Moreover, all four strains are more playful than their wild counterparts and have an increased tolerance for interanimal distance and diminished acrobatic capacities. Thus, it could be concluded that there are some common mechanisms that are changed via the domestication process. However, there are also some significant differences in how different strains defend themselves during play fighting. For example, some strains displayed similar frequencies of evasive tactics to wild rats. These significant strain differences, especially given that most differences have very large effect sizes, need to be taken into account when different laboratories use different strains for their studies. The conflicting results arising from the study of similar experimental factors may in part be because of the particular way in which strains react to the experimental manipulation (e.g., Aguilar et al., 2009; Arnold & Siviy, 2002; Janus, 1987; Parent & Meaney, 2008; Shimozuru et al., 2007; Siviy & Harrison, 2008; Veenema & Neumann, 2009).

Chapter 3: The Development of Strain Differences in the Play Fighting in Rats[*]

3.1 Abstract

During play fighting, rats attack and defend the nape, which if contacted is nuzzled with the snout. While all strains of rats use the same suite of defensive tactics to protect the nape, different strains use some tactics more frequently. This study tests two hypotheses for this strain difference: (1) each strain has a preference for using particular tactics and (2) strain differences in defense are a byproduct of strain differences in patterns of nape attack. Juvenile Long-Evans (LE) and Sprague-Dawley (SD) males, raised in same strain quads from shortly after weaning to the early juvenile period (i.e., 24-31 days), were tested with unfamiliar same-strain and different-strain partners (Experiment 1) and LE and SD males raised in mixed LE-SD quads were tested with both familiar (Experiment 2) and unfamiliar same-strain and different-strain partners. If hypothesis (1) were true, they would maintain strain-typical defense patterns irrespective of the strain of the attacking partner, whereas if hypothesis (2) were true, it would vary with the strain of the attacking partner. Hypothesis (1) was supported in the first experiment; all the rats maintained their strain-typical patterns regardless of the partner's strain. However, Experiments 2 and 3 supported neither hypothesis, as each animal displayed strain-divergent behaviour when playing with partners of either strain. Given that in Experiments 2 and 3, subjects were reared in mixed-strain groups, it is possible that,

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[*] Copyright © has been maintained by the author. Published as: Himmler, S.M., Lewis, J.M., & Pellis, S.M. (2014). The development of strain-typical defensive patterns in the play fighting of laboratory rats. *International Journal of Comparative Psychology, 27,* 385-396. (see appendix B)

during the early juvenile period, play fighting experiences shape strain-typical patterns of use of defensive tactics.

3.2 Introduction

Play fighting is one of the most commonly studied forms of play behaviour in mammals with laboratory rats being one of the most studied species (e.g., Bolles & Woods, 1964; Meaney & Stewart, 1981a; Panksepp et al., 1984; Pellis & Pellis, 1998a; Vanderschuren et al., 1997). However, there are many different strains of laboratory rats that are used in experimental research and these have been shown to differ in many features of their behaviour (e.g., Pisula et al., 2003; Pisula et al., 2012; Prusky et al., 2002), including aspects of their play behaviour (e.g., Reinhart et al., 2004; Siviy et al., 1997; Siviy et al., 2003; Siviy et al., 2011). Moreover, laboratory strains of rats are domesticated versions of wild rats and domestication is known to change many aspects of physiology and behaviour (e.g., Castle, 1947; Coppinger & Coppinger, 2001; Lockard, 1968; Pisula et al., 2012; Takahashi & Blanchard, 1982).

A recent pair of papers used a standardized testing and scoring scheme (B. T. Himmler et al., 2013a) to compare the play behaviour of four domestic strains to that of a strain of wild rats (B. T. Himmler et al., 2013c; S. M. Himmler et al., 2014b). In both domesticated and wild rats, play fighting involves competition for access to the partner’s nape of the neck, which if contacted, is gently nuzzled with the snout (B. T. Himmler et al., 2013c; S. M. Himmler et al., 2014b; Pellis & Pellis, 1987; Siviy & Panksepp, 1987).

Competition for the nape not only involves attacking the nape but also the recipient defending its nape from such contact (Pellis & Pellis, 1987). There are two main types of defense that rats can employ when defending against an attack, (a) evasion and (b) facing defense. Evasion involves the defender moving its nape away from its attacker by running, leaping, or pivoting away, and, in so doing, turning its face away from the attacker. Facing defense involves the defending animal turning to face its attacker, while simultaneously withdrawing the nape. In turn, facing defense can take one of two forms: rotating around its longitudinal axis or rotating around a vertical axis. Moreover, rotation around the longitudinal axis can also take one of two forms: rotating fully until the rat is lying supine, or partially, in which the forequarters are rotated toward its partner, with one or both of its hind feet planted on the ground (B. T. Himmler et al., 2013a). When rotating around the vertical axis, the defender maintains ground contact with all four paws. Rotation around the vertical axis and partial rotation around the longitudinal axis can end in the same configuration if the rats rear up onto their hind feet and face one another (B. T. Himmler et al., 2013a). Facing defenses, especially full rotation to supine, increase the occurrence and duration of body-to-body contact as the animals wrestle one-another, while evasion decreases body contact as the defender withdraws from the attacker. Like domesticated rats, wild rats use all these tactics during play fighting (B. T. Himmler et al., 2013c; S. M. Himmler et al., 2014b). However, wild rats use evasion more frequently and supine defense less frequently than do the domestic strains, but the domestic rats differ from one another in how they differ to the wild rats. The largest strain difference in defensive tactics among the strains compared was between Sprague-Dawley (SD) and Long-Evans (LE) rats. SD used evasion more frequently than facing defense and LE used facing defense more frequently than evasion. Also, when engaging in facing defense, LE used the full rotation to supine tactic more often than SD. Two hypotheses that could account for the strain differences in the use of defense tactics are tested in this paper.

The first hypothesis posits that different strains have a strain-typical bias for using some tactics more frequently than others. An analogy would be with sex-typical behaviour. In rats, females are less likely to mount and more likely to exhibit lordosis than males, which exhibit the opposite pattern. The male-typical pattern is established by perinatal hormonal influences on the neural mechanisms that regulate these behaviours (Baum, 1984). The same mechanism of sexual differentiation has been shown to account for the sex-typical differences the frequency of play fighting, with males usually engaging in more play (Meaney, Stewart, & Beatty, 1985). That there may be strain-typical preferences for using certain defense tactics is supported by findings that differences in neural function have been correlated with some aspects of the differences in the play between some strains (e.g., Siviy et al., 2011). Moreover, the bias to use the full rotation to supine defense in LE has been shown to be dependent on a specific area of the brain. Removal of the motor cortex selectively reduces the frequency of rotating to supine (Kamitakahara, et al., 2007). The rats with motor cortex damage attack and defend at the same frequency as intact controls, and like intact LE rats, use facing defense more frequently than evasion. The motor cortex damage-induced change selectively decreases the frequency of using supine defense and increases the frequency of partial rotation. With regard to facing defense, cortex damaged LE rats are like intact SD rats (B. T. Himmler et al., 2013c; S.M. Himmler et al., 2014b). Therefore, it is possible that the strain-typical differences in the use of supine defense and evasion in LE and SD may be due to differences in strain-typical neural biases. This is the brain-bias hypothesis.

The second hypothesis does not focus on brain mechanisms regulating the selection of preferred defensive tactics, but rather, on possible differences in the conditions that create the opportunity to use different tactics by different strains. For example, compared to wild rats, domestic strains of rats are less acrobatic and tolerate closer proximity when attacked (B. T. Himmler et al., 2013c, S. M. Himmler et al., 2014b). It remains to be determined whether these differences arise from differences in rearing, caging and feeding patterns or from genetic differences. Regardless of their origin, subtle differences in sensorimotor capabilities can have an impact on the execution of behaviour patterns in various contexts (e.g., Berridge, 1990; Whishaw et al., 2003). The differences between LE and SD rats may make the attack of an LE rat contextually different enough to that of an attack by an SD rat to make supine defense in the former and evasion in the latter the more effective tactics. There is precedent for such sensory and biomechanical factors influencing choice of defense tactics.

During serious fighting, short-bodied species, like hamsters, are more likely to use a rotation to supine tactic to block a bite to the rump, while long-bodied species, like voles, are more likely to use a rotation around a vertical axis (Pellis, 1997). The number of body movements and the time taken to block a bite in these species show that supine defense is faster and more efficient in short-bodied rodents and vertical rotation is more efficient in long-bodied rodents (Pellis, 1997). A sensorimotor difference affecting the successful use of some defense tactics used during play has been found between male and female LE rats. When attacked directly from the rear, rats are likely to use the vertical axis rotation tactic, but in this attack configuration, females begin to rotate to face when the attacker is further away than do males. This difference in timing of initiation means that females are more successful in facing the attacker before contact is made and so can block the attack in a face-to-face orientation. In contrast, the males, by beginning to turn later, give the attacker time to close the distance and pounce on the back and so contact the nape with the snout, leading the male to turn to supine to withdraw the nape (Pellis et al., 1993). That is, the successful execution of particular tactics may depend on biomechanical factors arising from small sensorimotor differences (Blumberg, 2005), such that attacks by members of a particular strain are more likely to create the context for particular kinds of defensive tactics to be used. This is the biomechanical hypothesis.

In order to test which of these two hypotheses may best explain strain differences in defensive tactics, the present study compared how SD and LE rats defended when attacked by either SD or LE partners. This was done in two ways. First, rats were housed in same-strain quads and then tested in the standard play paradigm (B. T. Himmler et al., 2013a) with unfamiliar partners of the same-strain and the opposite strain (Experiment 1). Second, rats were housed in mixed-strain quads and then tested with either familiar (Experiment 2) or unfamiliar (Experiment 3) same-strain and opposite-strain partners. If the brain hypothesis were correct, then irrespective of the rearing and testing paradigm, all rats should have used strain-typical patterns of defense. If the biomechanical hypothesis were correct, then rats should have used the strain-typical pattern of defense when attacked by same strain partners and some modified version when attacked by opposite strain partners. However, it is possible that rats could undergo experience-based learning, which would enable them to modify their pattern of defense to deal with the idiosyncrasies of the attacks by partners of the opposite strain better. Experiments 2 and 3 should detect such experience-based modifications. Nonetheless, while this modified brain hypothesis would account for experience-based changes in defending against an opposite strain attacker, it would still predict that when defending against a same-strain attacker, the pattern of defense should remain strain-typical. The modification should only occur when rats have had prior experience with opposite strain partners, especially familiar ones.

3.3 Method

3.3.1 Subjects

A total of 96 male rats (48 LE and 48 SD) were used. The rats were obtained from Charles River Laboratories (St. Constant, Quebec) around 23 days of age and housed at the Canadian Centre for Behavioural Neuroscience. All subjects were randomly housed in quads with two animals in each serving as the experimental subjects. The quad design was used to allow for a comparative measurement of the experimental animals playing with a partner of the same strain and a partner of the different strain (see below). The rats were maintained at a constant 21-23°C on a 12:12-hour light-dark cycle and were kept in 46cm X 25cm X 20cm polyethylene tubs, with processed corncob bedding. Food and water were provided *ad libitum*. All animals were handled and cared for in accordance with the Canadian Council for Animal Care regulations.

3.3.2 Procedure

Play was tested between 31 and 35 days, which is within the peak period for play behaviour in rats (Thor & Holloway, 1984) but before the age at which dominance hierarchies begin to form in males (Pellis & Pellis, 1991; Takahashi & Lore, 1983). For 3 days before play testing, all animals were habituated, in their quads, to the testing enclosure for 30 minutes each day. Short periods of social isolation preceding testing increases the frequency of play fighting (Niesink & van Ree, 1989; Panksepp & Beatty, 1980); the present study used 24 hours of social isolation (B. T. Himmler et al., 2013a). Rats were tested twice, with a 24-hour rest period between the two test days in order to avoid having animals socially isolated longer than 24 hours. Test trials lasted for 10 minutes each, providing enough time to capture most aspects of playful interactions (B. T. Himmler et al., 2013a). Both habituation and testing occurred in complete darkness as it has been shown that social behaviours such as play increase when in the dark compared to light conditions (B. T. Himmler et al., 2013a).

3.3.3 Experiment 1: Same-Strain Rearing and Play with Unfamiliar Partners

Forty-eight rats were divided in 12 quads with each quad composed of rats of the same strain. Two of the rats from each quad were designated the experimental subjects and were tested with unfamiliar rats from other quads – one from the same strain and one from the opposite strain. In this way, one experimental rat from each quad was tested with a same strain partner and one experimental rat was tested with an opposite strain partner. That is, no rat was exposed to the unfamiliar partner more than once, ensuring that the behaviour of the rats was not influenced by previous experience with those partners. Three groups were tested with the same strain first, and then a day later with the opposite strain partner, and the other three experimental subjects were tested in the reverse order, so counter-balancing for any order or age effects.

3.3.4 Experiment 2: Mixed-Strain Rearing and Play with Familiar Partners

Twenty-four rats were divided in six quads with each quad composed of two LE and two SD rats. One LE and one SD from each quad were designated the experimental subjects and were tested with the other two quad members. In this way, each experimental rat was tested with an LE and an SD partner. As in Experiment 1, the testing of the quads was counter-balanced.

3.3.5 Experiment 3: Mixed-Strain Rearing and Play with Unfamiliar Partners

Twenty-four rats were divided in six quads with each quad composed of two LE and two SD rats. One LE and one SD from each quad were designated the experimental subjects and were tested with two members from a different quad. In this way, each experimental rat was tested with an LE and an SD partner. As in Experiments 1 and 2, the testing of the quads was counter-balanced.

3.3.6 Equipment

Play trials occurred in a 50cm X 50cm X 50cm plexiglass box encased in a soundproofed chamber (61cm X 61cm X 84cm). The inside of the plexiglass box was filled with 1-2cm of CareFresh® bedding. A DVD103 Sony Handycam was used to film all play trials at a 45° angle using the night-shot option to film in the dark.

3.3.7 Behavioural Analysis

Playful interactions were scored for attack and defense (Pellis & Pellis, 1987). A playful attack was scored when the tip of the snout of one partner was either in contact with its partner's nape or when one partner made a targeted movement towards the nape of the other. The recipient of the attack could either respond to the attack using one of many defenses or simply ignore the attack. Playful defense of the nape could take one of two major forms: (a) evasion, which involves the defender moving its nape away from its attacker and does so by running, jumping, or pivoting away, and (b) facing defense, in which the defender moves its nape away by turning to face its attacker in order to block access to its nape by opposing its teeth between its partner and its own nape. Facing defense can take one of three forms: (a) complete rotation, which involves the defender rolling completely over onto its back, (b) partial rotation, which involves the defender rotating its forequarters while maintaining contact with the ground with one or both of its hind feet, and (c) other, which involves rotations or other movements in other dimensions. The first 2-3 video frames were used to determine the type of defensive tactic first attempted by the defending rat rather than recording the eventual outcome of an attack (B.T. Himmler et al., 2013a).

To assess possible cross-strain effects on overall playfulness, the frequency of nape attacks and probability of defense against attacks was recorded (B. T. Himmler et al., 2013a). To test the predictions arising from the two hypotheses, the probability of evasive defense as a proportion of total defense and the probability of supine defense as a proportion of total facing defense was calculated (B.T. Himmler et al., 2013c; S. M. Himmler et al., 2014b).

3.3.8 Statistical Analysis

The data were analyzed from both experiments using a two-way analysis of variance (ANOVA), with identity of experimental subject (LE or SD) and identity of the play partner (LE or SD) as independent factors; for significant analyses of variance, the Least Significant Difference test with the Bonferroni correction was used for subsequent pair wise comparisons. Differences were considered significant for p values of ≤ 0.05. For graphical representation of the data, values are given for group means and standard error.

3.3.9 Validation of Testing Protocols and Behavioural Analyses

The experimenter (Himmler) who performed the behavioural analysis for this study had previously scored with a high degree of inter-observer reliability in two previous studies (B. T. Himmler et al., 2013c; S. M. Himmler et al., 2014b). Given the consistency across studies in inter-observer reliability, it can be concluded that any significant differences in the current study were due to differences in manipulation of rearing rather than inconsistent behavioural scoring.

3.4 Results

3.4.1 Experiment 1

For all analyses of variance, three factors were compared, the identity of the performer, the identity of the partner and their interaction. A 2 X 2 ANOVA for the frequency of launching nape attacks revealed a significant effect for identity of attacker ($F(1, 20) = 5.780$, $p = 0.026$), but not for the identity of the partner; nor was there a significant interaction. SD rats launched more nape attacks than LE rats irrespective of the partner's identity (Figure 3.1A). There were no main effects for the probability of defense ($F(1, 20) = 2.816, p = 0.095$) (Figure 3.1B).

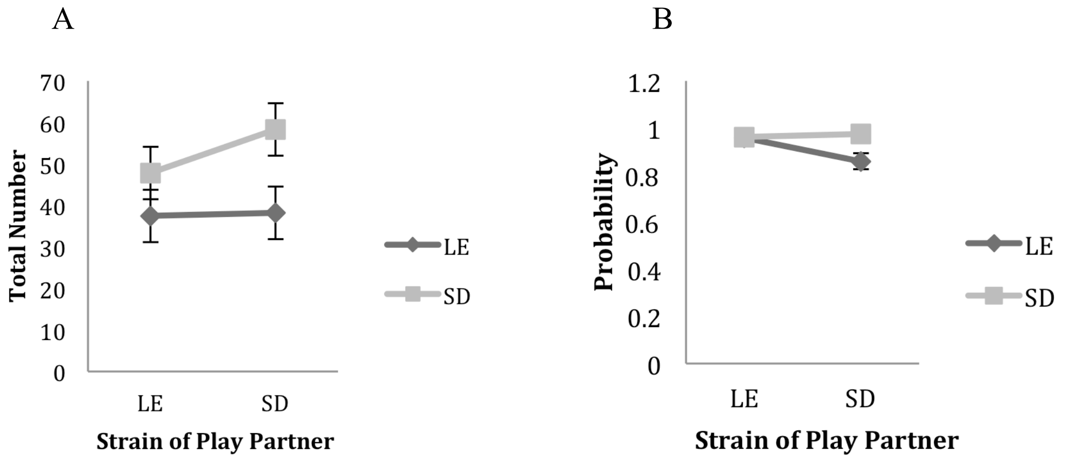

Figure 3.1. (A). The total number of playful attacks per 10 minute play trial. (B). The probability of defense against a playful attack.

A 2 X 2 ANOVA for the probability that a defending rat would use the complete rotation tactic revealed a significant difference for the identity of the defender ($F(1, 20) = 47.115$, $p = 0.0001$), but not for the identity of the partner, nor was there an interaction. LE rats were more likely to use the complete rotation tactic than SD rats irrespective of partner (Figure 3.2A). For the probability that the defending rat would use the evasion tactic there was a significant difference for the identity of the defender ($F(1, 20) = 90.658$, $p = 0.0001$), and for the ide B of the partner ($F(1, 20) = 12.674$, $p = 0.002$), but there was no significant interaction. SD rats were more likely to use evasive tactics than LE rats irrespective of the identity of the partner (Figure 3.2B). Moreover, Bonferroni corrected pairwise comparisons showed that being attacked by an SD partner was more likely to lead to evasive defense in both LE and SD rats ($p < 0.05$).

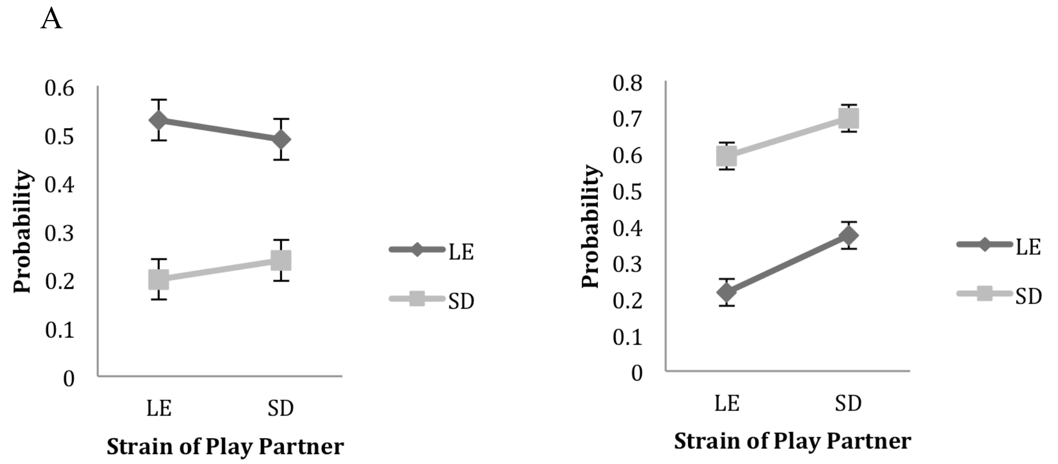

Figure 3.2. (A). The probability of using the complete rotation defense in response to a playful attack. (B). The probability of using evasive defense tactics in response to a playful attack.

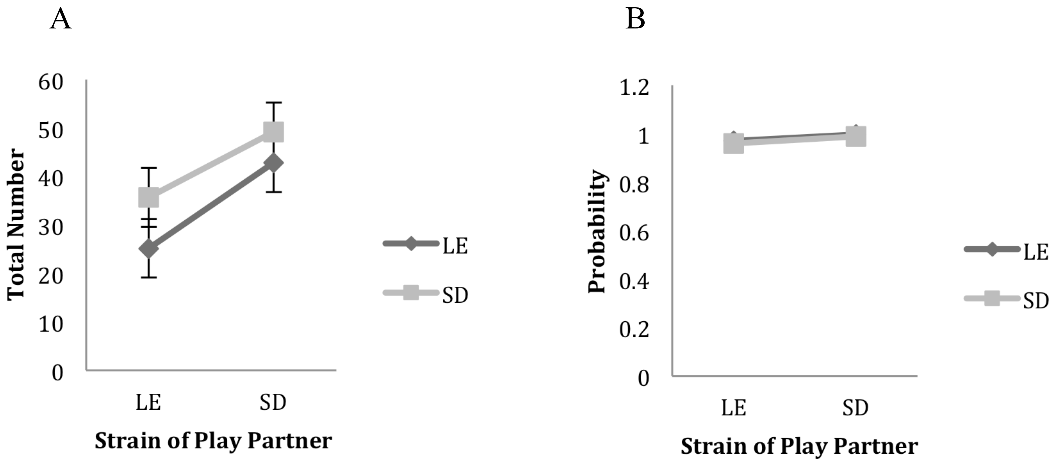

Figure 3.3. (A). The total number of playful attacks per 10 minute play trial. (B). The probability of defense against a playful attack.

3.4.2 Experiment 2

A 2 X 2 ANOVA for the frequency of launching nape attacks revealed a significant difference for the identity of the attacker (*F*(1, 20) = 6.617, *p* = 0.018), but not for the identity of the partner; nor was there a significant interaction. SD rats launched more nape attacks irrespective of the identity of the partner (Figure 3.3A). There were no main effects for the probability of defending against nape attacks (*F*(1, 20) = 0.017, *p* = 0.897) (Figure 3.3B).

Similarly, there were no significant differences for the probability that a defending rat would use either the complete rotation tactic (*F*(1, 20) = 1.075, *p* = 0.312) (Figure 3.4a) or evasion (*F*(1, 20) = 0.173, *p* = 0.682) (Figure 3.4b).

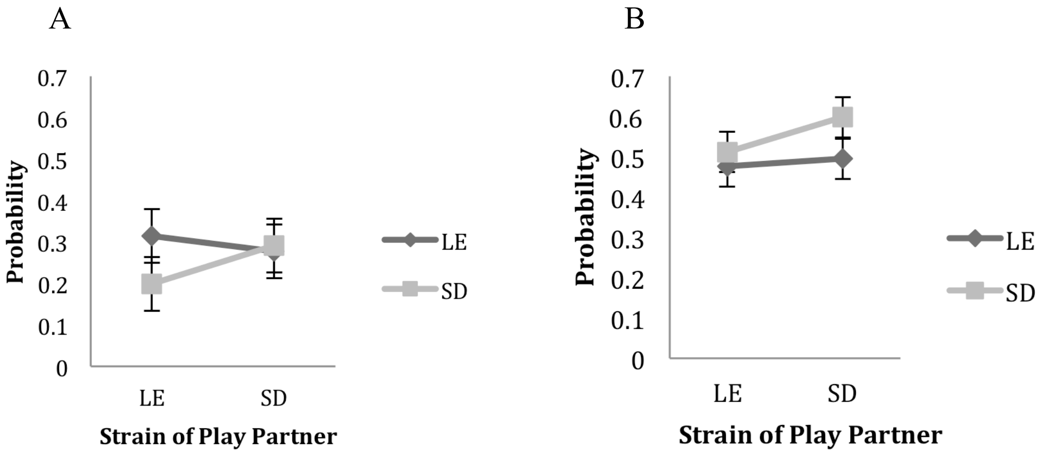

Figure 3.4. (A). The probability of using the complete rotation defense in response to a playful attack. (B). The probability of using evasive defense tactics in response to a playful attack.

3.4.3 Experiment 3

A 2 X 2 ANOVA for the frequency of launching nape attacks revealed a significant difference for the identity of the attacker (*F*(1, 20) = 12.629, *p* = 0.002), but not for the identity of the partner; nor was there a significant interaction. SD rats launched more nape attacks irrespective of the identity of the partner (Figure 3.5A). A

2 X 2 ANOVA for the probability of defending against nape attacks revealed a significant difference for the identity of the attacker ($F(1, 20) = 6.942$, $p = 0.016$), but not for the identity of the partner, nor was there a significant interaction. SD rats were more likely to defend against a playful attack from either strain of partner (Figure 3.5B).

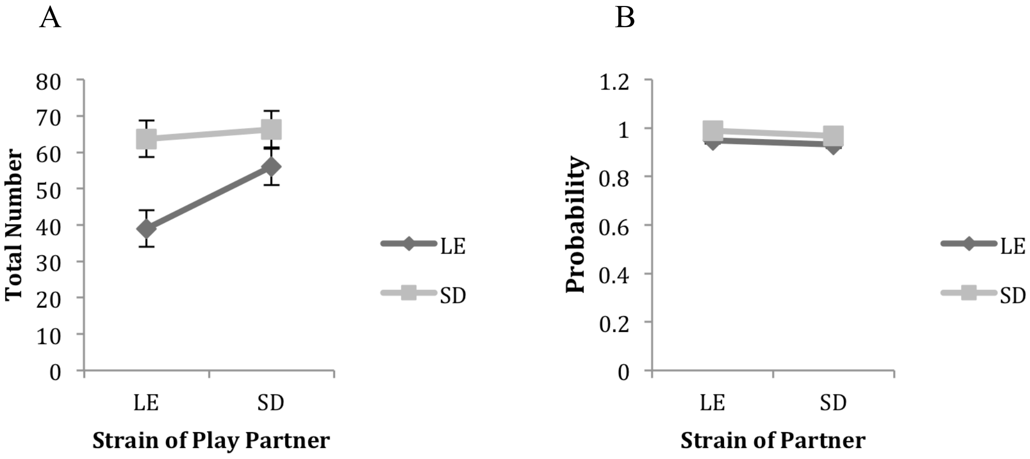

Figure 3.5. (A). The total number of playful attacks per 10 minute play trial. (B). The probability of defense against a playful attack.

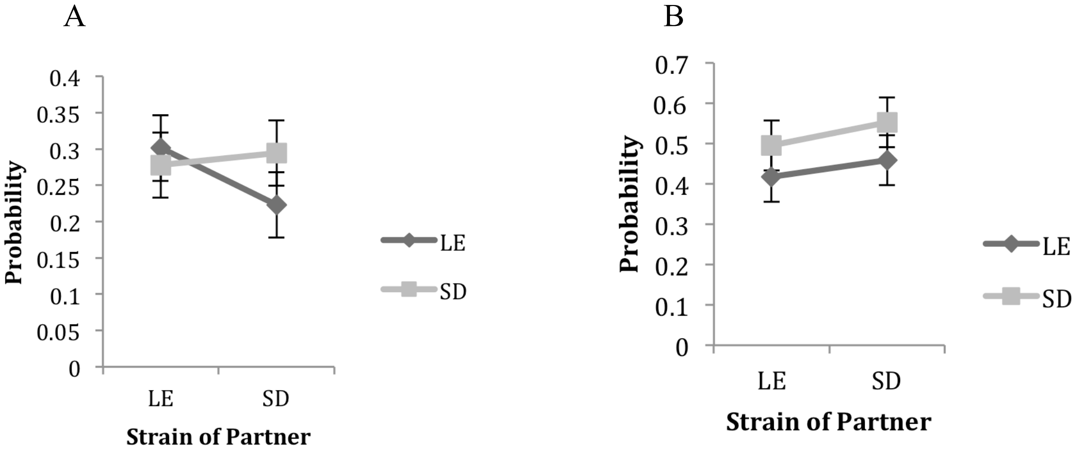

Figure 3.6. (A). The probability of using the complete rotation defense in response to a playful attack. (B). The probability of using evasive defense tactics in response to a playful attack.

There were no significant differences for the probability that a defending rat would use either the complete rotation tactic (*F*(1, 20) = 0.285, *p* = 0.600; Figure 3.6A) or evasion (*F*(1, 20) = 1.957, *p* = 0.177; Figure 3.6B).

3.5 Discussion

The present study was designed to test whether the strain-typical patterns of playful defense in rats is fixed by strain-typical brain mechanisms or arises from strain differences in how partners attack. For motivation to initiate play, as measured by the frequency of nape attacks (Panksepp, 1981; Thor & Holloway, 1983), there were no rearing and testing differences, in all cases SD rats launched more attacks than LE rats. For defensive responses to such attacks, SD rats tended to defend more frequently in all cases, with the difference reaching significance in Experiment 3. Thus, irrespective rearing experience and strain of the partner, with regard to attack and defense, the rate was higher in SD rats as shown in previous studies (B. T. Himmler et al., 2013c; S. M. Himmler et al., 2014b). It is how the rats defended themselves that specifically tested whether type of defense is based on a strain-typical preference or a byproduct of how different strains attack.

The findings from Experiment 1 were consistent with the brain hypothesis as all animals maintained their strain-typical patterns of playful defense regardless of the strain of the attacking partner. Although LE rats showed an increase in evasive tactics when playing with an SD partner, this frequency of evasion was not as high as in SD rats. This suggests that, while subtle differences in patterns of attack by different strains may have a small influence on some tactics, the biomechanics of attack is not sufficient to affect the strain-typical preference for using particular defensive tactics. Indeed, the values for percentages of using evasion and supine defense are in the strain-typical range established in previous studies (B. T. Himmler et al., 2013c, S.M. Himmler et al., 2014b). This being the case, it would mean that the diversification of defensive tactics among strains following domestication (B. T. Himmler et al., 2013c, S. M. Himmler et al., 2014b) would likely have involved differential changes in the neural mechanisms that regulate different facets of play behaviour (e.g., Kamitakahara et al., 2007; Siviy et al., 2011). That is, strain differences in defense involve differing biases for use of the different tactics of defense, with the neural mechanisms regulating those biases being changed accordingly. While the results from Experiment 1 clearly support this conclusion, the results from Experiments 2 and 3 suggest that more complex developmental processes in producing strain-typical patterns are involved.

The findings from the second and third experiments were not consistent with either the brain hypothesis or the biomechanical hypothesis. The biomechanical hypothesis predicted that, if strain differences are due to differences in attack styles, then these rats, just like those in Experiment 1, would defend in the strain-typical manner when tested with a same strain partner, but in a modified form when playing with an opposite strain partner. Given that Experiment 1 was consistent with the brain hypothesis, any change in behaviour when defending against opposite strain partners in Experiments 2 and 3 would be more consistent with a modified brain hypothesis – prior experience with the play of the opposite strain modifies how rats defend against strain discordant partners. Thus, defending in a strain-typical way with same strain partners and in non-strain typical ways with opposite strain partners could be consistent with both hypotheses. Yet the changes in defense seen in Experiments 2 and 3 were not predicted by any version of the two hypotheses.

In Experiments 2 and 3, the rats had eight days of cohabitation experience with both same and opposite strain partners before being tested, so providing them with experience of the differences in play typical of the two strains. Yet, while SD rats maintained the higher frequency of attacks and probability of defense compared to LE rats as was seen in same strain reared animals, rats from neither strain maintained their strain-typical patterns of defense. When playing with an LE or SD partner, LE rats displayed lower frequencies of complete rotation and higher frequencies of evasion than seen in LE rats from Experiment 1 and from the previous studies (B. T. Himmler et al., 2013c; Kamitakahara et al., 2007; Pellis & Pellis, 1987). Similarly, when playing with either a LE or SD partner, SD rats displayed higher frequencies of complete rotation and lower frequencies of evasive tactics than SD rats from Experiment 1 and from a previous study (S.M. Himmler et al., 2014b).

For Experiments 2 and 3, defensive behaviour patterns in both same-strain and different-strain pairings were not strain-typical, but rather, the playful defense of both LE and SD subjects converged at a level intermediate between the two strains (Experiment 1; B. T. Himmler et al., 2013c, S. M. Himmler et al., 2014b). This convergence was present whether the rats were tested with familiar (Experiment 2) or unfamiliar (Experiment 3) partners, increasing the likelihood that the difference with the results from Experiment 1 and from previous studies (B. T. Himmler et al., 2013c; S. M. Himmler et al., 2014b) was due to the effects of being housed with partners of both strains. These findings suggest that the strain-typical biases for using particular tactics can be modified by social experiences in the first week of the juvenile period.

For LE and SD rats, the strain typical pattern of defense emerges whether housed in same sex pairs, quads or whole, mixed sex litters (e.g., Experiment 1 in this study; B. T. Himmler et al., 2013c, S. M. Himmler 2014b; Pellis & Pellis, 1987, 1990, 1997). The critical difference between the rats in Experiments 2 and 3 compared to Experiment 1 appears to be that those rats experienced living with and interacting with rats of both strains from 24-31 days of age. Play fighting begins to emerge in the week preceding weaning at about 16-17 days of age (Bolles & Wood, 1964; Pellis & Pellis, 1997) and reaches its peak frequency of occurrence between 30-35 days of age (Meaney & Stewart, 1981; Panksepp, 1981; Pellis & Pellis, 1990; Thor & Holloway, 1984). Even though playful interactions begin as early as 16 days of age, they do not reach the fully strain-typical pattern until about 30 days of age. In LE rats, patterns of facing defense leading to supine are favored, with the strain-typical pattern of infrequent evasion and highly frequent complete rotation gradually maturing through the weaning to the early juvenile period (Pellis & Pellis, 1997). The early post-weaning period, when the rats were formed in mixed strain quads for Experiments 2 and 3, represents an age when the juvenile-typical pattern of play is still maturing and so may be susceptible to novel social experiences.

The juvenile period is critical for brain and behavioural development, making animals in this stage of life particularly sensitive to a variety of experiences, especially social ones (e.g., Baarendse et al., 2013; Einon & Morgan, 1977; Einon et al., 1978; Hall, 1998; Van den Berg et al., 1999; Von Frijtag et al., 2002). A series of studies on the effects of social isolation at different times during post-weaning development not only supports the view that the juvenile period is critical for neurobehavioural development, but that the first week of post-weaning is particularly critical for the development of social behaviour (Arakawa, 2002, 2003, 2007a,b). In addition, Meaney and Stewart (1981a) compared the play behaviour of adult rats reared in mixed sex social groups compared to same sex groups as juveniles. The behaviour of the females remained relatively consistent across rearing conditions, whereas that of the males differed. The males reared in mixed sex groups had a bigger age-related decrease in play than did those reared in same sex groups. In combination, these studies suggest that the type of partners experienced during the juvenile period can affect the development of social behaviour and that the early phase of post-weaning is likely a highly critical time for these experiences to affect brain and behaviour.

3.6 Conclusions

Strain differences in play behaviour appear to be determined by brain mechanisms that control preferences for particular patterns of defense, as animals that have been reared with animals of the same strain will exhibit their strain-typical patterns of play regardless of the strain of the partner (Experiment 1). However, brain mechanisms regulating strain differences appear to be vulnerable to change during the early juvenile period - indeed, so much so, that living with a different strain during the early juvenile period produces atypical strain behaviour during play (Experiments 2 and 3), further supporting that, during the juvenile period, behaviour is modifiable by novel social experiences. Thus, while it can be concluded that strain differences in playful defense do not arise from biomechanical differences but from brain mechanisms regulating biases in tactics used, it is possible that playful experience with same strain partners is necessary for those brain biases to be established.

Chapter 4: Future Directions

The most important finding from this thesis is with regard to how play develops. The development of juvenile-typical play in rats does not depend on the experience of immature forms of play during the peri-weaning period. These findings lead to the hypothesis that play in the juvenile period is so important for refining social skills that its pre-juvenile development is robust. That is, the role of play in the juvenile period is so critical to refining social skills, and the associated brain mechanisms, that its development is not susceptible to the influence of variable social experiences in maturing the typical form. While my findings on the development of the overt behaviour performed during play by juveniles are consistent with this hypothesis, there is one potential untested assumption with this hypothesis.

Although the data show that the overt behaviour used during play develops seemingly normally in the absence of peri-weaning play experience with peers, it may be possible that the peri-weaning experiences prime the brain mechanisms to be responsive to the experiences generated by juvenile play. There is precedence for such priming, as various pre-juvenile experiences can influence later responsive of the prefrontal cortex to new experiences and to subsequent responses to brain damage (Muhammad, Carroll, & Kolb, 2012; Muhammad, Hossain, Pellis, & Kolb, 2011). That is, the play-induced pruning of the medial prefrontal cortex that arises due to the experiences derived from playing with peers in the juvenile period (Bell et al., 2010; B.T. Himmler et al., 2013b) may depend on priming the medial prefrontal cortex during the peri-weaning period by play experiences with peers.

This possibility could be tested by rearing female Long Evans rats in one of two conditions, alone with a mother or with a mother and peers, from 15-27 days old (as in B.T. Himmler et al., 2015). Then between 28-60 days each of the two groups would be split, with half the animals being housed singly with an adult female only and the other half being housed singly with another same sex peer (as in B.T. Himmler et al., 2013). At 60 days old the brains would be harvested and used for Golgi analysis of dendritic branching and length in the medial prefrontal cortex (as in Bell et al., 2010). Previous studies have found that Long Evans rats deprived of play from weaning until early adulthood have more complex cells in the medial prefrontal cortex (Bell et al., 2010; B.T. Himmler et al., 2013b) (Figure 4.1A). From these studies it has been suggested that play promotes a reduction of complexity in these cells thereby promoting increased behavioural flexibility (B.T. Himmler et al., 2014; Pellis et al., 2010a).

If peri-weaning play experiences with peers are not important to these juvenile induced brain changes, it would be expected that the animals not reared with peers from 15-27 days old would show dendritic pruning if they had played with a peer in the juvenile period, similar to that seen in rats that have been reared with peers from birth to 60 days old (Figure 4.1B). However, if peri-weaning experiences are necessary to prime the medial prefrontal cortex to then be responses to the juvenile play experiences, it would be expected that no such pruning would occur (Figure 4.1C).

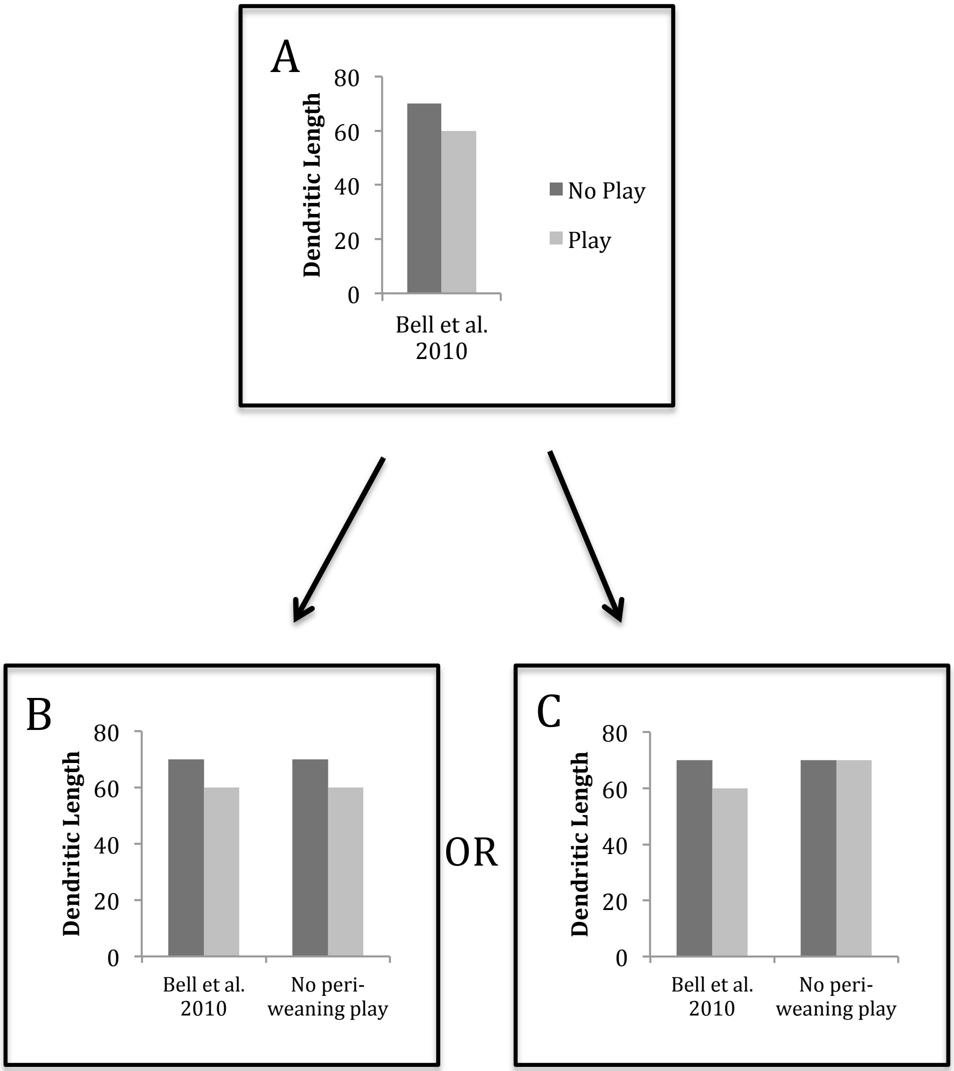

Figure 4.1. This figure illustrates the two possible outcomes of the above proposed study. (A) Displays results for dendritic length as found by Bell et al., 2010; (B) Shows what the results would look like if peri-weaning play experiences are unimportant for the development of the medial prefrontal cortex; (C) shows what the results would look like if peri-weaning play experiences are important to the development of the medial prefrontal cortex.

Even though peri-weaning experience with play is not needed for the normal development of juvenile-typical play, some aspects of that juvenile-typical play can be altered in rats by being reared in mixed strain housing in the week following weaning. This suggests that while no peri-weaning play experiences leads to normal juvenile play, experience with discordant forms of play do alter the development of juvenile-typical play (see Chapter 3). However, even though some aspects of playful defense are altered, the important experiences generated during juvenile play, as reflected by unchanged frequencies of role reversals, remain unchanged (see Chapter 1). These findings may be explained by two possibilities. (1) Given that role reversals are an index of the play-induced experiences that change the brain (Pellis et al., 2010a), and these are the same whether cross-reared or same strain reared, it is possible that these rearing condition-induced changes in play are a means of adapting the juveniles' play to ensure that their play can generate the appropriate experiences. Counter to this possibility is that same-strain reared rats playing with different strain rats are also able to generate the same frequency of role reversals (see Chapter 1), which suggests another possible explanation. (2) Within certain boundaries of modified expression of play some discordant experiences can deviate the normal development of play but not in a non-adaptive way. The results from the cross rearing experiment may be an artifact of the early age at which these discordant experiences were encountered, that is, an age when the brain is still malleable.

This could be tested by having Long Evans rats cross reared with Sprague-Dawley rats in blocks of 7 days, the effective age for changes in play (see Chapter 3), over different developmental periods in order to determine if the same cross-rearing effects are seen at all ages through development or if there is a critical period during which rats are susceptible to these behavioural changes. One set of animals could be cross-reared just as play is beginning to emerge at 15-21 days old. If these animals display similar changes in their play to the animals that have been cross reared from 24-31 then it would be the case that this earlier period is also susceptible to discordant play experiences. Once this has been established groups of rats could be cross-reared when 34-41 (Figure 4.2A), 44-51 (Figure 4.2B) and 54-60 (Figure 4.2C) days old – thus spanning the entire juvenile and adolescent period. If it is the case that the pre-juvenile period is the only period critical to the development of play it would be expected that animals cross reared later in life would show no changes in their play patterns. Alternatively, cross rearing could have a reduced effect over time with animals being cross reared at 54-60 days old displaying less changes in play patterns compared to those at 34-41 days old. By doing this it could be determined whether or not rats are subject to changes in their play patterns at particular ages or if play is a malleable behaviour throughout the lifespan.

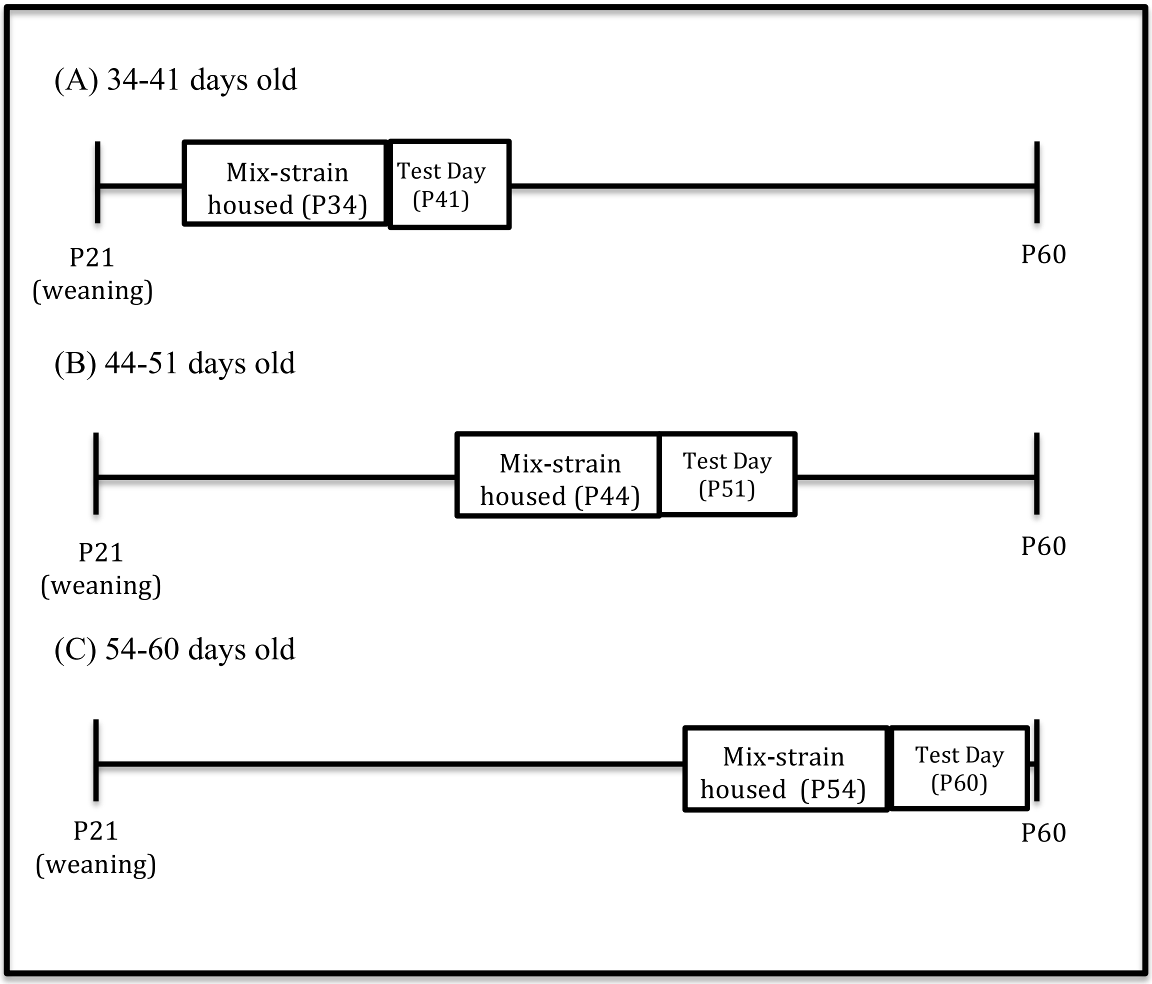

Figure 4.2. The figure shows the age blocks from the juvenile and adolescent periods over which exposure to non-strain cage mates would be tested.

Together these studies could help gain insight in to when play is modifiable by atypical social experiences with peers and the relationship between the development of playful behaviour and medial prefrontal cortex. In turn, better understanding of how play and brain mechanisms are influenced by play co-develop could provide clues on how play has evolved (Chapter 1; Pellis et al., 2014). In studying play, it is clear that a fully comprehensive understanding will only emerge by integrating all four of Tinbergen's questions.

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6. Appendix A

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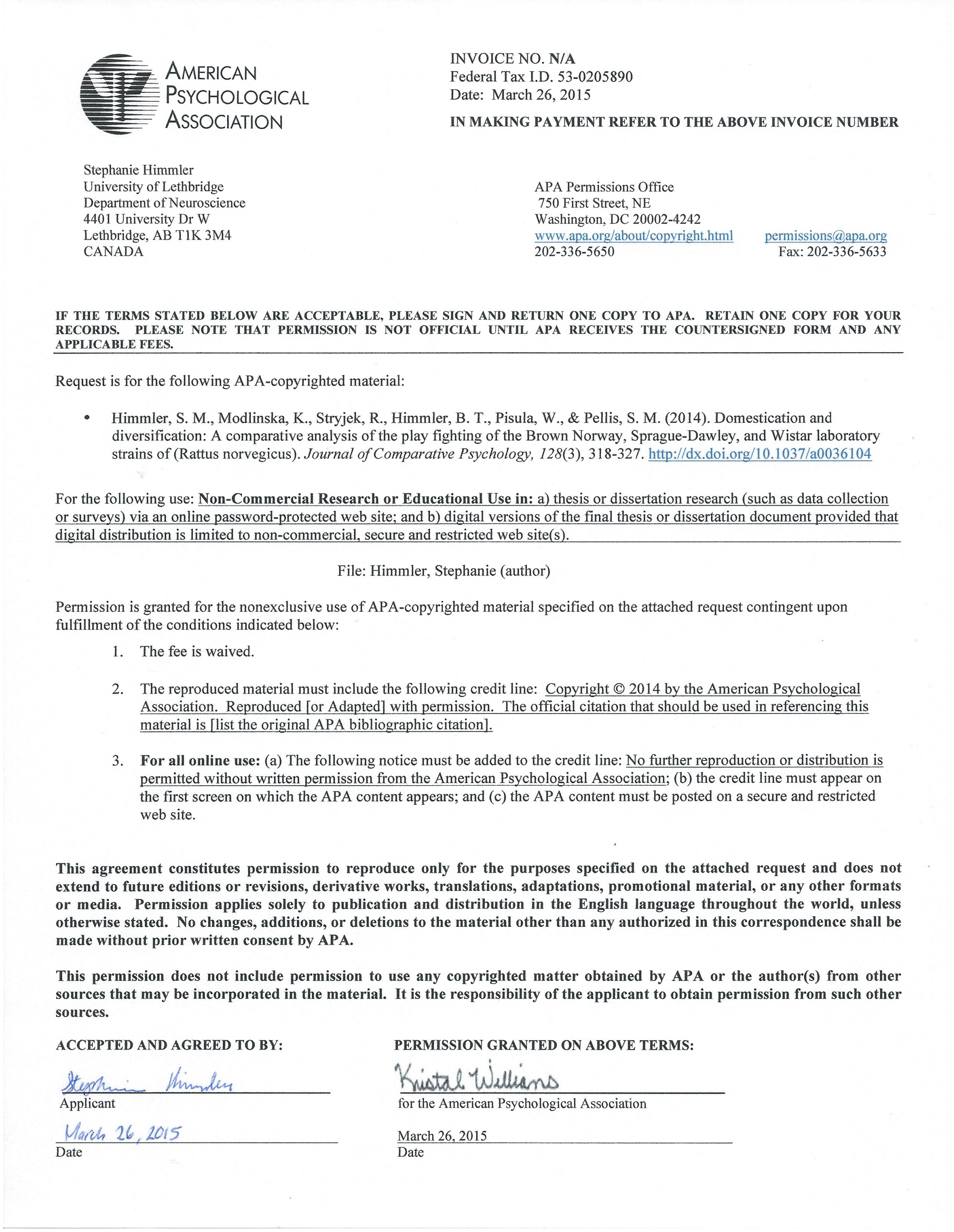

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