

THE PUBERTAL TRANSITION IN THE PLAY FIGHTING OF MALE RATS:
DEVELOPMENTAL BYPRODUCT OR ONTOGENETIC ADAPTATION?

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DEDICATION

To my family and friends, who were always supportive, even when they had no idea what I was doing or why I would even bother to do it.

ABSTRACT

Play fighting is a common behaviour among juveniles of many mammalian species, including rats (*Rattus norvegicus*). The mechanisms underlying the change that occurs in the play fighting of male rats at puberty were the focus of this thesis. It was found that castration at weaning disrupted the formation of male-typical dominance relationships, but did not alter the pubertal changes in play fighting, whereas neonatal castration prevented the shift from juvenile- to adult-typical behaviour at puberty. This transition is male specific and cannot be induced in females by exposing them to more extreme social contexts, involving interactions with unfamiliar males. The change in play by males at puberty is not, then, a byproduct of other sex differences, but results from a highly specific mechanism in early infancy. The significance of this sex difference is explored with respect to the functions of play fighting in rats and other species.

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First they tell you you're wrong, and they can prove it.
Then they tell you you're right, but it's not important.
Then they tell you it's important, but they've known it for years.
C. F. Kettering

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CHAPTER 1

GENERAL INTRODUCTION

What is play? No behavioral concept has proved more ill-defined, elusive, controversial, and even unfashionable.

E. O. Wilson, 1975, p. 164

What is Play?

The answer to this question has long eluded students of this enigmatic behaviour. Rarely do we encounter a behaviour so easily recognized, yet so hard to define. There are likely as many definitions as there are species which exhibit play. The term is often treated as a "garbage pail" (Burghardt & Bekoff, 1978, p. 318), with a number of behaviours which do not fit into other categories tossed in. Despite the difficulties inherent in studying play, we are still fascinated by it and believe that the knowledge gained will be well worth the effort. Hence, play research has been dubbed, "the ugly duckling of behavioral science" (Fagen, 1981, p. 33).

Many researchers believe that in order to study a behaviour you must first define it (Martin & Caro, 1985). This has not proven to be an easy task. Part of the problem in defining play lies in the term itself. Play often brings

with its anthropomorphic connotations. Play is the opposite of work, and because animals do not work, they cannot play, in the human sense of the word (Loizos, 1966). The word play also tends to be overused. By some definitions it encompasses all activities of juvenile animals and, thus, is too broad to be of any practical use (Martin, 1984; Martin & Caro, 1985). Other definitions, in contrast, are too narrow and, hence, difficult to generalize. Much of the confusion stems from the focus on either the structure or function of play (Fagen, 1974). Structuralists are concerned with the form, appearance, and physiology of play, whereas functionalists look for consequences and purposes (Fagen, 1974). Definitions based on structure are difficult, because of the vast variability in play. Species, sex, age, and other differences make it hard to develop a common classification (Bekoff & Byers, 1981). Defining play in terms of function is also difficult because the functions of play are not yet known (Bekoff & Byers, 1981) and the majority of hypotheses have very little empirical support (Martin & Caro, 1985). Play can not be considered a general class of behaviour with obvious consequences, nor is there a single characteristic that is universal to all the behaviours labelled play (Martin, 1984; Martin & Caro, 1985). There are, however, a number of definitions of play that are widely used in the

literature; Fagen (1981, Appendix A) gives an extensive list of examples. Two of the most commonly cited definitions are: (Play is) "a behavior that functions to develop, practice, or maintain physical or cognitive abilities and social relationships, including both tactics and strategies, by varying, repeating, and/or recombining already functional subsequences of behavior outside their primary context" (Fagen, 1981, p. 65); and "play is all motor activity performed postnatally that appears to be purposeless, in which motor patterns from other contexts may often be used in modified forms and altered temporal sequencing" (Bekoff & Byers, 1981, p. 300). Fortunately, a precise, agreed-upon definition of play may not be necessary in order to continue the study of it, because play is so easily recognized (Caro, 1988; Loizos, 1966; Martin, 1984). The definitions in use should be considered 'working definitions' which are likely to improve with additional information (Allen & Bekoff, 1994).

While play may not have a clear definition, a number of characteristics can still be used to distinguish it (see Bekoff & Byers, 1981; Fagen, 1981 for lists of examples). For example, both of the definitions cited above suggest that one of the defining characteristics of play is that it incorporates modified forms of behaviours seen in other contexts. The

behavioural components from sex, aggression, and other contexts may be exaggerated, repeated, reordered, fragmented, and incomplete in play (Loizos, 1966). When incorporated into play, these activities are divorced from their original motivations (Loizos, 1966).

Because play shares many characteristics with other behaviours, such as sex and aggression, it is often difficult to distinguish it from those behaviours. Therefore, many researchers look for play signals, facial expressions, postures, vocalizations, or perhaps even scents that are unique to play (Bekoff, 1978; Fagen, 1981). Examples include the primate open-mouth play face and the play bow of many canids. These signals are used by playmates to denote that what they have been doing, are doing, or are about to do is play (Fagen, 1981; Pellis & Pellis, 1996). They may be used to solicit or maintain play. However, many of these play signals may not be unique to play and may serve different functions both during play and in other behavioural contexts (Pellis & Pellis, 1996). Another defining characteristic of play is its lack of agonistic or threat signals, such as flattened ears and bared teeth (Aldis, 1975; Barber, 1991; Fagen, 1981; Smith, 1982). Once again, the presence or absence of signals is unlikely to be an unambiguous marker of play, as these threat signals are sometimes present in the play of many

species (Pellis & Pellis, 1996). Thus, play may not be as easily recognized as first thought. Play researchers are well served by looking for a variety of different signals and paying close attention to the context in which the behaviours are observed.

Play is highly context specific. Even very playful animals will not play if hungry, injured, scared, or ill, thus play is most often engaged in when all essential needs, such as food and shelter, are fulfilled (Fagen, 1981; Loizos, 1966; Martin & Caro, 1985). An individual may play differently depending on habitat; more chasing in an open field, but more wrestling in a more confined area, for example (Hole, 1988; Müller-Schwarze, 1984). Also, play may differ depending on the age, sex, or even species of the playmates (Fagen, 1981). As well, the behaviours observed between two young siblings playing near their mother is likely not the same as that seen between two adults when a limited resource is at stake, regardless of the similarities.

Despite the problems of definition three main categories of play have been described: locomotor, object, and social (Fagen, 1981; Hole & Einon, 1984; Smith, 1982). Locomotor play involves activities that move an animal about its environment. It is often a solitary endeavor, such as running,

jumping, leaping, crawling, dangling, or somersaulting (Smith, 1982). However, it may involve others either directly, that is chasing, or indirectly, such as when animals near each other begin to exhibit the same behaviour in a seemingly contagious manner (Bekoff, 1978; Bolles & Woods, 1964). Object play may also be done alone or with others (Fagen, 1981). The object may be the focus of play, like the ball of string batted around by a kitten or the toy involved in two dogs' game of tug-of-war. Inanimate objects may also be used as tools or props in play, such as the dolls children use when playing house. Social play, by definition, involves more than one participant, and it may include both locomotor and object play (Martin & Caro, 1985). It often contains elements of sex, aggression, and predation (Bekoff & Byers, 1981; Fagen, 1981; Martin, 1984), such as, pouncing, mounting, biting, rolling, butting, parrying, batting, and even grooming (Smith, 1982). The most frequently observed form of social play is play fighting or rough-and-tumble play. Many would consider this the most recognizable type of play, characterized by wrestling, pinning, and chasing (Aldis, 1975; Fagen, 1981). Play fighting involves one animal trying to gain an advantage over another, often biting while avoiding being bitten (Aldis, 1975; Symons, 1978). According to Fagen (1981), play fighting is nonagonistic and cooperative, and unlike agonistic fighting, it does not settle disputes

over resources, result in the permanent dispersal of participants, nor change relative dominance ranks.

Play Fighting in Rats

Similar to other mammals (Fagen, 1981), the most common type of play in laboratory rats (*Rattus norvegicus*) is play fighting (Poole & Fish, 1975). Play fighting in rats contains both attack and defense components (Pellis & Pellis, 1987), is most often performed by pairs of animals (Poole & Fish, 1975), and occurs in bouts (Panksepp, 1981). To initiate a play fight, one rat contacts the nape of its partner with its snout, often by pouncing. This contact rarely involves biting, rather the attacker simply rubs its snout into the fur of its partner's nape. Meanwhile, the attacked animal attempts to block this nape contact and launch its own attacks at the original attacker's nape (Pellis & Pellis, 1987). Because both rats are attacking, defending, and jockeying for position, the resulting sequence of behaviours is often referred to as wrestling (Fig. 1).

The most common defenses used in response to a nape attack are evasions and facing defenses (Pellis, Pellis, & Whishaw, 1992). When evading, the defending animal turns away from the attacker and runs, leaps,

Figure 1. An example of a play fighting bout, or 'wrestling', in juvenile rats. The attacking animal contacts its partner's nape (a,b). The defending animal rolls over onto its back in order to avoid this contact (c), and is 'pinned' in this position by the attacker (d,e). Both animals attempt to contact the other's nape and defend against this contact (f-l). After freeing itself (m,n), the defending animal playfully attacks the original attacker (o).

Taken from Pellis & Pellis, 1987.

Figure 1 was removed due to the unavailability of copyright permission. The figure is described in the caption on page 8 of this thesis. Taken from **Pellis & Pellis, 1987**.

or swerves out of the attacker's reach (Fig. 2A). Facing defenses include: a) complete rotations, where upon contact, the recipient rotates around its longitudinal axis, cephalocaudally, to supine (Fig. 2B); b) partial rotations, where upon contact, the recipient rotates around its longitudinal axis, cephalocaudally, but stops at the pelvis, so that it maintains a standing position on one or both hindfeet (Fig. 2C); and c) other behaviours, where upon contact, the recipient adopts some alternative form of facing defense, such as standing upright (Pellis, Pellis, & McKenna, 1994) (Fig. 3). There are both sex and age differences in the frequency of play fight initiation (Fig. 4A). Males tend to playfully attack more often than females, and both sexes exhibit a peak in this behaviour during the juvenile phase of development (Meaney & Stewart, 1981a; Olioff & Stewart, 1978; Pellis & Pellis, 1990, 1991a; Poole & Fish, 1975; Thor & Holloway, 1984). Regardless of age or sex, however, nape attacks are rarely ignored (Pellis & Pellis, 1990). Nearly all play fight initiations are responded to defensively (Fig. 4B).

It is well known that most play occurs in juveniles. In many species, adults rarely, if ever, play, especially with each other (Bekoff & Byers, 1981). Adult rats play, but considerably less than they did prior to puberty (Adams & Boice, 1989; Pellis & Pellis, 1990, 1991a). Play fighting in this species begins

Figure 2. The most common types of defense in the play fighting of rats.

A. Evasion: the attacking animal contacts its partner's nape (a,b), and the partner (defending animal) swerves away to avoid this contact (c).

B. Complete rotation: following nape contact (a), the defending animal rotates cephalocaudally (b,c) until it is lying on its back, where it is 'pinned' by the attacker (d).

C. Partial rotation: upon contact of the nape (a,b), the defending animal begins to rotate, similar to a complete rotation, but stops short and keeps one or both hindfeet firmly planted on the ground (c).

Note that all playful attacks are directed towards the nape, regardless of the attacker's orientation to the defender: A. front, B. back, C. side.

Taken from Pellis, Pellis, & Whishaw, 1992.



Figure 2 was removed due to the unavailability of copyright permission. The figure is described in the caption on page 11 of this thesis. Taken from Pellis, Pellis, & Whishaw, 1992.

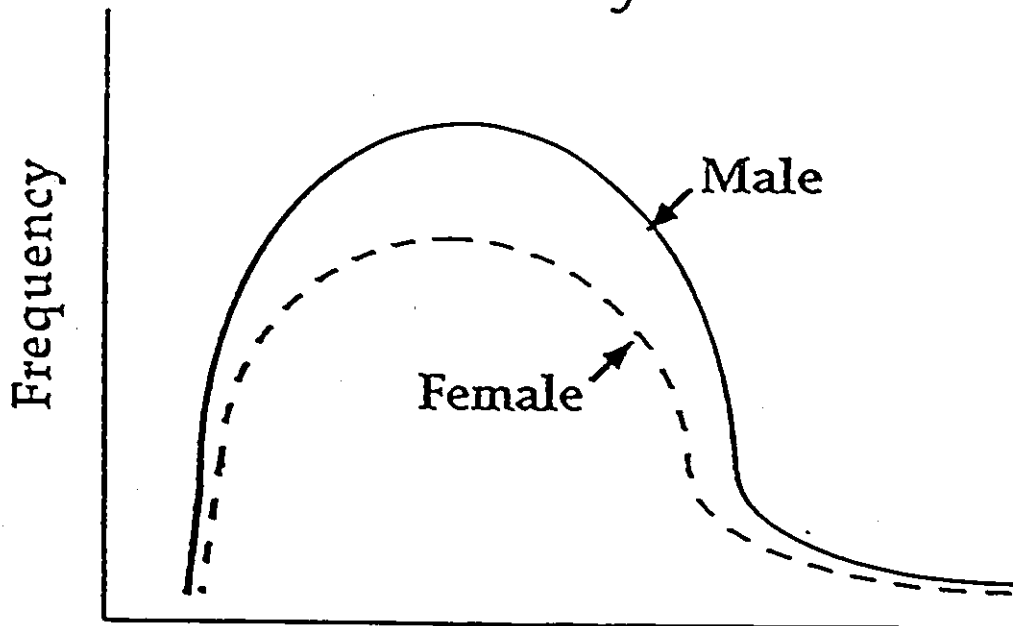
Figure 3. An example of an 'other' facing defense used by rats during play fights. Following nape contact (a), the defending animal partially rears and simultaneously rotates cephalocaudally to face the attacker (b,c). The defending animal then launches its own playful attack on its partner (d,e).

Taken from Pellis, Pellis, & McKenna, 1994.

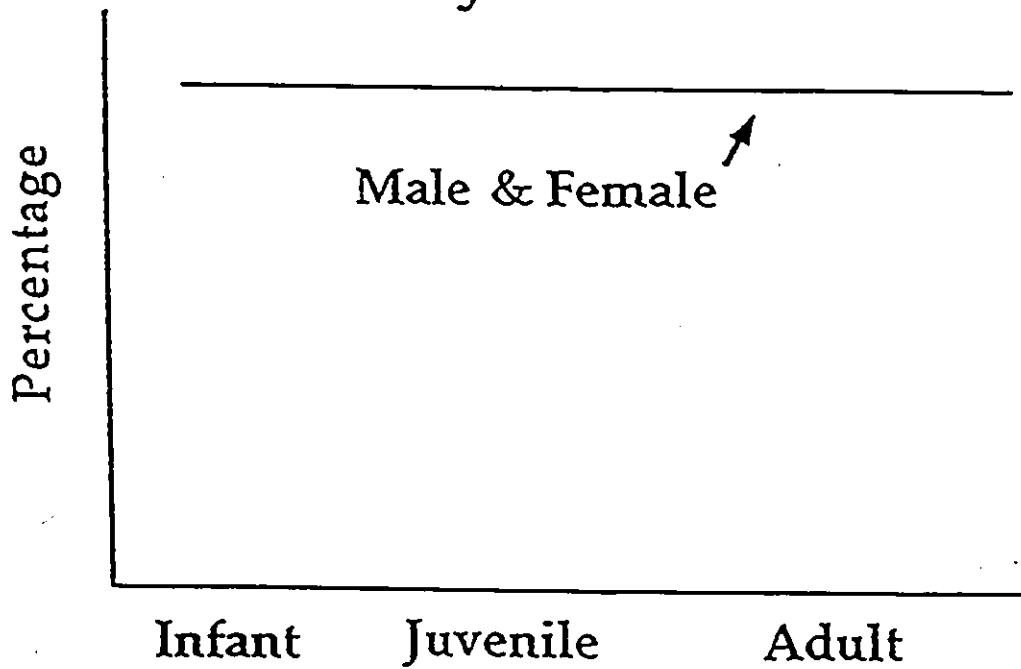
Figure 3 was removed due to the unavailability of copyright permission. The figure is described in the caption on page 13 of this thesis. Taken from Pellis, Pellis, & McKenna, 1994.

Figure 4. Diagrams of the frequency of playful attacks, or play fight initiations, for male and female rats as infants, juveniles, and adults (A), and the likelihood of male and female rats responding defensively to playful attacks as infants, juveniles, and adults (B).

A . Amount of Play



B . Probability of Defense



around 18 days of age (Bolles & Woods, 1964), peaks between 30-40 days and then declines markedly at puberty, between 50-60 days (Meaney & Stewart, 1981a; Panksepp, 1981; Takahashi & Lore, 1983; Thor & Holloway, 1985) (Fig. 4A). Play fighting in rats not only changes quantitatively with age, it also changes qualitatively. As infants, male and female rats predominantly use partial rotations when defending against playful attacks (Pellis & Pellis, in press a). Beginning shortly after weaning, at about 25 days of age, they switch to using more complete rotations, and by 30 days, this is the most frequently used defense (Pellis & Pellis, in press a). Upon reaching sexual maturity, males revert back to using mostly partial rotations, while females retain the juvenile-typical complete rotation frequencies (Meaney & Stewart, 1981a; Pellis & Pellis, 1987, 1990; Takahashi & Lore, 1983). Thus, females start with adult-typical defenses, then switch to juvenile-typical defenses and retain this pattern as adults (Fig. 5A). Males, in contrast, first utilize adult-typical defenses most often, switch to using mostly juvenile-typical behaviours, and then switch back to the adult-typical pattern (Fig. 5B). Because of the many physical and behavioural changes that an animal undergoes during development, it may often seem to be a totally different animal at every age (Bekoff, 1981). Thus, in order to fully understand a

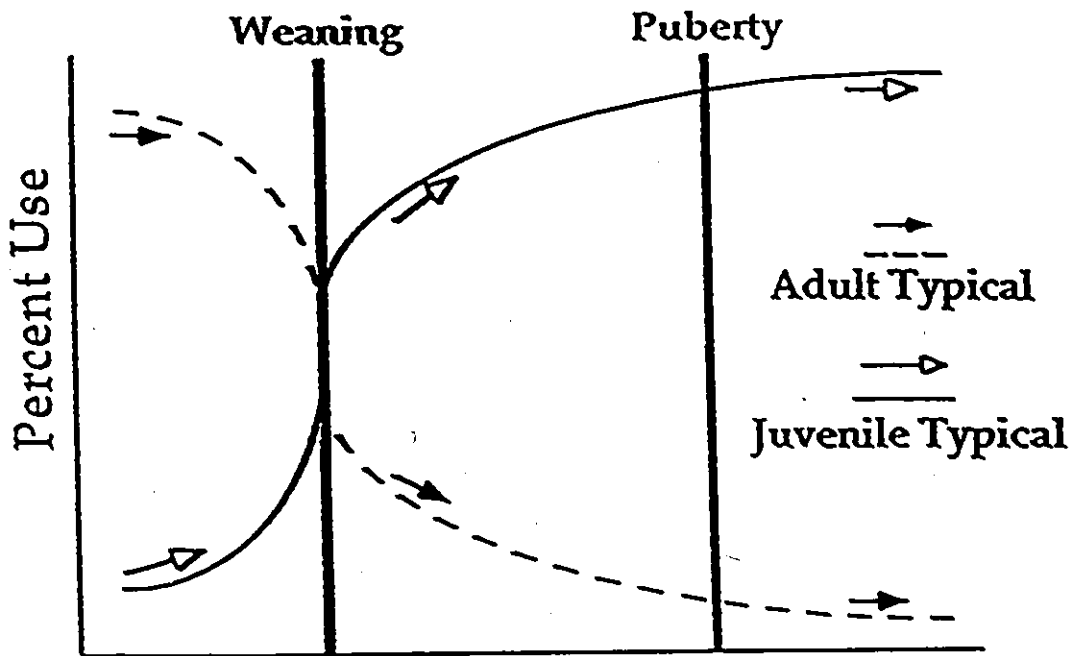
Figure 5. An illustration of the patterns of playful defense used by female (A) and male (B) rats as infants, juveniles, and adults. Weaning and puberty mark transitions in the predominant type of defense used.

Juvenile-typical defense = complete rotations

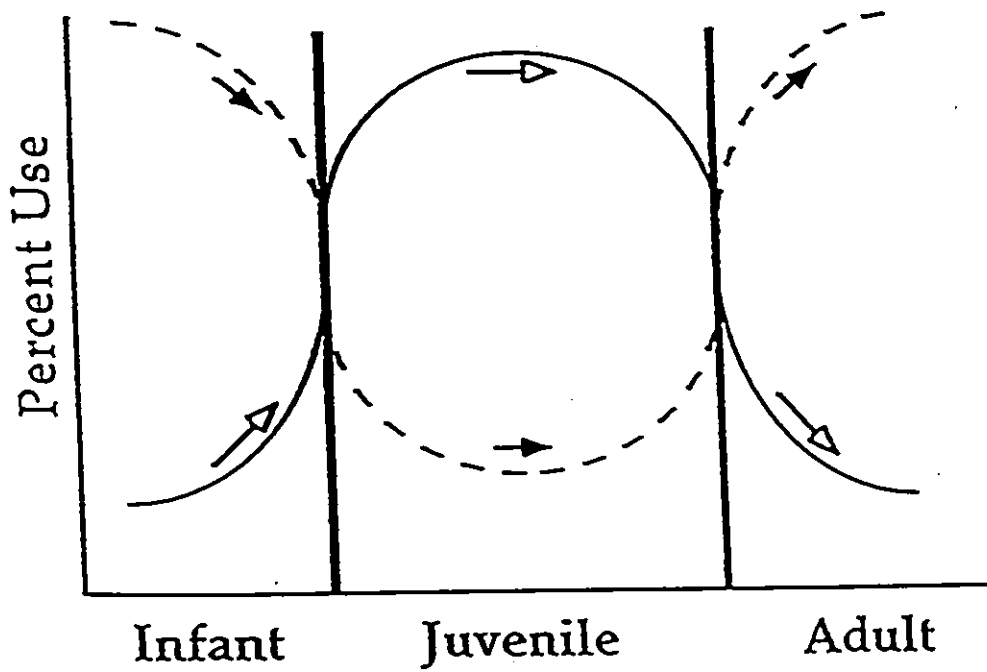
Adult-typical defense = partial rotations and 'other' defenses

Note that both males and females use both types of defense at all ages, but with varying frequencies.

A . Females



B . Males



behaviour like play, it is important to examine it from a developmental perspective.

Mechanisms Underlying Developmental Changes in the Play of Rats

The sex differences in frequency of play fighting have been shown to be generated by the actions of gonadal hormones in the perinatal period (Beatty, Dodge, Traylor, & Meaney, 1981; Meaney, 1988). Male rats undergo two major testosterone surges, one begins a few days before birth and continues for a few days after birth, while the other occurs at puberty (Ward & Weisz, 1980). It has been shown that castrating a male rat within the first week after birth decreases the amount of play fighting it engages in to female-typical levels, but castration after this critical period has no effect on the frequency of play fighting (Beatty et al., 1981; Meaney & Stewart, 1981b; Taylor, Frechmann, & Royalty, 1986). Injecting males with testosterone propionate at birth increases their play fighting to levels higher than that of normal males without affecting their defense (Pellis, Pellis, & Kolb, 1992). As well, injecting female rats with testosterone propionate increases their play fighting frequencies to male-like levels (Meaney & Stewart, 1981a; Thor & Holloway, 1986), and makes their playful defense more like that of males

(Pellis & McKenna, 1992; Pellis et al., 1994). Indeed, hormones can exert their influence on play even before the animals are born. Intra-uterine position and maternal stress, for example, can affect the amount of testosterone a fetus receives, and hence alter the organization of neural systems which feminize and masculinize later behaviours. For example, a female fetus positioned between two male fetuses will receive more testosterone than a female not between two brothers (vom Saal, 1989). This extra testosterone will affect organizational systems which may defeminize some behaviours and masculinize others making the female act more like a typical male. Maternal stress can feminize and/or demasculinize a male rat by decreasing the amount of circulating testosterone in the fetus prior to birth, and this can affect juvenile levels of play fighting (Ward & Stehm, 1991). Unlike the sex differences in frequency of play fighting, the sex differences in styles of defense during the developmental transitions (Fig. 5) have not been analyzed.

The transition in predominant playful defense following weaning marks the beginning of the juvenile play phase. Because this shift is common to both males and females (Pellis & Pellis, in press a), it is not likely to be the result of hormonal actions in a sex differentiated manner.

The change at puberty, in contrast, is specific to males and thus, may be linked to the testosterone surge at 50-60 days. Given this correlated change in both hormones and behaviour at puberty, it may be thought that the hormonal surge induces this change in behaviour. However, there are other sex-typical changes that occur at puberty which may also be involved in the change in playful defense by males. Upon reaching sexual maturity, male rats that have been housed together begin to establish dominance relationships, often in the absence of overt aggression (Blanchard, Spencer, Weiss, Blanchard, McEwen, & Sakai, 1995; Lore & Stipo-Flaherty, 1984; Pellis & Pellis, 1992, 1993). The behaviours of these animals change as they assume the roles of dominants and subordinates. Dominant males switch to using more adult-typical, partial rotations when defending during play fights. Subordinate males, on the other hand, retain the juvenile-typical defense of rolling into a supine position (Pellis & Pellis, 1992, 1993). Female rats demonstrate neither the pubertal shift in defense, nor male-typical dominance relationships (Pellis & Pellis, 1990). Therefore, there are at least three possible explanations for the relationships between the pubertal testosterone surge, the transition in playful defense, and the formation of dominance relationships: a) the surge in testosterone may cause the

formation of dominance, which in turn causes changes in play fighting; b) the surge in testosterone causes both the shift in defense and the formation of dominance, but the two behavioural changes are independent; or c) the three changes at puberty are correlated but not all may be causally connected. It is possible that all males undergo the pubertal surge, which stimulates the change in play fighting behaviour. As the animals interact, one becomes dominant and the other is forced to be subordinate, thus reverting to the juvenile-typical defense as a sign of submission (Panksepp, 1981).

The Objectives of this Thesis

The mechanisms involved in the pubertal transition in playful defense have not been previously explored. Therefore, three experiments were conducted in order to examine the effects of gonadal hormones and the influence of the identity of the play partner on this pubertal shift in defensive behaviour. Chapter two focuses on the effects of pre-pubertal castration on play fighting and dominance relationships in male rats. Previous work has shown that castration following the first week after birth has no effect on the frequency of play fighting (Beatty et al., 1981; Meaney & Stewart, 1981b). However, whether or not there are qualitative changes in

this play has not been studied. So, in the experiment outlined here, the aims were to discern if there were structural differences in the play of intact and post-weaning castrated rats, over time, and to test the link between dominance and the pubertal shift in defensive behaviour. In chapter three, the emphasis is on the pubertal shift in playful defense itself, and whether or not neonatal castration has an effect on it. Based on previous studies, it was expected that neonatal castrates should play less (Beatty et al., 1981; Meaney & Stewart, 1981b; Taylor et al., 1986), but once again, it was unknown if play fighting would be altered qualitatively. The experiment described in chapter four looks at how male and female rats play with both familiar and unfamiliar male and female partners. The focus here is on the females, to see if the male-typical pattern of play fighting can be induced by putting females in a more stressful social context, that of interacting with unfamiliar rats, especially males.

In chapter five, the results of these experiments are used to evaluate possible functions of play. It will be argued that the historical focus of play research on the long-term benefits of play has ignored the possibly more important functions derived from play, those of immediate adaptive value. The analysis of the mechanisms underlying the pubertal shift in defense by

males gives new insights into the immediate benefits that male rats may derive from post-pubertal play fighting. In addition, understanding this transition highlights the uniqueness of the juvenile play phase, and raises the possibility that play by juveniles benefits them directly at the time it is performed. That is, does play make juveniles better juveniles, and subadults better subadults, or does it simply make them better adults?

CHAPTER 2*

Dominance and Age-Related Changes in the Play Fighting of Intact and
Post-Weaning Castrated Male Rats (*Rattus norvegicus*)

Play fighting is a common form of play in the juveniles of many species (Fagen, 1981), including rats (Poole & Fish, 1975). In rats, the play fighting of both males and females involves one animal contacting the nape of its partner with its snout, and the partner using various defensive maneuvers to avoid such contact (Pellis & Pellis, 1987). If successful, the attacker rubs its snout into the fur of the recipient's nape. In the juvenile phase, the recipient is most likely to defend the nape by rotating into a supine position. While lying on its back, the animal may then push and kick to block the attacker's further attempts to gain access to the nape (Pellis, 1988). At puberty, the frequency of play fighting decreases in both sexes (Thor & Holloway, 1984) and the pattern of playful defense by males changes (e.g., Meaney & Stewart, 1981a; Pellis & Pellis, 1987, 1990; Takahashi & Lore,

*This chapter is modified from a paper published in *Aggressive Behavior*, 1996, 22(3):215-226.

1983). With the onset of puberty, the recipient is more likely to rotate only partially to supine, with the hindfeet remaining in contact with the ground. From this position, it can then adopt an alternative tactic, such as rearing to a boxing position or moving against the partner in a lateral orientation (Pellis, 1989; Pellis & Pellis, 1987, 1990). Playful attacks, however, involve nape contact at all ages (Pellis & Pellis, 1987).

Males typically engage in play fighting more often than females (Meaney, Stewart, & Beatty, 1985), and exhibit the above described age-related change in playful defense. Females, on the other hand, continue to exhibit the same pattern of play fighting post-pubertally as they did pre-pubertally (Pellis & Pellis, 1990). The sex differences in frequency of play fighting (Thor & Holloway, 1984), and possibly in the age-related change in pattern of playful defense (Pellis, Field, Smith, & Pellis, in press) arise from the action of androgens during the perinatal period (Meaney, 1988). Reduction of androgen exposure after this critical period, such as by castration at weaning, has been shown not to affect juvenile levels of play (Meaney, 1988), nor the age-related decline in frequency of play fighting (Thor & Holloway, 1984). Even though circulating androgens in the juvenile phase and adulthood are not necessary for some of the changes in

the play of males, it is still possible that androgens at the pubertal stage may be important for inducing some of the age-related changes in the playful defense of males. This could be achieved in two ways. The gonadal hormones could induce maturational changes in the use of defensive motor patterns. For example, Groothuis and Meeuwissen (1992) have shown that testosterone directly influences the maturation of agonistic displays in black-headed gulls. Alternatively, gonadal hormones at puberty may be necessary to induce the establishment of dominance-subordination relationships, which in turn could be responsible for the changes in the content of play fighting (Panksepp, Siviy, & Normansell, 1984; Pellis, Pellis, & McKenna, 1993). As adults, castrated males are less aggressive than intact males (Albert, Walsh, Gorzalka, Siemens, & Louie, 1986; Beatty, 1992; Leshner, 1981), and are often ranked lower in their social groups (Albert et al., 1986; Monaghan & Glickman, 1992). Furthermore, dominant animals often have increased testicular androgen production (Brain & Benton, 1983). Therefore, in the absence of testosterone-induced dominance relationships, the pubertal transition in playful defense may be absent.

When reared together, male rats do not seem to establish dominance relationships through overt aggression (Blanchard, et al., 1995; Lore & Stipo-

Flaherty, 1984). Nonetheless, differences in dominance appear to be reflected in asymmetrical patterns of play fighting. Playful contacts are initiated more often by subordinates (Pellis & Pellis, 1991b), and they continue to perform the more juvenile-like defensive pattern of rolling completely to supine when they are contacted, whereas dominants are more likely to adopt more adult-like styles of defense (Pellis & Pellis, 1992). When interacting together, adult subordinates perform the more adult-like patterns of defense, while when interacting with a dominant, they roll over to supine (Pellis et al., 1993). This demonstrates that the pattern of play is modulated by the relationship of the participants. Therefore, asymmetries in the pattern of play fighting are a useful measure of dominance-subordination relationships (Pellis & Pellis, 1991b, 1992, 1993).

Whether gonadal hormones act directly to enhance the maturation of male defensive patterns during play fighting or indirectly to induce the formation of dominance-subordination relationships, castration at weaning should abolish the transition to more adult-like defensive patterns at puberty. The present study suggests that neither hypothesis is correct. Rather, while the dominance-related asymmetries in post-pubertal play fighting depend upon the presence of gonadal hormones, the age-related

shift in the pattern of playful defense does not.

Materials and Methods

Subjects

Forty male Long-Evans hooded rats (*Rattus norvegicus*) from nine litters born and reared in the animal colony of the Psychology Department at the University of Lethbridge, Lethbridge, Alberta, Canada were used. The animals were housed in an air-conditioned room (21-23°C) on a 12:12 light:dark cycle (lights off at 2000 hours). They were weaned when about 21 days of age. Food and water were provided *ad libitum*.

Procedures

When 22-26 days old, twenty of the animals were castrated. The animals were anaesthetized using isoflurane anesthesia (AErrane; Anaquest, Mississauga, Ontario). Once the animal was anaesthetized, the scrotum was cleaned with antiseptic soap and then a single, vertical, midline incision was made through the scrotum. The testes were bluntly dissected from the tunica and the spermatic cord was ligated and transected

and the testes and the fat pad along the cord were removed. The tunica on either side were closed with a single interrupted absorbable suture. A series of interrupted, single sutures was used to close the scrotal incision. The remaining twenty animals were given an open and close sham surgery, consisting of a single, midline scrotal incision. Following surgery, all animals were given an intramuscular injection of 15,000 units of penicillin (Perlong XL; rogar/STB, inc., London, Ontario). The animals were then divided into ten littermate pairs of castrates and ten littermate pairs of intact, with each pair housed in a 40 X 24 X 18 cm stainless steel hanging cage. Animals were ear punched and their back markings recorded for identification.

Testing took place when the animals were approximately 31, 61, 91, 121, and 151 days of age. Each pair of rats was habituated to the 46 X 46 X 50 cm test enclosure for 15 minutes per day, for three days prior to testing. The enclosure had a wooden floor covered with a 2.5 cm layer of processed corn cobs, two wooden walls, and two clear plexiglass walls. The day prior to testing, each rat was socially isolated in a 17 X 18 X 24 cm cage from its partner for 24 hours, so as to increase the frequency of play in the test period (Panksepp, 1981; Pellis & Pellis, 1990). As the animals play less frequently when older, two trials were conducted at 121 and 151 days old (isolate, film,

isolate, film). These two trials were then averaged, to ensure an adequate sample of behaviour. Rats were videotaped from above for 10 minutes using a Sony Hi-8 camcorder. All habituating and testing occurred during the light half of the animals' cycle and was done under red light provided by a 100 W lamp 60 cm above the test enclosure. After each test period, all rats from both groups were weighed.

Behavioral Analysis

Videotapes were analyzed using a Sony Hi-8 videorecorder capable of slow motion and frame-by-frame advancement. Offensive and defensive behaviors were scored according to the body area contacted and the type of response. For this purpose, the body was divided into five areas: head (tip of the snout to ears), nape (behind ears to edge of hood), upper back (thoracic region), lower back (lumbar region), and rump (sacral region) (Pellis et al., 1994). An attack was scored when one pairmate brought the tip of its snout either into contact with or within one centimeter of its partner. This was measured as the number of attacks/animal/ten minutes (Pellis, Pellis, & Whishaw, 1992). Such proximity of the snout with the partner's body has been shown to be highly correlated with initiating play fighting (Pellis &

Pellis, 1987; Pellis, Pellis, & Dewsbury, 1989). This differs from the more investigatory approaches, where the anogenital area is most likely the area to be contacted (Pellis, Pellis, & Whishaw, 1992).

Withdrawal of the nape area by the recipient of an attack was scored as a defense by that animal. The number of defensive responses by each rat was contingent on the number of attacks received and so is expressed as a probability rather than as an absolute number. The contacted animal could use one of several defensive tactics: (i) *Evade*. The recipient ran, leaped, or swerved away, so that its face moved away from the attacker (see Fig. 2A); (ii) *Facing defense*. The recipient withdrew the nape, but in so doing, turned to face the attacker. These defenses were categorized as one of three types: (a) complete rotation, where upon contact, the recipient rotated around its longitudinal axis, cephalocaudally, to supine (see Fig. 2B); (b) partial rotation, where upon contact, the recipient rotated around its longitudinal axis, cephalocaudally, but stopped at the pelvis, so that it maintained a standing position on one or both hind feet (see Fig. 2C); and (c) other behaviors, where upon contact, the recipient adopted some alternative form of facing defense such as standing upright (see Fig. 3). Results were analysed using repeated measures analysis of variance.

Results and Discussion

Age-related Changes in the Pattern of Play Fighting

There was an age-related decrease in the frequency of playful attacks per pair (Fig. 6A; $F(4,72) = 43.61, p < 0.0001$), but no significant differences between the castrated and intact pairs ($p > 0.05$). These findings are consistent with previous studies for both intact and post-weaning castrated males (Pellis & Pellis, 1991a; Thor & Holloway, 1985). The probability of responding to a playful attack with a playful defense did not differ significantly between groups ($p > 0.05$), nor did it change significantly with age (Fig. 6B; $p > 0.05$). Again, these findings are consistent with previous studies on intact rats (Pellis & Pellis, 1991b) and studies of aggression which demonstrate that castration does not inhibit an animal's ability to defend itself (Leshner, 1981). Therefore, with respect to the frequency of attacks and the probability of defense, the intacts and castrates exhibited the same behavioral profiles during the pubertal transition.

Even though the nape is the preferred target of contact during play (Pellis & Pellis, 1987), the recipient may respond before nape contact is achieved (Pellis et al., 1994). When defense is initiated before nape contact is

Figure 6. The mean frequency (\pm SE) of playful attacks (A) and the mean probability (\pm SE) of playful defense (B) are shown for both intact (N = 10 pairs) and post-weaning castrated (N = 10 pairs) groups over all ages tested.

Figure 6 was removed due to the unavailability of copyright permission. The figure is described in the caption on page 35 of this thesis. Taken from Smith, Field, Forgie, & Pellis, 1996.

achieved, the recipient is more likely to adopt different patterns of defense than when the nape is actually contacted (Pellis et al., 1989). Therefore, to compare patterns of playful defense without the confound of differential use of defensive tactics at different body locations, only the types of defense adopted in response to nape contact were used. Based on the data from individual pairmates, all three types of facing defense changed with age (Fig. 7). Significant age-related differences were found for complete rotations ($F(4, 152) = 5.13, p < 0.001$), partial rotations ($F(4, 152) = 4.43, p < 0.005$), and 'other' defenses ($F(4, 152) = 4.96, p < 0.001$). Evasion remained unchanged throughout the study ($p > 0.05$). Consistent with previous studies, complete rotations decreased at puberty, while partial rotations and 'other' increased (Pellis & Pellis, 1987, 1990). There were, however, no significant differences between intact and castrates ($p > 0.05$), nor any significant group by age interactions ($p > 0.05$). Therefore, whether castrated or intact, male rats underwent the same changes in patterns of playful defense at puberty.

Dominance-related Changes in the Pattern of Play Fighting

Previous studies have shown that by 90 days of age, pairs of male rats

Figure 7. Mean probability (\pm SE) of a complete rotation (A), partial rotation (B), evasion (C), or 'other' defense (D) being used in response to an attack to the nape, expressed as a percentage. N = 40 animals.

Figure 7 was removed due to the unavailability of copyright permission. The figure is described in the caption on page 38 of this thesis. Taken from Smith, Field, Forgie, & Pellis, 1996.

reared together from weaning exhibit asymmetries in their play fighting behaviour that reflect a dominance-subordinance relationship (Pellis & Pellis, 1991a, 1992; Pellis, Pellis, & Kolb, 1992; Pellis et al., 1993). The male initiating the most playful attacks is the subordinate, and is also the pairmate that gains the least weight and continues to perform the more juvenile-like defense by rotating completely to supine when playfully attacked by its partner (Pellis & Pellis, 1991b, 1992). Therefore, in the present study, when 90 days old, the pairmates were categorized as dominant or subordinate based on whether they initiated more or less playful attacks. For both groups, the pairmates initiating the most playful attacks at 90 days tended to initiate the most playful attacks at younger and older ages as well (Fig. 8). The proportion of playful attacks ending at body areas other than the nape increased with age (Fig. 9; $F(4, 152) = 8.53, p < 0.0001$). Furthermore, while there were no group differences ($p > 0.05$), there was a trend towards an age by group interaction ($p = 0.065$), with the frequency of non-nape attacks increasing more for intact males than castrates after puberty (Fig. 9). More non-nape attacks, as measured in the present procedure, arise from the recipient initiating its defense before the attacker can reach the nape (Pellis et al., 1994). Therefore, so as not to bias the assignment of dominance

Figure 8. Mean frequency (\pm SE) of playful attacks to all body areas, for the animals that attacked the most and least in each pair, in the (A) intact (N = 20) and (B) post-wearing castrated (N = 20) groups.

Figure 8 was removed due to the unavailability of copyright permission. The figure is described in the caption on page 41 of this thesis. Taken from Smith, Field, Forgie, & Pellis, 1996.

Figure 9. The mean percentage (\pm SE) of playful attacks where body areas other than the nape were contacted is shown for both intact (N = 20) and post-weaning castrates (N = 20).

Figure 9 was removed due to the unavailability of copyright permission. The figure is described in the caption on page 43 of this thesis. Taken from Smith, Field, Forgie, & Pellis, 1996.

in the two groups, all attacks were used, not just those scored specifically as nape attacks.

Because the behaviour of one pairmate is contingent on that of its partner, the data for dominants and subordinates could not be treated as independent (Pellis & Pellis, 1993). Only pairs where this difference in playful attacks was greater than 5% were used for dominance measures, as differences of less than 5% are not reliably predictive of dominance (Pellis & Pellis, 1991b). By this criterion, one intact pair was dropped from the analysis. Therefore, to compare intact and castrates, a measure of difference between pairmates was used. The percentage of complete rotations by the dominant (least frequent attacker) was subtracted from that of the subordinate (most frequent attacker). This percentage difference was predicted to be greater than zero, as previous studies have shown that subordinates perform more complete rotations than their dominant pairmates (Pellis & Pellis, 1992). Pairmate differences in weight were calculated by subtracting the weight of the subordinate from that of the dominant, dividing by the weight of the dominant, and then multiplying by 100. This percentage difference was predicted to be greater than zero, as previous studies have shown that dominants gain more weight than do

subordinates (Lore & Stipo-Flaherty, 1984; Pellis & Pellis, 1991b) and lose less weight due to stress (Blanchard & Blanchard, 1990). As the designations of dominant and subordinate were made at 90 days, and individual pairmates were so treated at all other ages, irrespective of their current frequency of initiating attacks, this procedure tested the robustness of the description of dominance based on relative playful attack frequency at this age. If such a description did not predict dominance, then the weight and defensive reactions of pairmates should have varied independently at different ages. Furthermore, if castrates exhibited as strong a within-pair dominance asymmetry as intact, then the difference scores should have been either similar, or have fluctuated randomly across trials.

For complete rotations, there was a significant group effect ($F(1, 17) = 7.61, p < 0.05$), with the intact showing a much higher positive difference across all trials (Fig. 10A). There was also an age-related effect, and although not significant ($p > 0.05$), it did indicate that there was a tendency for the asymmetry to increase for intact after puberty, which is similar to results found for intact in previous studies (Pellis & Pellis, 1992). Similarly, for weight there was a significant group effect ($F(1, 17) = 5.89, p < 0.05$), with the intact showing a much higher positive difference across all trials (Fig.

Figure 10. (A) Mean percentage differences (\pm SE) in complete rotations within pairs for the post-weaning castrate (N = 10 pairs) and intact (N = 10 pairs) groups. For each point, the probability of the least frequent attacker exhibiting a complete rotation was subtracted from the probability of the most frequent attacker. Complete rotations in response to all body areas are included.

(B) Mean percentage differences (\pm SE) in weight within pairs for the post-weaning castrate (N = 10 pairs) and intact (N = 10 pairs) groups. Each point was calculated by subtracting the weight of the most frequent attacker from that of the least frequent attacker and dividing this total by the weight of the least frequent attacker.

Figure 10 was removed due to the unavailability of copyright permission. The figure is described in the caption on page 47 of this thesis. Taken from Smith, Field, Forgie, & Pellis, 1996.

10B). In this case, however, there were no age-related changes in the magnitude of the difference between groups. Therefore, for the intact, being the most frequent attacker at 90 days predicted subordination in respect to continued use of juvenile defense patterns and in gaining less weight than the dominant pairmate. The values for castrates were closer to zero, suggesting that frequency of playful attack did not predict subordination or dominance as measured by these indices. In addition, the mean weight differences within pairs, regardless of who attacked the most, showed that, while the two groups did not differ significantly ($p = 0.087$), the intact animals tended to have greater differences than castrates. As well, they exhibited more variance, as indicated by the error bars (Fig. 11). There was, however, a strong age effect ($F(4, 72) = 14.22, p < 0.0001$). This suggests that while the two groups began with similar low weight differences at 30 days, the differences became greater following puberty, as weight differences became indicative of dominance relationships. The finding that castrate pairs show less difference in pairmate weights than the intact supports the view that they do not form dominant-subordinate relations.

Figure 11. Mean within pair weight differences (\pm SE) , in grams, for intact (N = 10 pairs) and post-weaning castrated (N = 10 pairs) males are shown for all ages tested.

Figure 11 was removed due to the unavailability of copyright permission.
The figure is described in the caption on page 50 of this thesis. Taken from
Smith, Field, Forgie, & Pellis, 1996.

General Discussion

As castration at weaning did not alter the age-related changes in the frequency and structure of play fighting in rats, it is concluded that the motivational and organizational changes in play fighting accompanying puberty in male rats do not arise from the activational effects of gonadal hormones. This suggests that both the sex-typical differences in play frequency (Meaney et al., 1985) and the age-related changes in the pattern of play by males (Meaney & Stewart, 1981a; Pellis & Pellis, 1987, 1990) result from the organizational influences of gonadal hormones on neural systems in the perinatal period (Pellis, et al., in press). Gonadal hormones at puberty and early adulthood are, however, necessary to establish aggression and dominance in males (Beatty, 1992). In intact males, the most frequent playful attacker demonstrated the behaviours indicative of subordinate status: less weight gain and more juvenile defensive patterns (Pellis & Pellis, 1991b, 1992). In castrates, however, the absence of gonadal hormones at puberty abolished dominance formation and the accompanying play asymmetries. These hormones, then, have only an indirect effect on play fighting through their actions on dominance relationships. This supports

the conclusion that, differences in play fighting between males and females are not due to sex differences in dominance patterns (Pellis et al., in press; Pellis, Pellis, & Whishaw, 1992). Dominance only modifies the age-related sex differences in play fighting that are organized in the perinatal period by gonadal hormones.

The dominance asymmetry in the play of adult male rats has been hypothesized to function as a 'friendship maintenance' mechanism, which provides the means by which subordinates can actively maintain familiarity with the dominant in multi-male colonies (Pellis & Pellis, 1991b, 1992). When playing together, subordinates establish the typical age-related changes but do not show asymmetry in their behaviour. When the dominant male in a triad is removed, one of the subordinates becomes dominant. The pairmate that had shown the most playful attacks then switches so that its play behaviour follows the subordinate pattern when interacting with the same, but now dominant, pairmate (Pellis et al., 1993). Unlike rats, in Syrian golden hamsters it is the dominant who initiates the most play fights and gains the most weight (Pellis & Pellis, 1993). Similarly, in the absence of dominance relationships, it is the most aggressive female rat who initiates the most play fights, and hence its partner who performs

proportionally more supine defenses (Pellis & McKenna, 1992). In these cases, play does not appear to be used for friendship maintenance. The castrates in this study did not show the asymmetry in body weight and play fighting typical of intact male rats, even though they exhibited the normal changes in play fighting at puberty. Therefore, these results are in accordance with the view that the play asymmetries in adult male rats arise as a direct response to the establishment of dominance-subordination relationships.

In some species, such as tree shrews, hamadryas baboons, and rabbits, the presence of a dominant retards endocrinological and behavioural maturation in subordinates (i.e., 'psychic castration'), who then retain a more juvenile appearance and behaviour (Wickler, 1968). That adult male subordinate rats can play in a more adult manner with other subordinates, but in a more juvenile manner when playing with dominants (Pellis et al., 1993), and that castrates can behave in the adult manner, but not asymmetrically (present study), suggests that the subordinate male's retention of juvenile-like play patterns is an adaptive response to its subordinate status and not a result of immaturity. Furthermore, it has been shown that while castration decreases aggressiveness, it does not prevent

the animal from defending itself (Leshner, 1981) or its territory (Albert et al., 1986), nor does it automatically make the animal submissive (Leshner, 1981). It appears that post-weaning castrates possess a fully matured behavioral repertoire for play and aggression, but lack the 'motivation' to spontaneously develop dominance relationships when in familiar pairs or groups.

CHAPTER 3*

The Post-pubertal Change in the Playful Defense of Male Rats

Depends Upon Neonatal Exposure to Gonadal Hormones

Play fighting is a common behaviour of juvenile mammals (Fagen, 1981). Play fighting in rats reaches its peak occurrence between 30-40 days, and then wanes at puberty (Bolles & Woods, 1964; Thor & Holloway, 1984). It does not completely disappear, however, but continues at a lower frequency well into adulthood (Adams & Boice, 1989; Pellis & Pellis, 1990, 1991a). In addition to these age-related changes in the frequency of play fighting, the play of male rats, but not females, undergoes changes in form following puberty (Meaney & Stewart, 1979; Takahashi & Lore, 1983). In this paper, a possible mechanism for this pubertal change in the content of play fighting by male rats is analyzed.

The play fighting of rats involves one animal contacting the nape of another with its snout, while the partner uses various defensive

*This chapter is modified from a paper that has been submitted to *Physiology and Behavior*.

maneuvers to avoid this nape contact (Pellis & Pellis, 1987). During the juvenile phase, the most likely defensive tactic to be used is to rotate to a supine position in order to withdraw the nape. While lying on its back, the defender may push and kick to block the partner's further attacks to the nape (Pellis, 1988). Post-pubertally, male rats are more likely to rotate only partially to supine, while retaining contact on the ground with at least one hindfoot. From this position, other, more adult-like tactics can be used, such as moving against the attacker in a lateral orientation or rearing into a boxing stance (Pellis & Pellis, 1987). Therefore, with the onset of puberty, males increase their use of adult-like patterns of playful defense (Meaney & Stewart, 1981a; Pellis & Pellis, 1990; Takahashi & Lore, 1983).

A possible reason for this change in playful defense is that post-pubertally, male rats begin to develop dominance-subordination relationships (Lore & Stipo-Flaherty, 1984; Pellis & Pellis, 1991b, 1992), and this change may be a reflection of such relationships. However, a study of male rats castrated at weaning showed that the pubertal change in type of playful defense can be dissociated from the onset of dominance relationships (Smith, Field, Forgie, & Pellis, 1996). The castrated males still exhibited the male-typical change in playful defense at puberty, but they did

not develop dominance-subordinance relationships. This study also suggested that the pubertal shift in playful defense does not depend on the presence of gonadal hormones at puberty (Pellis et al., in press), whereas the establishment of dominance relationships amongst male rats does (Beatty, 1992).

The play fighting of males castrated at birth was compared to that of intact males, in order to determine whether the age-related change in playful defense is dependent on the actions of gonadal hormones early in postnatal development. In addition, given that male-typical dominance relationships require neonatal gonadal exposure, as well as circulating hormones in adulthood for their development (Beatty, 1992), the presence of dominance-subordinance asymmetries in adulthood were analyzed as confirmation of the efficacy of the castrations.

Materials and Methods

Subjects

Sixty-six male, Long-Evans hooded rats born and reared in the animal colony of the University of Lethbridge Psychology Department, Lethbridge,

Alberta, Canada were used. The animals were housed in an air-conditioned room (21-23°C) on a 12:12 light:dark cycle (lights off at 19:30 hours). They were weaned when about 21 days of age. Food and water were provided *ad libitum*.

Procedures

Within 3-4 hours after birth, pups were removed from the dams and transported to another room. Forty-four male animals were anaesthetized with hypothermic anaesthesia, by placing each animal onto ice until it no longer responded to a tail or foot pinch. For 22 of these rats, a 2-3 mm midline, abdominal incision was made, and the testes were removed by blunt dissection. The abdominal wall was closed with a single, interrupted, silk suture, and a series of interrupted, single sutures was used to close the abdominal incision. The other 22 animals were only exposed to the anaesthetic. The animals were marked with either a plantar tattoo or by toe clipping, and then warmed by the surgeon's hands and a heating lamp. When revived, they were returned to the dams along with their female siblings. A third group (N = 22) of males that had received no treatment was also used. Following weaning at 21 days, the animals were divided into

11 littermate pairs of castrates, 11 littermate pairs of sham-treated controls, and 11 littermate pairs of untreated controls. Each pair was housed in a 40 X 24 X 18 cm stainless steel hanging cage. Animals were ear punched and their back markings recorded for identification. The untreated control group was used in addition to the sham-treated control group as previous studies have shown that various manipulations during the neonatal period may affect normal androgen production, and hence, the normal masculinization processes for some anatomical (Pellis, Pellis, & Kolb, 1992) and behavioural systems (Matuszczyk, Silverin, & Larsson, 1990).

Testing took place when the animals were approximately 30, 60, and 90 days of age. Each pair of rats was habituated to the 46 X 46 X 50 cm test enclosure for 15 minutes per day, for three days prior to testing. The enclosure had a wooden floor covered with a 2.5 cm layer of processed corn cobs, two wooden walls, and two clear plexiglass walls. The day prior to testing, each rat was socially isolated in a 17 X 24 X 18 cm cage from its partner for 24 hours, so as to increase the frequency of play in the test period (Panksepp, 1981; Panksepp & Beatty, 1980; Pellis & Pellis, 1990). During the test trials, the rats were videotaped from above for 10 minutes using a Sony Hi-8 camcorder. All habituating and testing occurred during the light half

of the animals' cycle and was done under red light provided by a 100 W lamp 60 cm above the test enclosure. After each test period, all rats from the three groups were weighed.

Behavioral Analysis

Videotapes were analyzed using a Sony Hi-8 videorecorder capable of slow motion and frame-by-frame advancement. An attack was scored when one pairmate brought the tip of its snout either into contact with or within one centimetre of its partner's nape. Playful attack was measured as the number of attacks/animal/ten minutes (Pellis, Pellis, & Whishaw, 1992). Withdrawal of the area contacted, or about to be contacted by the recipient of an attack was scored as a defense by that animal. The number of defensive responses by each rat was contingent on the number of attacks received and so was expressed as a probability rather than as an absolute number (Pellis et al., in press; Pellis & Pellis, 1990).

The contacted animal could use one of several defensive tactics to withdraw its nape (Pellis, Pellis, & Whishaw, 1992). As in previous studies (Pellis & Pellis, 1990, 1992), it was found that rotating around the longitudinal axis to turn to face the attacker was the predominant pattern of defense at all ages in all groups (> 65%). These rotatory defenses were of two

types: (a) complete rotation, where upon contact, the recipient rotated around its longitudinal axis, cephalocaudally, to supine (see Fig. 2B), and (b) partial rotation, where upon contact, the recipient rotated around its longitudinal axis, cephalocaudally, but stopped at the pelvis so that it maintained a standing position on one or both hind feet (see Fig. 2C). The defenses other than the rotatory ones did not change with age and thus, will not be discussed further.

Previous studies have shown that the biggest change in increasing the use of partial rotations and decreasing the use of complete rotations occurs at about 60 days (Pellis & Pellis, 1990; Smith, Field, et al., 1996). Then, with increasing age, as the dominance-subordinance relationships between intact pairmates develop (Pellis & Pellis, 1991b), the subordinate increases its relative use of the complete rotation tactic (Smith, Field, et al., 1996). Therefore, the comparisons between groups for the relative age-dependent changes in complete and partial rotations were made between 30 and 60 days. The probability of performing the complete and partial rotations at 30 days was subtracted from that at 60 days. Positive values indicated that the occurrence of the behaviour increased from 30 to 60 days, whereas negative values indicated a decrease.

Dominance within pairmates was determined at 90 days. Previous studies have shown that the subordinate male is the one that initiates the most playful attacks, gains the least weight and continues to perform the more juvenile-like defense of rotating completely to supine when playfully attacked by its partner (Pellis & Pellis, 1991a, 1991b, 1992; Pellis et al., 1993). Therefore, at 90 days, the subordinate in each pair was determined by identifying the most frequent playful attacker and the dominant was the least frequent attacker.

The percentage of complete rotations by the dominant was subtracted from that of the subordinate. Similarly, pairmate differences in weight were calculated by subtracting the weight of the subordinate from that of the dominant, dividing by the weight of the dominant, and then multiplying by 100. If the pairmates formed dominance relationships, the measures for supine defense and weight should be positive (Lore & Stipo-Flaherty, 1984; Pellis & Pellis, 1991b, 1992). Due to a lack of homogeneity of variance, data were analyzed using Friedman's two-way analysis of variance by ranks with following Wilcoxon matched-pairs signed-ranks tests, and Kruskal-Wallis one-way analysis of variance by ranks with following Mann-Whitney U tests, rather than parametric tests (Siegel, 1956).

Results and Discussion

The frequency of playful attacks was highest in the juvenile phase (30 days) and then decreased with age (Fig. 12A). There were no significant group differences in frequency of playful attack ($p > 0.05$), although the neonatal castrates had the lowest value in the juvenile phase, which is consistent with other studies (Meaney & Stewart, 1979; Pellis & Pellis, 1991a; Thor & Holloway, 1986). Overall, at 30 days, the rats launched between 60-80 attacks per 10 minutes, but then decreased to between 35-45 attacks by 60 days. In contrast, the probability of responding to a playful attack did not change with age (Fig. 12B). Furthermore, there were no significant differences among groups ($p > 0.05$). Therefore, significant changes in patterns of defense or in patterns of dominance (see below) could not be explained by group differences in frequency of play attack or in probability of defense.

For intact males, the complete rotation defensive tactic decreased at puberty, whereas the partial rotation defensive tactic increased. In contrast, the castrates did not switch their pattern of defense at puberty (Fig. 13). There were significant group differences for both complete ($H = 7.41, df 2,$

Figure 12. The mean frequency (\pm SE) of playful attacks per 10 minutes (A) and the mean probability (\pm SE) of defense (B) are shown for both control and neonatally castrated groups over all ages tested. N = 33 pairs

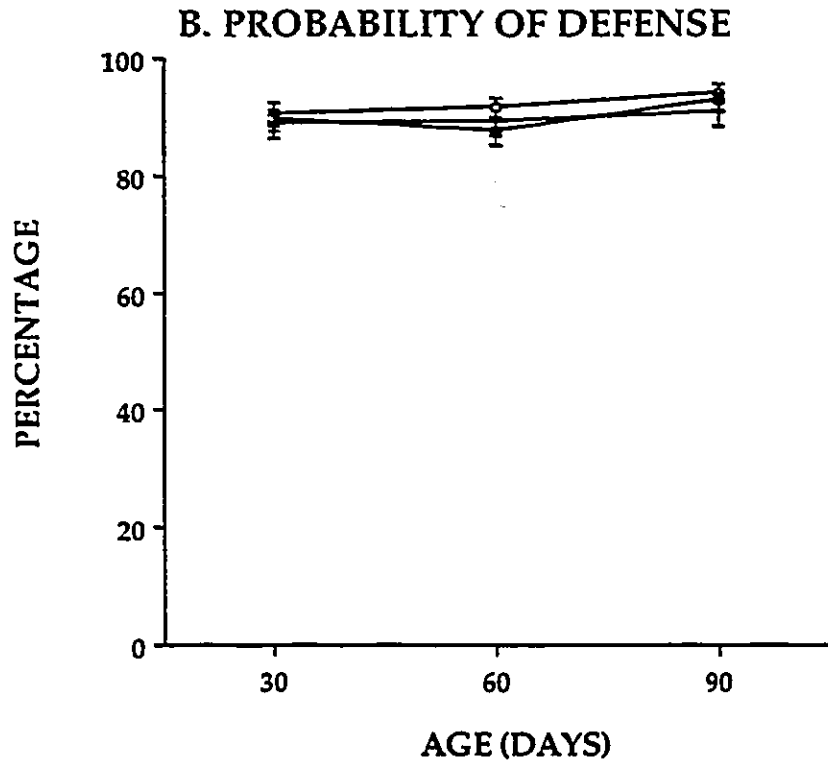
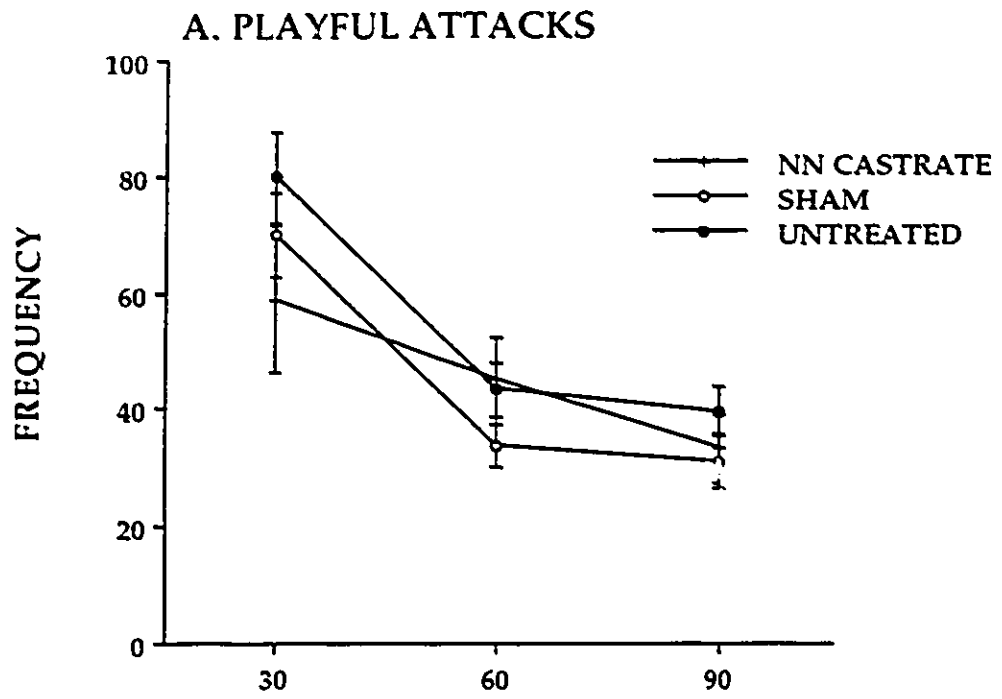
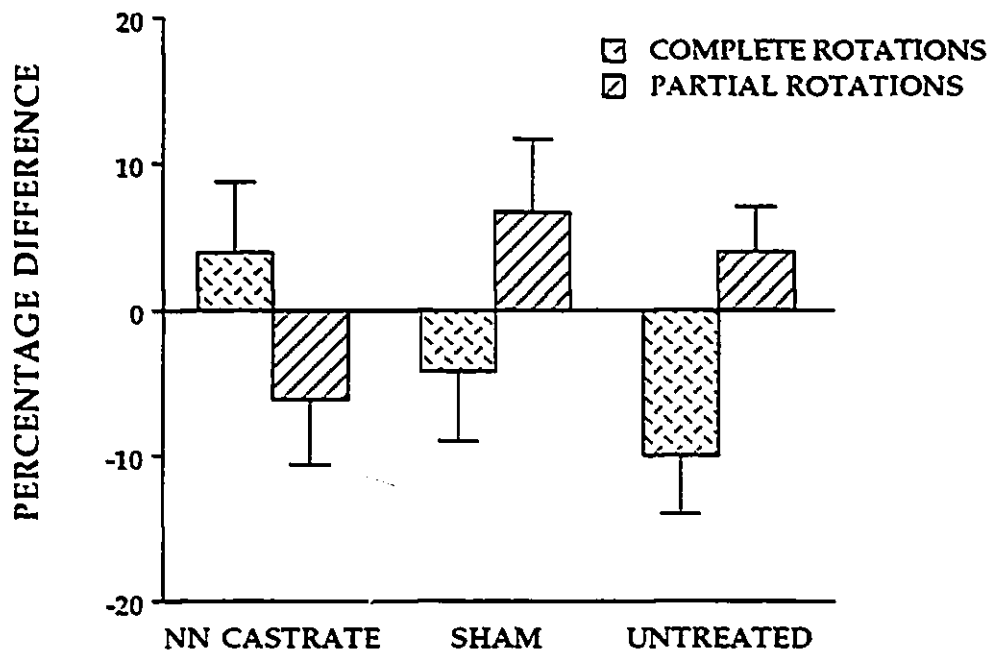


Figure 13. Mean percentage differences (+ SE) between 30 and 60 days of age for complete and partial rotations within pairs for the control and neonatally castrated groups (N = 33 pairs). For each pair, the 30 day probability was subtracted from the 60 day probability of exhibiting a particular defense. Positive values indicate that use of the behaviour increased from 30 to 60 days, while negative values indicate a decrease in use.

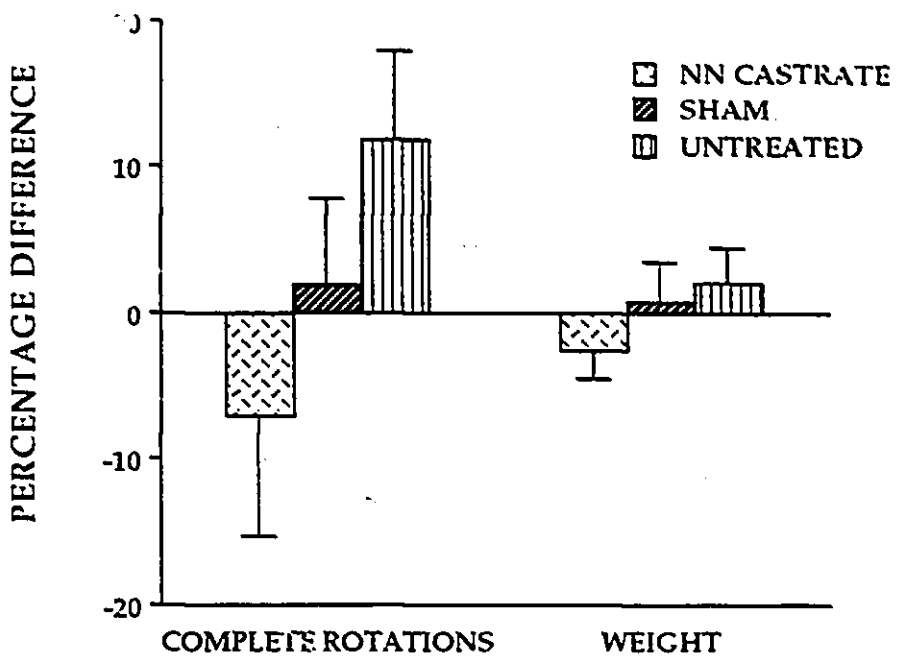


$p < 0.05$) and partial rotations ($H = 6.73$, $df = 2$, $p < 0.05$). However, this difference was only significant between the neonatal castrates and the untreated controls (complete rotations $U = 30$, $N = 11$, $p < 0.025$ 1 tailed; partial rotations $U = 34$, $N = 11$, $p < 0.05$ 1 tailed), with the sham controls being intermediate. Thus, the neonatal castrates retained the juvenile defense of rotating completely to supine following puberty, while the control groups adopted more adult-typical responses (i.e., partial rotations).

The measurements of dominance-related asymmetries in the use of complete rotations at 90 days (Fig. 14) showed that there were significant group differences for complete rotations ($H = 7.46$, $df = 2$, $p < 0.05$), with the neonatal castrates being significantly different from the untreated controls ($U = 29$, $N = 11$, $p < 0.05$ 1 tailed). While not significant ($p > 0.05$), pairmate weight differences followed the same trend.

The present study clearly shows that the pubertal shift in playful defense by male rats is dependent upon the organizational effects of gonadal hormones in the perinatal period. Such hormones do not need to be present at puberty to activate this change in behaviour (Smith, Field, et al., 1996). Females, furthermore, do not exhibit this shift (Pellis & Pellis, 1990). Therefore, the pubertal change in playful defense appears to be a sex-specific

Figure 14. Mean percentage differences (+ SE) in complete rotations and weight within pairs for the neonatally castrated and control groups at 90 days of age. For each pair (N = 33), the probability of the least frequent attacker exhibiting a complete rotation was subtracted from the probability of the most frequent attacker. For weight, values were obtained by subtracting the weight of the most frequent attacker in each pair (N = 33) from that of the least frequent attacker, dividing this total by the weight of the least frequent attacker and multiplying by 100%.



difference in the developmental pattern of play fighting. Similarly, the higher frequency of play fighting by males (Meaney & Stewart, 1981a; Meaney et al., 1985; Olioff & Stewart, 1978; Poole & Fish, 1975), which is mostly due to a higher frequency of launching playful attacks (Thor & Holloway, 1986), is also dependent upon the actions of gonadal hormones in the perinatal period (Meaney, 1988). The differences in frequency of play fighting have been shown to depend upon the action of gonadal hormones on the amygdala (Meaney, Dodge, & Beatty, 1981; Meaney & McEwen, 1986). The target tissue responsible for the pubertal transition in playful attack is, at present, undetermined.

Similarly, the functional significance of this pubertal transition in playful defense by males is also undetermined. However, that this transition requires the action of gonadal hormones in the perinatal period (this study), but is independent of gonadal hormones at puberty and also of the onset of dominance relationships (Smith, Field, et al., 1996), suggests that males, but not females, face a predictably new functional context at the pubertal age. One possibility is that males are more likely to emigrate from their natal colonies than are females (Calhoun, 1963), and a 'rougher' form of play fighting to assess the competitive ability of strange males that are

encountered may be needed. As has been suggested for other species, a rougher form of play fighting may be a substitute for serious fighting, as a means of testing the strength of potential opponents (e. g. Croft & Snaith, 1991; Geist, 1982). Essentially, the young males may be behaving as dominants until proven to be subordinates (Geist, 1971; Jackson, 1988). If such testing is important for post-pubertal males, but not females, then males, but not females, should use the partial rotation tactic when encountering unfamiliar rats. This appears to be the case (Smith, Forgie, & Pellis, 1996a).

CHAPTER 4*

The Effects of Familiarity and Sex on the Development of
Defense During Play Fighting in Rats (*Rattus norvegicus*)

Play fighting is a common activity for the juveniles of many species of mammals (Fagen, 1981), including rats (Meaney & Stewart, 1981a; Panksepp, 1981; Poole & Fish, 1975). It usually involves one animal trying to gain an advantage over its partner (Aldis, 1975; Symons, 1978). In rats, play fighting involves competition for access to the nape (Pellis & Pellis, 1987; Siviy & Panksepp, 1987), which if successfully contacted is nuzzled with the snout (Pellis, 1988). In contrast, serious fighting involves bites directed at the opponent's lower flanks and dorsum (Blanchard, Blanchard, Takahashi, & Kelley, 1977; Pellis & Pellis, 1987). Therefore, although the juvenile pattern of playful fighting resembles serious fighting (Taylor, 1980), it is quite distinct. Play fighting in rats has its peak frequency of occurrence during the juvenile phase, between 30-40 days of age and then declines in

*This chapter is modified from a paper that has been submitted to *Developmental Psychobiology*.

frequency (Thor & Holloway, 1984). It does not, however, completely disappear with the onset of sexual maturity at 50-60 days of age. Rather, it persists, albeit at a lower frequency, well into adulthood in both males and females (Adams & Boice, 1989; Pellis & Pellis, 1990, 1991a). However, not only does the frequency of play fighting decrease at puberty, its pattern also changes.

With the onset of puberty, male rats use more adult-typical behaviour patterns in their play fighting (Meaney & Stewart, 1981a; Takahashi & Lore, 1983). The most common form of defense adopted by a juvenile, in response to a nape attack, is to roll onto its back. From this supine position, the defending animal can block the partner standing over it from gaining access to the nape (Pellis & Pellis, 1987). This pattern of defense, and the ensuing on-bottom and on-top positions of the participants, is so frequent that such a 'pinning' configuration is often used as a marker for play fighting (Panksepp, 1981; Panksepp et al., 1984). Following puberty, the defender is more likely to rotate only partially, keeping one or both hindfeet in a standing position. From this position, the defending animal can rear into an upright posture or push into the attacker from a lateral orientation (Pellis & Pellis, 1987). Other defensive tactics

occur at lower frequencies and generally do not change significantly with age (Pellis & Pellis, 1990). Both male and female rats, of all ages, utilize both rotational tactics, but the probability of using one over the other varies with age and sex.

In the juvenile phase, the complete rotation tactic is used most frequently in the play fighting of both sexes. At puberty, however, males, but not females, switch and exhibit the partial rotation defensive tactic more often (Pellis & Pellis, 1990). This pubertal shift in pattern of defense occurs independently of the onset of the dominance relationships which develop between male littermates following puberty (Lore & Stipo-Flaherty, 1984; Pellis et al., 1993; Smith, Field, et al., 1996). Therefore, the failure of females to establish competition based dominance-subordinance relationships (Panksepp et al., 1984; Ziporyn & McClintock, 1991) cannot account for the absence of this pubertal shift in defense pattern in females.

Previous studies on males have shown that playful encounters between animals are more likely to escalate into serious fighting following puberty (Hole & Einon, 1984; Pellis & Pellis, 1991b; Poole & Fish, 1976; Takahashi & Lore, 1983). Furthermore, individuals tend to be more aggressive towards unfamiliar, as opposed to familiar partners (Barnett,

1958; Thor, 1979), and males do not begin to aggressively attack intruders until after puberty (Takahashi, 1986). Given that most studies on play fighting in rats have focused on the juvenile phase (Panksepp et al., 1984; Thor & Holloway, 1984), and that the few studies of post-pubertal play in females involved familiar male and female playmates (Pellis & Pellis, 1990), it is possible that an appropriate stimulus context for expression of the pubertal shift in females has not been provided. The hormonal mechanisms underlying the pubertal shift in males further supports this possibility.

In males, post-weaning castration does not interfere with the occurrence of the pubertal shift in playful defense (Smith, Field, et al., 1996), whereas neonatal castration does (Pellis et al., in press; Smith, Forgie, & Pellis, 1996b). These findings suggest that gonadal hormones during the perinatal period are necessary for the organization of this male-typical developmental play fighting pattern. Given that most sex differences involve differences in the thresholds at which particular behaviour patterns are elicited (Baum, 1987; Meaney, 1988), the ability to switch to the predominant use of partial rotations may be present in post-pubertal females, but may require a higher level of stimulation to manifest itself.

Therefore, it is possible that when interacting with unfamiliar conspecifics, both male and female rats may be more likely to utilize the adult-typical defensive patterns following puberty. Encountering a stranger, especially a male, should induce a more 'tense' social context and hence, stimulate the adoption of adult-typical patterns of playful defense. This study was conducted in order to determine whether, under more extreme social situations, female rats could be induced to exhibit the male-typical pubertal transition in playful defense.

Methods

Subjects

Six littermate quadrads (N = 24), each composed of two male and two female Long-Evans hooded rats, born and reared in the animal colony of the Psychology Department at the University of Lethbridge, Lethbridge, Alberta, Canada were used. Following weaning at 21 days of age, each littermate quadrad was housed in a 66 X 24 X 18 cm stainless steel hanging cage, in an air-conditioned room (21-23° C) on a 12:12 light:dark cycle (lights off at 1930 hours). Food and water were provided *ad libitum*. Animals were ear

punched and their back markings were recorded for identification.

Procedure

Testing sessions were conducted when the animals were 30, 60, and 90 days of age. Each quadrat was habituated to the 46 X 46 X 50 cm test enclosure for 15 minutes per day, for three days prior to testing. The enclosure had a wooden floor covered with a 2.5 cm layer of processed corn cobs, two wooden walls, and two clear Plexiglas walls. The day prior to testing, each rat was socially isolated in a 17 X 24 X 18 cm cage from its cagemates for 24 hours, so as to increase the frequency of play in the test period (Panksepp, 1981; Pellis & Pellis, 1990). Pairs of rats were videotaped from above for 10 minutes using a Sony Hi-8 camcorder. All habituating and testing occurred during the light half of the animals' cycle and under red light provided by a 100 W lamp 60 cm above the test enclosure. After each test period, all rats were weighed.

Two quadrats were tested at a time, with each rat filmed with every other rat in both groups. This yielded twenty-eight combinations for every two quadrats: two familiar (cagemates) male pairs, two familiar female pairs, four unfamiliar (from paired quadrats) male pairs, four unfamiliar female pairs, eight familiar male-female pairs, and eight unfamiliar male-

female pairs. Although the unfamiliar animals met each other more than once over the course of the study, they were still much less familiar with each other than the individuals that lived together. Furthermore, it has been demonstrated that rats have a very short social memory and therefore, are unlikely to remember each other following the weeks between test periods (Gheusi, Bluthe, Goodall, & Dantzer, 1994). By conducting the experiment in this way, we hoped to better approximate conditions in the wild, where animals not only encounter members of their home colony, they also come into contact with unfamiliar animals from other colonies, especially at the edges of their home ranges (Calhoun, 1963).

To avoid pregnancies in the heterosexual quadrads, the males (N = 12) were vasectomized under isoflurane anesthesia (AErrane; Anaquest, Mississauga, Ontario) when 50 days of age. The animal's scrotum was shaved and cleaned with antiseptic soap. A single, vertical, midline incision was made through the scrotum, and a second within the tunica to expose the vas deferens. Two ligatures were tied around the vas deferens, spaced 1 cm apart. The vas deferens was then severed between the ligatures. The tunica was closed with a single, interrupted, absorbable suture. The same procedure was then repeated on the other side. A series of

interrupted, single silk sutures was used to close the external scrotal incision. Following surgery, the animals were given an intramuscular injection of 15,000 units of penicillin (Penlong XL; rogar/STB, inc., London, Ontario).

Behavioral Analysis

A) Play Fighting

Videotapes were analyzed using a Sony Hi-8 videorecorder capable of slow motion and frame-by-frame advancement. Offensive and defensive behaviors were scored according to the body area contacted and the type of response. For this purpose, the body was divided into five areas: head (tip of the snout to ears), nape (behind ears to edge of hood), upper back (thoracic region), lower back (lumbar region), and rump (sacral region) (Pellis et al., 1994). An attack was scored when one pairmate brought the tip of its snout either into contact with or within 1 cm of its partner. This was measured as the number of attacks/animal/ten minutes (Pellis, Pellis, & Whishaw, 1992). Such proximity of the snout with the partner's body has been shown to be highly correlated with initiating play fighting (Pellis & Pellis, 1987; Pellis et al., 1989). The mean frequencies of playful attacks for males and

females when interacting with familiar males (FM), unfamiliar males (UM), familiar females (FF), and unfamiliar females (UF) were calculated.

Withdrawal of the nape area by the recipient of an attack was scored as a defense by that animal. The contacted animal could use one of several defensive tactics: (i) *Evade*. The recipient ran, leaped, or swerved away, so that its face moved away from the attacker (see Fig. 2A). (ii) *Facing defense*. The recipient withdrew the nape, but in so doing, turned to face the attacker. These defenses were categorized as one of three types: (a) complete rotation, where upon contact, the recipient rotated around its longitudinal axis, cephalocaudally, to supine (see Fig. 2B); (b) partial rotation, where upon contact, the recipient rotated around its longitudinal axis, cephalocaudally, but stopped at the pelvis, so that it maintained a standing position on one or both hind feet (see Fig. 2C); and (c) other behaviors, where upon contact, the recipient adopted some alternative form of facing defense such as standing upright (see Fig. 3). The mean probabilities of adult-typical defenses, expressed as percentages, were calculated for all pair combinations. Absolute numbers were not used, as the number of defensive responses by each rat was contingent on the number of attacks received. Repeated measures analyses of variance (ANOVA) were used to analyze both attack

and defense data. Because these 'other' behaviours were often performed in conjunction with partial rotations, they were grouped together as adult-typical behaviours. Previous studies have shown that evasions do not change with age (Pellis & Pellis, 1990; Smith, Field, et al., 1996) and as that was the case in this study, they will not be discussed further here.

B) Agonistic Behaviour

During some play fights, one or both pairmates would become agonistic, that is, an animal could bite its partner's lower flanks, piloerect, or perform a lateral display (Blanchard et al., 1977; Pellis & Pellis, 1987; Takahashi & Lore, 1983). Proportions, expressed as percentages, were calculated by dividing the number of trials in which one member of the pair exhibited some form of aggression by the total number of trials for each type of partner combination. The proportions were compared using a test for significance of difference between two proportions (Bruning & Kintz, 1987) for both sexes in order to determine differences in the frequency of aggressive behaviour between 30 and 90 days, and at 90 days between familiar and unfamiliar pairmates and between male and female pairmates.

Results

Males (Fig. 15A) initiated fewer playful attacks with increasing age ($F(2,88) = 38.15, p < 0.0001$). Although not significant ($p > 0.05$), males were more likely to playfully attack females than other males and less likely to attack unfamiliar males, especially at 90 days. Females (Fig. 15B) also exhibited a change with age ($F(2,88) = 60.95, p < 0.0001$), with the frequency of playful attacks declining following puberty. Furthermore, a significant age by partner interaction ($F(6,88) = 2.748, p < 0.05$) indicated that post-pubertally, females were more likely to playfully attack females, as opposed to males.

With age, males (Fig. 16A) increased their use of adult-typical playful defenses ($F(2,88) = 32.53, p < 0.0001$). While this age-related change occurred with all partner combinations, there was a tendency for males to utilize these tactics more often with unfamiliar pairmates, especially at 90 days. This trend, however, was not significant ($p > 0.05$). Females, in contrast, showed little change with age (Fig. 16B). That is, they continued to use the complete rotation tactic at juvenile-typical levels following puberty. They did, however, tend to use the adult-typical tactics less often when interacting with unfamiliar males, but this trend was not significant ($p >$

Figure 15. The mean frequency (\pm SE) of playful attacks by (A) male (N = 12) and (B) female (N = 12) rats at all ages tested. FM = familiar male, UM = unfamiliar male, FF = familiar female, UF = unfamiliar female

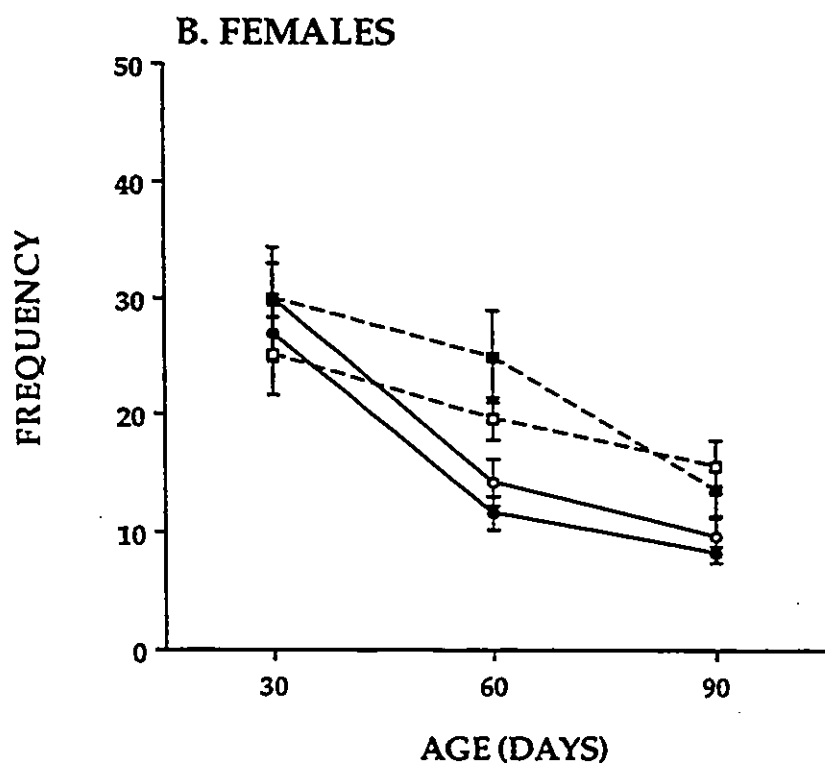
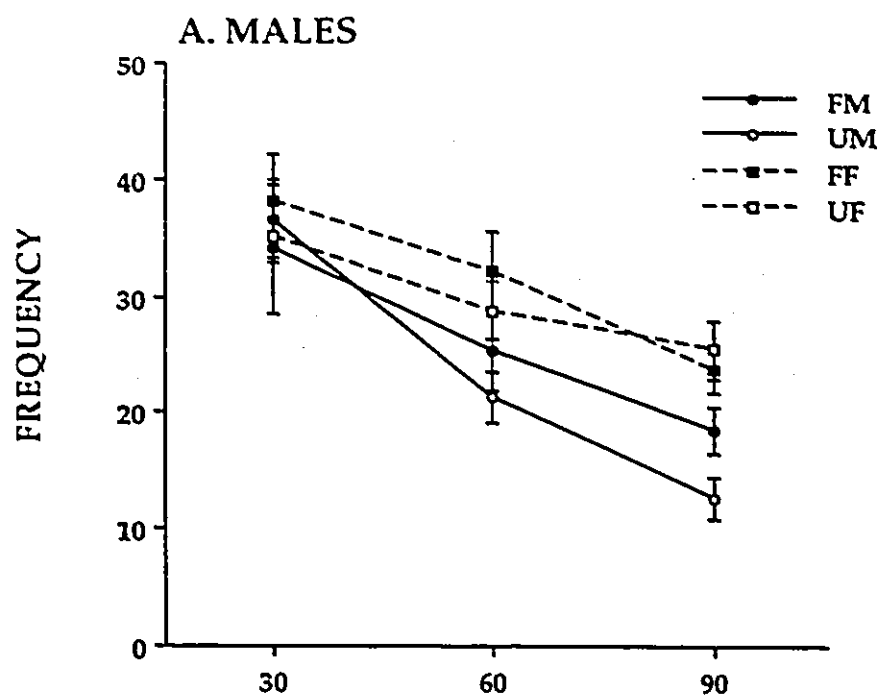
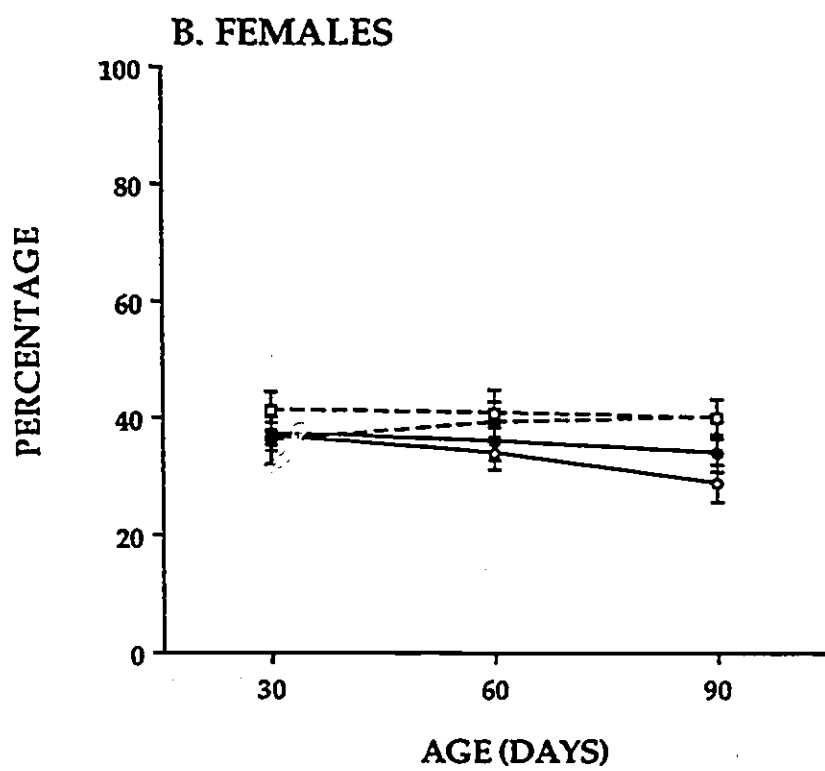
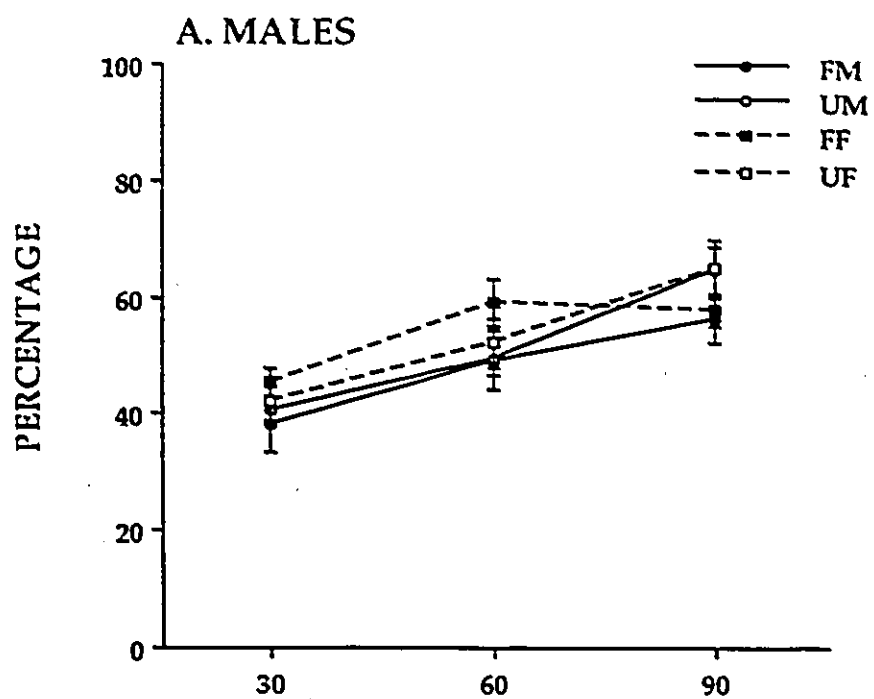


Figure 16. The mean probability (\pm SE) of using adult-typical defenses in the play fighting of (A) males (N = 12) and (B) females (N = 12). FM = familiar male, UM = unfamiliar male, FF = familiar female, UF = unfamiliar female



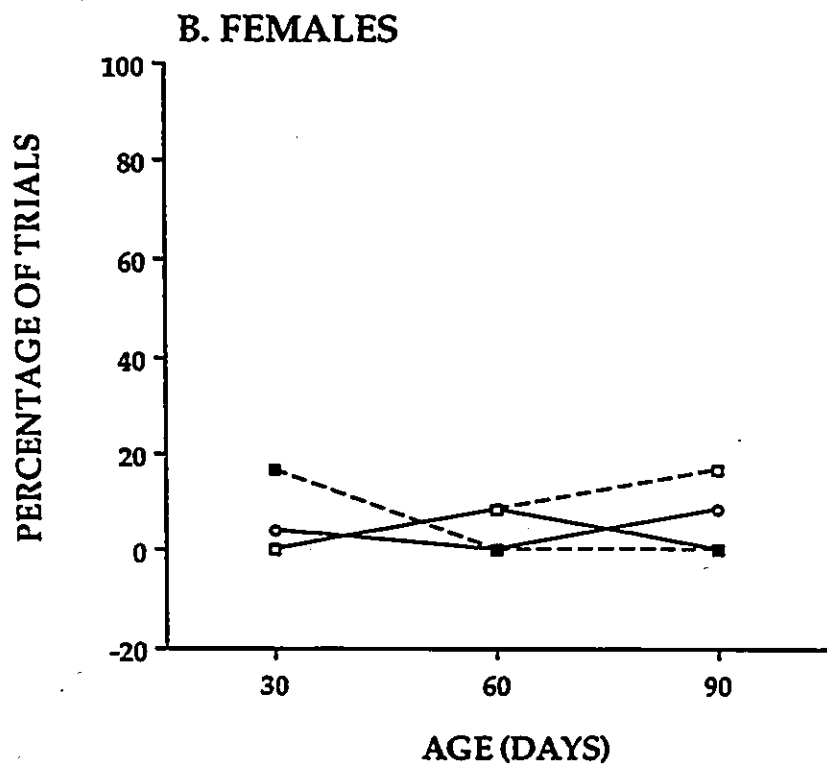
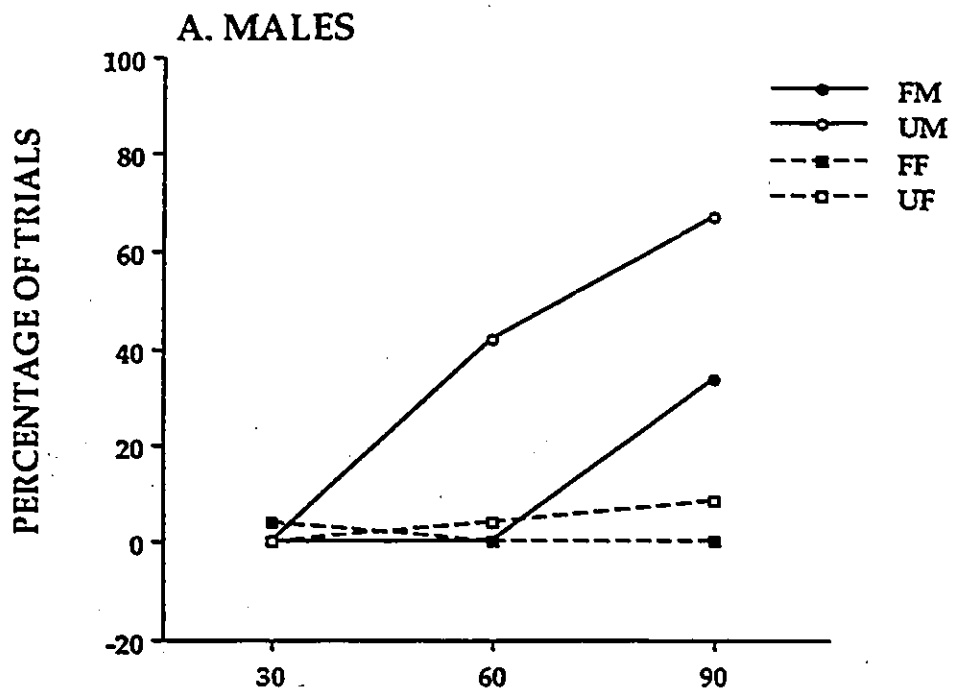
0.05).

Overall, males were more likely to escalate play fighting into agonistic behaviour post-pubertally than were females (Fig. 17). Between 30 and 90 days, there was a significant increase in the proportion of trials containing agonistic behaviour for males ($Z = -12.05, p < 0.0001$), but not for females ($p > 0.05$). Analysis of the agonistic behaviour directed towards males versus that towards females at 90 days showed that males were more agonistic towards males than towards females ($Z = 13.85, p < 0.0001$), whereas for females there was no significant difference ($p > 0.05$). With regard to familiarity of the partner, both males and females were more likely to be agonistic towards unfamiliar partners (males: $Z = -3.40, p < 0.0005$ females: $Z = -2.95, p < 0.005$), although this effect was most dramatic for males (Fig. 17A versus 17B). Therefore, post-pubertally, males were more likely than females to escalate encounters to agonism, and such escalation was most likely with an unfamiliar male partner.

Discussion

During the juvenile phase of development, the identity of an

Figure 17. The percentage of trials in which the rats exhibited agonistic behaviour. (A) Males (N = 12) were more aggressive with other males, especially unfamiliar males, than they were with females, especially after puberty. (B) Females (N = 12) seldom incorporate agonistic behaviours into their play fighting, regardless of with whom they are interacting. FM = familiar male, UM = unfamiliar male, FF = familiar female, UF = unfamiliar female



individual rat's play partner seems to have no effect on the initiation of play fights or the defense used in response to an attack. That is, all play partners are treated equally. Indeed, pre-pubescent males presented with an unfamiliar juvenile male in their home cage are more likely to initiate play than aggression (Takahashi, 1986). In this study, males were less likely to engage strange adult males in play, and more likely to escalate to serious fighting if they did play. Similarly, after puberty, both sexes were more likely to initiate play fighting with females than males, and both sexes tended to escalate play into serious aggression with unfamiliar partners, especially if the partner was male. Therefore, both post-pubertal males and post-pubertal females could distinguish potential play partners in terms of their sex and familiarity when encountering them in a neutral arena.

Significant age-related differences in play partner preference, as shown in an earlier study (Meaney & Stewart, 1981a), may have been obscured by features of the test paradigm used in this study. By artificially increasing the frequency of play fighting by using prior social isolation and a short (10 minutes) testing period, the levels of play fighting were probably at a ceiling level, masking subtle differences in partner preference. Nonetheless, the tendency for post-pubertal males and females to solicit

play more frequently from females than males may be linked to the development of sexual behaviours (Meaney & Stewart, 1981a) and the presence of sex-related odours (Brown, 1979; Gheusi et al., 1994; Thor, 1979). Because there were no significant differences in terms of which animals were most frequently solicited for play, male or female, familiar or unfamiliar, it is unlikely that the the age-related change in defense by males and its absence in females, can be attributed to differences in frequency of playful solicitation or by play partner preferences.

Males exhibited a pubertal shift in their playful defense with both familiar and unfamiliar partners, whether male or female. Females did not change their pattern of defense, irrespective of the familiarity or sex of their play partners. Indeed, if anything, older females were more likely to utilize the juvenile-typical response of rotating to supine when interacting with strange males. This sex difference in playful defense persisted, even though both sexes were more likely to escalate to agonistic behaviour when playing with strangers. That is, even though post-pubertal males and females could discriminate amongst pairmates in terms of sex and familiarity, and modify their responses accordingly, only males shifted their pattern of playful defense from predominantly complete to predominantly partial rotations.

Interactions with social partners that created greater 'social tension' and that were more likely to lead to agonism, did not induce females to change their defensive behaviour.

The present study suggests that the pubertal transition in pattern of playful defense by males is a sex-specific feature of life-history changes in play fighting. Females do not exhibit this shift with familiar play partners (see also Pellis & Pellis, 1990), nor with strangers. This is true even though females are more likely to behave agonistically towards strangers. The present data show that although females are more aggressive towards unfamiliar partners when play fighting, they tend to defend themselves even more like juveniles. Therefore, while it may still be the case that this sex difference in defensive behaviour is the result of different thresholds of elicitation of adult-like defenses, it is unlikely that in more naturalistic rat colonies, females would encounter situations where they would exhibit the male-typical pubertal transition. This sex difference suggests possible differential functions of post-pubertal play fighting in rats.

Adult males, but not females, modulate their pattern of playful defense with respect to dominance-subordinance relationships (Pellis & Pellis, 1991b). Subordinate males predominantly use the complete rotation

defense when attacked by a dominant animal (Pellis & Pellis, 1992), but will tend to use partial rotations when attacked by another subordinate (Pellis et al., 1993). In the present study, it is likely that each quadrat had a dominant and a subordinate male. Dominants and subordinates would thus have been paired with both same status and different status unfamiliar males. No differences were evident in the behaviours of dominant-subordinate, dominant-dominant, and subordinate-subordinate pairs (Smith, Fantella, & Pellis; work in progress). That is, even though subordinates behave in the juvenile-typical manner when play fighting with the dominant male in the home colony, they behave in the adult-typical manner when play fighting with strangers. Because male rats are more likely to disperse from the natal colony, they are more likely to encounter strangers (Barnett, 1958; Calhoun, 1963). By employing adult-typical patterns of play fighting, they may be able to test the competitive abilities of conspecifics without the risk of injury inherent in overt aggression (Pellis & Pellis, 1987). As suggested by Jackson (1988), when animals first encounter each other, especially on neutral ground, both act as dominants until one is proven to be subordinate (see also Geist, 1971). Indeed, in the present study, and that reported for punares by Thompson and Cranford (1985), the pairs only escalated to agonistic

behaviour after first engaging in play fighting. It is possible that interactions with strangers involve two stages. At first the animals engage in play fighting in the adult-typical manner. If the play fighting fails to establish the relative competitive strengths of the interactants, one or both may escalate to serious fighting. When encountering unfamiliar females, males were less likely to escalate to aggression than when interacting with unfamiliar males, which suggests that by continuing to adopt the juvenile-typical rotation to supine, females may be signalling submission.

Adult female rats tend to retain the more frequent use of juvenile-like behaviour patterns, similar to when adult males behave in a subordinate manner (Pellis & Pellis, 1990; Pellis et al., 1993), regardless of with whom they are interacting. This suggests that females do not use play fighting as a means of 'social testing'. They are either less likely to encounter unfamiliar individuals (Barnett, 1958; Calhoun, 1963), or use different strategies to gain acceptance from a stranger (Militzer, 1995; Thor, 1979). Militzer (1995) has suggested that males use aggression and good bodily condition to achieve dominance, whereas females use appeasement behaviours. Adult male and female rats may thus be using play fighting for different functions. Males may be using play, post-pubertally, as a quasi-

agonistic pattern of interaction to assess potential dominance relationships with unfamiliar conspecifics (this study) and familiar males of comparable rank (see also Croft & Snaith, 1991; Paquette, 1994, for similar views on other species), and as a friendship maintenance tactic with familiar dominants (Pellis et al., 1993). Females are more likely to utilize play fighting as an appeasement behaviour in all social contexts in order to avoid agonism and gain access to limited resources.

CHAPTER FIVE

GENERAL DISCUSSION

The experiments conducted for this thesis have revealed several new facts about play fighting in rats. Chapter two showed that the pubertal shift in playful defense is not dependent on the formation of dominance relationships. Post-weaning castrates shifted to adult-typical defensive patterns, but did not exhibit the asymmetries indicative of dominance. This experiment also reinforced the view that gonadal hormones are necessary at puberty to activate the systems involved in the establishment of dominance-subordination relationships (Panksepp et al., 1984; Pellis et al., 1993), but it turned out that they are not necessary to activate the pubertal shift in playful defense. That is, the pubertal shift in defense is independent of both circulating gonadal hormones and dominance-subordination relationships. The study of neonatal castrates in chapter three provided evidence for the organizational effects of gonadal hormones, during the perinatal period, on the pubertal shift in playful defense. Not only did the castrated animals fail to establish dominance relationships, they also failed to show the male-typical pubertal shift in playful defense. Finally, chapter

four illustrated that the pubertal shift in play fighting is male specific. It cannot be induced in females through the use of more extreme social situations, involving interactions with unfamiliar males. That is, females continue to behave in a juvenile-typical manner in all contexts. These findings suggest that the transition in playful defense is a pre-programmed male-typical feature of play fighting, and the play fighting of male and female rats is likely to serve different functions, especially post-pubertally. The issue of function has been a highly contentious one since research into play began, but in order to fully evaluate the findings of this thesis, the question of function needs to be considered.

Why Play?

One of the most commonly used defining characteristics of play is its lack of obvious purpose and benefits (Martin & Caro, 1985). Play may be considered to be simply a byproduct of immaturity, something that animals engage in while awaiting sexual maturity (Coppinger & Smith, 1989; Pellis, 1993). However, most authors believe that animals do not exhibit a behaviour unless its benefits exceed its costs (Fagen, 1981). Thus, most researchers work under the assumption that play is an adaptive trait that

has evolved to provide some essential function(s). According to Owen Aldis (1975), "although play is often considered to be a frivolous pastime of little consequence, play probably has an important survival value for many species..." (p. 1). In their review, Martin and Caro (1985) indicated that the costs of play may not be as great as generally assumed, and thus, the benefits need not be great either. To account for the lack of observable benefits at the time of performance, several researchers have suggested that the benefits of juvenile play are not realized until adulthood (Martin, 1984; Martin & Caro, 1985; Smith, 1982). A less popular, yet equally possible, view is that play serves immediate benefits (Barber, 1991; Bekoff & Byers, 1981). Immediate benefits may simply have been overlooked because they were small and/or subtle (Martin & Caro, 1985).

A common hypothesis about the delayed benefits of juvenile play is that, as an immature or incomplete form of such behaviours as sex and aggression, play serves as practice for adult behaviour patterns (Fagen, 1981; Martin & Caro, 1985; Moore, 1985; Smith, 1982). For example, by play fighting, young animals can learn how to fight, without the risk of injury and retaliation (Symons, 1978). Studies of socially isolated animals are often used as evidence supporting this theory (Martin & Caro, 1985). Socially

isolated animals often show deficits in their behaviour (Hård & Larsson, 1971; Meehan & Henry, 1981). However, these animals are not only prevented from playing, they are also unable to perform other social behaviours, thus confounding the results (Bekoff, 1976). As well, their abnormalities may not be as severe as often thought, because several of these studies failed to find significant differences between isolates and control animals (Martin & Caro, 1985). The practice theory is also flawed in that the animals may be practicing the wrong behaviours in play or rehearsing behaviours that were already mastered (Müller-Schwarze, 1984; Pellis & Pellis, in press b). For example, it is unlikely that rats are practicing for sex when they play (Moore, 1985), as the positions assumed are not the same (Pellis & Pellis, in press b). Females do not adopt a supine position for mating (Nelson, 1995), hence they should be practicing lordotic postures during play. Males may be gaining some practice for sex through play, as they often exhibit mounting (Pellis & Pellis, 1990) and use the same targets of contact as in sex (Calhoun, 1962; Pellis, 1988). But, the play of males is usually considered practice for fighting. If this is true, the males are attacking the wrong targets. In play fighting and sex, the nape is the primary target of attack, whereas in serious fighting it is the flanks and lower

dorsum that are bitten (Blanchard et al., 1977; Pellis, 1988; Pellis & Pellis, 1987). It has also been shown that in several species, including spotted hyenas (Drea, Hawk, & Glickman, 1996) and coyotes (Bekoff, 1978), the young exhibit serious fighting before they start play fighting. Thus, play would not serve as practice for behaviours that are already well established and being used for life and death situations! The same applies to behaviours that are complete the first time they are shown. Pre-weaning rat pups, for instance, exhibit all the behaviours typical of adult play shortly after their eyes open (Pellis & Pellis, in press a). The practice theory also cannot account for adult play, as adults should already be adept at the behaviours they need (Martin & Caro, 1985). Therefore, in many cases, play is unlikely to serve as practice for adult behaviours. While some of these behaviours may be improved as a result of play, this is more likely to be a byproduct or side benefit, not the main function of play.

Other suggested delayed benefits of play are motor training, socialization, and cognitive or sensorimotor training (Bekoff & Byers, 1981). The exercise or physical training hypothesis suggests that through play an animal can increase its strength, endurance, skill, and overall physical health. Socialization hypotheses postulate that play helps young animals

learn communication skills, establish and maintain social bonds, and control aggression. Cognitive training is purported to result from play, as animals improve their ability to deal with the nonsocial environment, through learning and the development of the nervous system. While these hypotheses may prove to be true, at present, there is little empirical evidence to support any of them, and in many cases, the benefits that are exhibited are in the short-term, and quickly fade with age (Bekoff & Byers, 1981; Martin & Caro, 1985).

Immediate benefits of play, especially play fighting, have been proposed for a variety of species (Bekoff & Byers, 1981; Martin & Caro, 1985; Müller-Schwarze, 1984). Different types of play may serve different functions depending on age, sex, species, and play partner. For instance, adolescent boys may use a rough form of play fighting in order to establish dominance relationships (Neill, 1976; Pellegrini, 1995), and then use more gentle forms to maintain this dominance (Neill, 1976). Adolescent chimpanzees may also use play fights to increase or maintain dominance (Paquette, 1994). Among New World deer, such as mule and white-tailed deer, sparring matches usually occur between males of unequal size and dominance rank (Geist, 1981). The younger and/or subordinate buck may use these encounters to develop and maintain coalitions with the

dominant. Old World deer, in contrast, may be using the same behaviour as a means of assessing fighting ability and establishing dominance between well-matched males (Geist, 1982). Barber (1991) contends that play is a promoter of adaptive energy loss, which results in enhanced ability to elude predators, and resist cold stress and pathogens.

Rats may also derive immediate benefits from play fighting. The pre-programmed, male-specific shift in playful defense, at puberty, suggests that males may have to alter their behaviour at this time in order to cope with new conditions. Around puberty, the males of many rodent species disperse from their natal territories (Calhoun, 1963; Nelson, 1995). These dispersing males are likely to encounter a number of unfamiliar conspecifics, including competing males. The dominant males of neighbouring colonies will readily attack dispersers that intrude on their territories (Blanchard, Fukunaga, Blanchard, & Kelley, 1975; Blanchard & Blanchard, 1977). Even though dispersing males are often more aggressive than non-dispersers (Nelson, 1995), it is often in their best interest to avoid overt aggression, in order to reduce the risk of retaliation and to be accepted by the group (Meehan & Henry, 1981). Even minor injuries obtained in a fight can be detrimental and even lead to death (Lore & Schultz, 1993). Thus,

unfamiliar adult males may engage in play fights as a means of social testing. The animals can test each others' strengths and weaknesses while minimizing the chances of an agonistic attack. In contrast, it has previously been suggested that within colonies of animals, familiar adult male rats may play in order to maintain friendships and reinforce dominance relationships (Pellis et al., 1993). Subordinate males may be soliciting play fights with the dominant in order to ensure that he is 'remembered' and not aggressed against, whereas, dominant males may play fight in order to reaffirm their position without using overt aggression (Pellis & Pellis, 1991b, 1992; Pellis et al., 1993).

Female rats are less likely to disperse than males, or do not emigrate as far (Calhoun, 1963) and thus, do not need to change their play fighting behaviour (Pellis & Pellis, 1990). Indeed, by maintaining a juvenile-typical form of playfighting, females may be using play as an appeasement behaviour for gaining resources and maintaining social bonds at all ages (Militzer, 1995). If females do emigrate, they are usually readily accepted (Thor, 1979) and hence, do not need to test relationships with unfamiliar conspecifics. When play occurs in adolescents and adults, it is often more clear what the immediate benefits are. However, because there are a variety

of immediate benefits for play fighting as an adult, it is likely that juvenile play may also serve different purposes; some immediate and some delayed.

Why do Juveniles Play?

By definition, "juveniles are animals that would be likely to survive the death of their caretaker or loss of parental provisioning (e. g., yolk sac) but have not yet matured sexually" (Pereira, 1993, p. 19). A number of species have a distinct juvenile phase, which is often distinguishable by characteristic colours, behaviours, or other characteristics (e. g., Estes, 1991; Flood, 1984; Mahon, 1994). This phase of development is important because it serves as a transition between dependence on caretakers and sexual maturity and independence. It may be brief or very protracted, depending on rates of physical growth and behavioural development. Pagel and Harvey (1993) have proposed that mammalian juvenile periods may have evolved: a) as a consequence of the time needed to acquire adult size and of seasonal constraints; b) because natural selection favours individuals that spend extra time before adulthood learning the skills that affect later reproductive success as well as, age-specific mortality; or c) as some combination of the two. Thus, the juvenile period itself may be a byproduct

of development or an adaptation for survival. If being a juvenile is in fact beneficial, it would seem to be an advantage to prolong this juvenile phase as long as possible.

In rats, and a variety of other species, the onset of the juvenile phase corresponds with weaning (Pagel & Harvey, 1993; Rubenstein, 1993). Prior to weaning, the young are dependent on their mother and can not leave. After weaning, however, it is in the mother's best interest to force the weaned young to disperse so that she can concentrate her energy on the next litter (Trivers, 1974). Dispersing can be a highly risky endeavor, as it exposes the disperser to predation, starvation, and conspecific aggression (Nelson, 1995). Because they are often tolerated by colony males and their mothers (Calhoun, 1963; Thor, 1979) and not forced to leave (Stafford & Stout, 1983) until they reach puberty and acquire the 'androgen' odours that distinguish them as males (Thor, 1979), it is in the dispersing males' best interest to stay with their families as long as possible. Delaying dispersal may be a way for young to prolong the amount of time they spend in their parents' care and, thus, increase their chances of survival. By stalling for time, they can become larger, stronger, and more adept at the skills needed to compete for territories and mates while still enjoying the comforts of home and their

parents' protection (Emlen, 1994). Males that do not disperse are likely to be highly subordinate (Meehan & Henry, 1981) and either 'suck up' to the dominant male or avoid him (Barnett, 1975; Pellis & Pellis, 1991b, 1992; Pellis et al., 1993).

Play is an important part of the juvenile period for many species. Changes in the behaviours of juvenile animals often coincide with transitions in environment and social relationships. Kittens, for example, switch from predominantly engaging in social play to more object play following weaning (Bateson, 1981). Early weaning tends to accelerate this change. Bateson (1981) suggests that behaviour must be reorganized as different systems concerned with different functions gain prominence. Thus, kittens play more with each other when in enforced contact, in order to simulate adult social encounters, but play more with the physical environment when it is time for them to gather new information about food and surroundings. Cuvier's gazelles show distinct time courses in their play, with social play peaking in the advent of new social situations (Gomendio, 1988). These animals play more vigorously during periods of social uncertainty and may use play in order to integrate into new social groups. The behaviour of spotted hyenas changes markedly when they are transferred from their natal to communal dens (Drea et al., 1996). Cubs of

this species are highly aggressive right from birth and do not play until dominance has been established between siblings and it is time to move to the communal den. Along with the onset of play is a decrease in aggression. Play serves the immediate benefit of helping to establish affiliative relationships and promote social cohesion by forming, strengthening, and maintaining social bonds.

Play in rats also shifts following weaning. Both males and females begin to form aggregates (Calhoun, 1963), play fight more frequently and change from predominantly utilizing partial rotation defenses to more complete rotations (Pellis & Pellis, in press a). Juveniles, females, and subordinate adult males all use the complete rotation as their predominant mode of defense in play fighting. If one considers this a submissive behaviour (Panksepp, 1981), then all three categories of these animals may be using play for the same immediate benefits, maintaining social cohesion and avoiding agonism. As noted, subordinate, males may be using play in order to maintain a close association with a dominant male (Pellis & Pellis, 1991b, 1992; Pellis et al., 1993). As females are less likely than males to migrate far from their natal home, they may be using play to maintain familiarity with the female kin they live with. Juveniles may be acting

submissively in order to stay with their parents and siblings as long as possible. Parents are more likely to be tolerant of submissive offspring and siblings are more likely to maintain bonds with those they play with (Calhoun, 1963). An animal that becomes too aggressive may quickly be chased off. Prior to weaning, rat pups can play more aggressively without fear of being forced out of the nest. A mother will rarely evict her offspring before they can survive on their own, as this decreases her reproductive fitness. As well, the pups need not be as cautious under the watchful eye of their mother. The same has been found in human children, who tend to play more vigorously when there is an adult nearby (Lore & Schultz, 1993). Thus, by playing as juveniles, in this submissive manner, these animals may be gaining the immediate advantage of delaying dispersal.

By playing as a juvenile, an animal may be becoming a better juvenile, not just a better adult. "Juveniles are forerunners specialized for the tasks of surviving the wait until reproduction and of using that time wisely " (Pereira & Fairbanks, 1993, p. 4). Being a juvenile can be an exceptionally risky endeavor. As individuals become less dependent on their mothers, they are forced to fit into an adult world when they have neither the size nor experience to do so easily (Janson & van Schaik, 1993).

While play may serve to enhance the skills an animal needs as an adult, its benefits also help to make better juveniles, ones that are competent and more likely to survive than ones that do not play (Martin & Caro, 1985). They are equipped to cope with the problems of being a juvenile not just the ones they will have to contend with as adults. Play behaviour in the juvenile phase, then, may be what Gould and Vrba (1982) call an 'exaptation'. It may not have originally evolved to do so, but the play fighting of juvenile rats may now aid in their ability to remain in the natal colony for longer than they would otherwise. Being a juvenile for a certain length of time may increase a rat's chances of being successful, in terms of reproductive fitness; playing as a juvenile may make a rat more likely to survive long enough to reproduce.

Conclusion

The work reported in this thesis supports the view that play fighting has both delayed and immediate benefits (Bekoff, 1978; Bekoff & Byers, 1981; Martin & Caro, 1985). In rats, the age and sex differences in playful defense suggest that play fighting may serve a variety of functions (Table 1). Other authors have demonstrated that juvenile play may enhance later sexual

Table 1. Delayed and immediate benefits of play fighting in rats as suggested by other authors and the work of this thesis. Bold face indicates work from this thesis.

Functions of Play Fighting in Rats

DELAYED BENEFITS

adult males	enhancement of sexual skills Larsson, 1978, Moore, 1985
adult males	possible enhancement of skilled use of some defensive tactics Pellis & Pellis, in press b

IMMEDIATE BENEFITS

adult males - familiar	maintenance of friendships, reinforcement of dominance relationships Pellis, Pellis, & McKenna, 1993
adult males - unfamiliar	social testing
females	appeasement, social bonding (also see Miltzer, 1995)
juvenile males	delay dispersal, avoid agonism

skills (Larsson, 1978; Moore, 1985) and possibly the skilled use of some defensive tactics (Pellis & Pellis, in press b) Immediate benefits have been suggested by Pellis and colleagues (1993), who determined that familiar adult males in established groups may use play fighting to maintain friendships and reinforce dominance relationships. As well, Militzer (1995) proposed that females use appeasement behaviours in order to establish and maintain social bonds. It is likely that play functions as an appeasement behaviour and thus, serves to enhance these social bonds. The experiment outlined in chapter four of this thesis suggests that both male and female rats alter their behaviour when interacting with strangers. Females become even more submissive towards unfamiliar males, that is they exhibit more complete rotations, whereas males are less likely to engage in this submissive behaviour. These males are likely using a rougher form of play fighting to test and push each other in order to determine who is 'boss' without provoking a serious fight. Thus, the change in playful defense at puberty in males and the lack of such a change in females, may be adaptive strategies for dealing with the rigors of dispersal in two distinct ways. Chapters two and three demonstrated that the shift in playful defense in males is organized neonatally and relies on gonadal hormones at birth but

not at puberty; it is pre-programmed for use in later life. Because juvenile males are no longer dependent upon their mothers, but not ready to compete for mates or their own territories, they may be using play to delay dispersal. By acting submissively, they are more likely to be tolerated by colony adults.

While a number of authors consider complete rotations or 'pins' to be submissive postures (e. g., Panksepp, 1981; Poole & Fish, 1975), few have viewed it as an adaptive advantage. By behaving submissively, an animal can avoid agonism and gain acceptance into a group. For subordinate adult males, being submissive may be a way of making the most of the situation; it is too risky to challenge the dominant or try to establish a new territory, so by 'sucking up' to the dominant they increase their chances of survival. Females may be using submission as an alternative tactic. Males often use aggression to gain resources and establish themselves in a group, whereas females may use appeasement and more subtle determinants of status (Militzer, 1995; Ziporyn & McClintock, 1991). Juvenile males may also act submissively to avoid agonism and maintain group cohesion, but for them it may be a tactic to minimize the risks involved with being a juvenile and increase their chances of survival. So, while the benefits of play in rats are

still being debated, this work proposes alternative ways in which play can help improve fitness by focussing on the immediate rather than the delayed benefits of this behaviour. Considering submission in play as an adaptation (or exaptation) provides a novel view of the problem of why animals play, and may give new insights into the development of the life-history patterns and social systems of rats and other species.

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