PLAY FIGHTING IN KINDLING-PRONE (FAST) AND KINDLING-RESISTANT
(SLOW) RATS: POTENTIAL GENETIC CONTROLS OVER THE COMPONENTS
OF PLAY

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ABSTRACT

Play fighting in kindling-prone (FAST) and kindling-resistant (SLOW) rats: .

Potential genetic controls over the components of play.

Even though the behavioral components of play fighting have been well characterized in

the rat, little is known about the underlying neurobehavioral mechanisms that control

them. FAST and SLOW lines of selectively-bred rats were used to determine whether the

components of play fighting were dissociable. Differences in their respective play

profiles suggest that there are genetic differences in the expression of different

components of play. The effects of gene-environment interactions on the components of

play suggest that playful attack and playful defense may be differentially labile, and that

socially-relevant environmental manipulations may have a greater impact on social

interactions in adulthood than in the juvenile phase. Furthermore, these findings suggest

that the genetic constraints on each of the components of play fighting give each

component a unique pattern of context-dependent change. Future experiments using

FAST and SLOW, as well as other selectively-bred lines of rats may provide insight into

the proximate mechanisms regulating play fighting.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 2.

Chapter 3.

CHAPTER ONE

General Introduction

What is play fighting?

Play behavior is not easily defined (Bekoff & Byers, 1981; Burghardt, 1998; Fagen, 1981) in terms of both its functional purpose (or lack thereof), and the wide range of behavioral patterns it may encompass. Bekoff and Byers (1981) have offered a definition that advocates a descriptive way of thinking about play which emphasizes the structure of play sequences rather than the putative functions of play: "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" (pp. 300-1). Of importance for this thesis is the additional caveat: "If the activity is directed toward another living being it is called social play".

Complementary to Bekoff and Byers' descriptive account of play, Burghardt (1999) has more recently developed five criteria that a behavioral sequence must meet to be classified as play: (1) it does not appear to be adaptive or contribute to one's survival, (2) it appears to be spontaneous, voluntary, intentional, pleasurable, rewarding, (3) it differs (in at least one respect) from the "serious" performance of an ethotypic behavior, (4) it is observed repeatedly, but not as a fixed or stereotyped pattern of behavior, and (5) it is observed in healthy animals when they are in a relatively low-stress environment. Importantly, these criteria do not make any assumptions about the functions of play, and yet they are applicable to play behavior observed in various mammalian species (e.g., rats).

1

It is fairly well established that play is a distinct behavioral entity that is not simply a juvenile representation of more adult-typical behaviors (Fagen, 1981). Social play, specifically play fighting, has become the most frequently studied and reported form of play in the animal literature (Pellis & Pellis, 1998a), which should not be unexpected considering that it has been documented in most mammalian and many avian species, as well as in some reptiles (Burghardt, 1999). Despite the fact that no definition for play contains undisputed objective criteria (Pellis & Pellis, 1998a), there is near-universal agreement in recognizing instances of play fighting (Panksepp, Siviy, & Normansell, 1984). During a play fight, partners compete with each other to gain some advantage (e.g., contacting a particular part of the opponent's body) (Aldis, 1975; Pellis, 1988), but unlike serious fighting, the interactants provide the opportunity for the partner to gain an advantage (Pellis & Pellis, 1998b). These characteristic role reversals (i.e., of attacker and defender) between play partners are important for preventing the interaction from escalating to a serious fight, and are obvious during a bout of rough-and-tumble play, even to a naïve observer. The study of play fighting then, can be guided to some extent by the definitions of play provided by Bekoff and Byers (1981), and Burghardt (1999), as well as an intuitive understanding of play fighting.

Play fighting in the rat

What do we know?

The most commonly used model species for the study of play fighting is the laboratory rat (Panksepp, 1998; Siviy, 1998; Vanderschuren, Niesink, & Van Ree, 1997). Because rats begin to play fight approximately 18 days after birth (Bolles & Woods, 1964), and

2

continue to play into adulthood, albeit at a reduced frequency (Pellis & Pellis, 1990, 1991a), they (as a model species) are well suited to investigate the development of play fighting. In particular, the behavioral components of play fighting have been well characterized in the rat. That is, previous studies have (1) identified behavioral characteristics that distinguish play fighting from serious fighting (e.g., target of attack) (Pellis & Pellis, 1987; Siviy & Panksepp, 1987a, 1987b), (2) generated detailed descriptions of the specific components of play fighting (i.e., attack and defense), and (3) documented the way in which components of play fighting change throughout development (Foroud & Pellis, 2002, 2003; Pellis, Field, Smith, & Pellis, 1997; Pellis & Pellis, 1997).

A play fight between rats begins when one partner (i.e., the attacker) tries to make contact with and gently nuzzle the other partner's (i.e., the defender) nape of the neck (Pellis, 1988). In contrast, during a serious fight, the attacker will direct bites to the defender's lower flanks and rump (Pellis & Pellis, 1987). Because the target of playful attack does not change throughout development, we have a clear behavioral marker that allows us to differentiate whether or not rats are in fact play fighting. The frequency of launching playful attacks peaks during the juvenile phase (i.e., 30-40 days) (Thor & Holloway, 1984), and it has been shown that the age-related decrease in play fighting can be attributed to a reduction in the number of playful attacks launched between play partners (Pellis & Pellis, 1990).

3

Once the attacker has launched a playful attack, the defender can use a variety of defensive tactics to protect the nape from being contacted (Pellis & Pellis, 1990). While the probability of defending against playful attacks remains relatively high at all ages (i.e., between 80-90%) (Pellis et al., 1997), the use of different tactics of defense changes in a characteristic, age-related manner (Pellis & Pellis, 1990, 1997). When defending, rats can either move away from the attacker (i.e., evade) or turn to face the attacker to protect the nape. To evade nape contact, the defender can run, leap, or simply turn away from the attacker. In facing defense, the defender turns to face the attacker, thus interposing its face between the attacker's snout and its own nape. The facing maneuver can involve one of two tactics; turning around the mid-body while remaining standing (Pellis, Pellis, & McKenna, 1993), or by rotating cephalocaudally around the longitudinal axis of the body (Pellis & Pellis, 1987). Rotatory defenses account for 60% or more of all defensive actions (Pellis & Pellis, 1990), with its two variants undergoing age-related changes in predominance (Pellis & Pellis, 1987, 1990, 1997). During the first variant of rotatory defense, the rat only partially rotates, and so keeps one or both hind feet in contact with the ground, and in the second, the rat rotates completely to a supine position (i.e., all limbs lose contact with the ground) to face the attacker (Pellis & Pellis, 1987).

Juvenile and adult rats differ in terms of the frequency with which they use different tactics of defense. The most common form of defense used during the juvenile phase of development is complete rotation (Pellis & Pellis, 1990, 1997); a tactic that is thought to be more conducive to the continued physical contact that is typical of complex play fighting (Pellis & Iwaniuk, 1999; Pellis & Pellis, 1998a). Evasion and partial rotation are

4

more frequently used in infancy and post-pubertally (Pellis & Pellis, 1990, 1997); the former putting an abrupt end to the attacker's solicitation for playful contact, and the latter effectively blocking nape contact altogether (Pellis & Pellis, 1987, 1998b).

The characteristic, age-related changes in play fighting exhibit sex differences. Both sexes show preference for the partial rotation tactic in infancy and the age-related change to a preference for the complete rotation tactic with the onset of the juvenile phase (Pellis & Pellis, 1997). Only males, however, exhibit the shift to the preference for the partial rotation tactic at puberty (Pellis, 2002; Pellis et al., 1997; Smith, Forgie, & Pellis, 1998). Furthermore, post pubertally, males are more likely to modulate their pattern of playful attack and defense depending on the identity of the partner with whom they are playing (Pellis & Pellis, 1991b, 1992; Pellis et al., 1993; Smith, Forgie, & Pellis, 1998). For example, in adulthood, pairs of male rats will form dominance-subordinance relationships (Lore & Stipo-Flaherty, 1984), and during play fighting, the subordinate male will launch more playful attacks than the dominant male, and use more juvenile-like defensive tactics (i.e., complete rotation) when attacked by the dominant (Pellis & McKenna, 1992; Pellis & Pellis, 1991b, 1992; Pellis, Pellis, & Kolb, 1992; Smith, Fantella, & Pellis, 1999). In contrast, female rats do not form the same kinds of dominance relationships as male rats (Barnett, 1975; Ziporyn & McClintock, 1991), and the asymmetry observed during play fighting between adult male rats is not observed in females (Pellis & Pellis, 1990, 1992).

It seems that the attack and defense components of play fighting are clearly dissociable on a behavioral level. Furthermore, it has been suggested that the components of play

5

fighting are motivationally distinct (Pellis & Pellis, 1991a), being mediated by different neural systems (Siviy, Love, DeCicco, Giordano, & Seifert, 2003). For example, when rats are socially-isolated prior to testing, the frequency of playful solicitations (i.e., playful attacks) increases (Panksepp & Beatty, 1980; Pellis & Pellis, 1990; Siviy et al., 2003), whereas playful defense remains constant irrespective of pre-testing social experience (Siviy et al., 2003). Similarly, studies that have examined the effects of various drugs on the components of play fighting (e.g., amphetamine, haloperidol, clonidine, RX821002) have shown that pharmacological agents do not have a uniform effect on all components of play behavior (e.g., Field & Pellis, 1994; Marshall, Pellis, Pellis, & Teitelbaum 1989; Siviy & Baliko, 2000). For instance, amphetamine (Field & Pellis, 1994) and haloperidol (Marshall et al., 1989) reduce playful attack at lower doses than they do defense; suggesting that the effects these drugs have on attack and defense may be relatively independent.

What's missing?

Not surprisingly, most of what we know about the neurobiology of play fighting has come from studies of the laboratory rat (Panksepp, 1998; Siviy, 1998; Vanderschuren et al., 1997). Indeed, there exists an extensive list of drug, hormone, and lesion experiments on play fighting in the rat (see Vanderschuren et al., 1997 for a thorough review), and yet we still do not fully understand the neurobehavioral mechanisms that control the components of play behavior. One limitation of much of the work done, is that researchers have examined only one aspect of play - pinning. Pinning refers to a posture in which one animal is on its dorsal surface and the other animal is on top in what appears

6

to be a 'dominant' posture (Panksepp et al., 1984). As the primary dependent measure of play, pinning provides an index for overall playfulness (Siviy & Baliko, 2000). However, pinning behavior alone does not provide a complete picture of what goes on between play partners during a bout of play fighting. A select few studies have dissociated and examined individually, the attack and defense components of play fighting. In addition to the previously described method for dissociating playful attack and playful defense (Pellis & Pellis, 1987), another method of behavioral analysis has distinguished between pins (similar to complete rotation tactic of defense), and dorsal contacts (similar to playful attack of the nape) (e.g., Ferguson & Cada, 2004; Panksepp, Burgdorf, Turner, & Gordon, 2003; Siviy & Panksepp, 1987a, 1987b). If playful attack and playful defense are in fact controlled by different neural systems, then it would be beneficial for future studies to examine both components of play fighting (i.e., attack and defense) individually, and in both play partners, following any type of experimental manipulation.

An additional limitation of the literature on play fighting is that few studies have examined potential genetic controls over the components of play fighting. While it may be the case that mice have been the tools more typically used (in terms of animal models) to study genetic controls over behavior, mice have only a rudimentary form of social play as compared to rats (Pellis & Pasztor, 1999). In fact, relative to other muroid rodent species, play fighting in the rat is more complex in that it involves more playful attacks, higher probabilities for defending against playful attacks, and intricate role reversals between attacker and defender (Pellis & Pellis, 1998a; Pellis, Pellis, & Dewsbury, 1989). Thus, rat strains that exhibit consistently different levels of play would be more beneficial

7

than mice for understanding the genetics behind mammalian playfulness (Siviy et al., 2003).

At present, the few studies that have compared play behavior between rat strains (e.g., Ferguson & Cada, 2004; Siviy, Baliko, & Bowers, 1997; Siviy et al., 2003), suggest that the components of play fighting are dissociable and that there is likely genetic variation that contributes to differences in play behavior. Further between-strain comparisons of play behavior in rats may prove to be an invaluable, non-invasive technique for investigating questions about genetic influences on play fighting, as well as how gene-environment interactions may affect the development of play. Assuming that social play behaviors share similar characteristics in all mammals, including humans, characterizing the play profiles of selectively bred rats can be thought of as a first step toward understanding the neurobehavioral mechanisms that control play not only in the rat, but also in other mammals.

Selective breeding and behavior

In recent years, it has been increasingly common to breed animals selectively on the basis of differences in behavior in order to investigate the neurobiological mechanisms that may underlie behavioral differences (Ellenbroek & Cools, 2002). Selective breeding begins by measuring a trait of interest in a large founder population. Two-way artificial selection can then be used to create 'low' and 'high' lines that widely differ for the chosen trait (Koch & Britton, 2005). That is, the contrasting lines are derived by breeding those rats from the founder population that demonstrate extreme values for the trait of

8

interest. At each subsequent generation, the progeny are phenotyped for the particular trait, and the 'best' rats (for the trait) are bred to create the next generation. The selection process continues until there is no overlap in the mean distributions for the trait between the two lines.

The major value of inbred or selectively-bred strains of rats emanates from their close genetic uniformity and the following assumptions: (1) that environmental factors account for within-strain variation, and (2) that genetic factors account for between strain variation (Koch & Britton, 2005). In rats, two-way artificial selection has been used extensively to create models that allow researchers to examine and characterize differences in relatively complex traits such as emotionality (Blizard & Adams, 2002), anxiety (Landgraf & Wigger, 2002), alcohol preference (Murphy et al., 2002), and arousal to novelty (Viggiano, Vallone, Welzl, & Sadile, 2002), to name a few. Entire issues of *Behavior Genetics* (e.g., 1981, volume 11, issue 5; 2002, volume 32, issue 5) have been published to inform the scientific community of the utility of selective breeding programs with rats, and the subsequent progress that has been made in the study of mammalian behavior genetics.

If one were to breed rats selectively for the purpose of studying the phenotypic expression of playful behavior, what specific behavioral features should be the basis for selection? Because play peaks in most species during the juvenile phase of development, some investigators have hypothesized that more frequent and elaborate play should be observed in species that exhibit relatively prolonged periods of juvenile development

9

(e.g., Fagen, 1981; Ortega & Bekoff, 1987; Pereira & Altmann, 1985). In support of this hypothesis, comparative analyses of play fighting between species of muroid rodents have indicated that there is an association between play complexity and juvenility (Pellis & Iwaniuk, 2000). That is, social play complexity is significantly predicted by the degree of prenatal growth in body size. Species that are born closer to their adult size (e.g., mice) show less complex play than species that require a greater proportion of postnatal growth to reach maturity (e.g., rats) (Pellis & Iwaniuk, 2000). However, these are broad cross-species comparisons, which may not be informative about variation in the components of play fighting within a single species. Therefore, given the apparent association between play and juvenility, a plausible starting point for making within-species comparisons of play would be to select for rats that demonstrate juvenile-like traits in adulthood.

As mentioned previously, rats have a relatively long period of postnatal development and they engage in complex and exuberant play fighting. However, if one were inclined to use selectively-bred rats to analyze play fighting, then it would be necessary to ask (1) what behavioral features are typical of juvenile rats, and (2) is it conceivable to select for features of juvenility in adult rats? Fortunately, there are suitable answers to both of these questions. In general, juvenile mammals are thought to be more hyperactive, impulsive, distractible, and less fearful than adult mammals (Spear, 2000). Coincidentally, there are two selectively-bred lines of rats (called FAST and SLOW), that show differences in both behavioral and neural features of juvenility (McIntyre, Poulter, & Gilby, 2002; Racine, Steingart, & McIntyre, 1999). Even though the selective breeding program from which these rats have stemmed was intended to examine the genetic underpinnings to seizure

predispositions (e.g., in epileptics), the lines have provided findings concerning many behavioral attributes that are under genetic control; this is to be expected in light of the fact that selective breeding can often result in the expression of comorbid features (Parmigiani, Palanza, Rodgers, & Ferrari, 1999).

Why FAST and SLOW rats?

Selective breeding of the FAST and SLOW lines began with a parent population derived from a mixture of Long-Evans and Wistar rats that showed strong genetic control in the rate of amygdala kindling (Racine et al., 1999). Briefly, the technique of kindling involves repeated sub-threshold electrical or chemical stimulation in the brain (e.g., amygdala), ultimately resulting in progressive and permanent changes in neural excitability that lead to the development of convulsive seizures (McIntyre, Poulter, et al., 2002). For 11 generations, the two lines of rats were selectively bred for their susceptibility to amygdala kindling. That is, the kindling-prone or FAST line was generated by breeding the rats that required the fewest amygdala stimulations to elicit a Stage-5 convulsive seizure (Racine, 1972), whereas breeding the rats that required the most amygdala stimulations generated the kindling-resistant or SLOW line. Despite the relaxation of further selection procedures, the two selected-lines (which will be referred to as 'strains' from this point forward) continue to display their unique kindling rates. Of importance for this thesis are the enduring differences between the two strains for a number of juvenile-like behavioral attributes (presumably associated with differential temporal lobe excitability) (McIntyre, Poulter, et al., 2002).

11

The initial concept for using the FAST and SLOW strains of rats to study questions about play fighting was prompted by an interest in the unique behavioral profile of the FAST rats. That is, FAST rats appear to retain juvenile-like behaviour and brain qualities in adulthood. Research carried out in various laboratories has shown that there is a tendency for impulsivity and hyperactivity in FAST rats (McIntyre, Poulter, et al., 2002). For instance, during open field testing, FAST rats show persistent activity (hyperactivity) after several days of testing, indicating that they habituate poorly to the environment (Mohapel & McIntyre, 1998). Similarly, Anisman and McIntyre (2002) have shown that during learning tasks (e.g., Morris water maze), FAST rats are more easily distracted than SLOW rats by irrelevant cues in the environment. The impulsive nature of FAST rats is further exemplified by their behavior in sexual contexts. Specifically, FAST males have difficulty withholding their approach responses to non-estrous females (i.e., they immediately attempt to mount females that are not emitting the appropriate receptive cues) (McIntyre, Poulter, et al., 2002).

Conversely, at all ages, SLOW rats exhibit a more adult-typical behavioral phenotype. For example, SLOW rats show greater fear- and anxiety-related responses than FAST rats in both familiar and novel environments, in reaction to foot shock, in fear-based learning (e.g., inhibitory and active avoidance) (Mohapel & McIntyre, 1998), and during play fighting (Reinhart, Pellis, & McIntyre, 2004). Stress-related responses also differ between FAST and SLOW rats. In the context of physical restraint, SLOW rats adopt a relatively passive, immobile posture throughout the test period, whereas FAST rats

12

persistently struggle during the entire test period (Anisman et al., 1997); behavior that is reminiscent of the previously mentioned hyperactivity/impulsivity of FAST rats.

With regard to differences in the brain, FAST rats retain a juvenile-like pattern of neural organization in adulthood, as compared to SLOW rats. Poulter and coworkers (1999) have shown that embryonic $GABA_A$ subunits (i.e., $\alpha2$, $\alpha3$, and $\alpha5$) are highly up-regulated in the amygdala, piriform cortex, and perirhinal cortex of adult FAST rats, and that consequently, there is low expression of the predominant adult subunit (i.e., $\alpha1$). SLOW rats exhibit the opposite profile (i.e., under-expression of the embryonic subunits and over-expression of the adult subunit) (Poulter et al., 1999). These different receptor subtypes are associated with unique behavior at a physiological level (i.e., spontaneous miniature inhibitory postsynaptic currents), and the contrasting GABA response profiles in the two strains are presumed to be integral components in their differential behavioral attributes (McIntyre, Hutcheon, Schwabe, & Poulter, 2002).

It seemed reasonable to investigate how the FAST and SLOW strains of rats would behave in a social play situation, primarily because of the documented differences in their behavioral and neural profiles (specifically for juvenile-like traits). Conjointly, that FAST and SLOW rats were selectively-bred on the basis of differential amygdala excitability (function) reinforced further the adequacy of these animals for testing hypotheses about potential genetic controls over components of play fighting. Several lines of research suggest that the amygdala is an important neural substrate that modulates complex social behaviors (e.g., play) (Baron-Cohen et al., 2000; Emery et al., 2001; Meunier,

Bachevalier, Murray, Malkova, & Mishkin, 1999; Prather et al., 2001). Because there are

known differences between FAST and SLOW rats for various social behaviors, one

would expect that genetically-induced functional changes of the amygdala and other

brain regions connected to the amygdala that are thought to be important for regulating

social interactions (i.e., prefrontal cortex), may also lead to changes in the organization of

play fighting.

In addition to the evidence suggesting that the amygdala may be important for

modulating complex social behaviors in general, there is more specific evidence that

suggests the amygdala is critically involved in the sexual differentiation of social play

(e.g., Meaney, Dodge, & Beatty, 1981; Meaney & McEwen, 1986), and that cortical

brain regions connected to the amygdala may be involved in age- and partner-related

modulation of play fighting (Pellis, Pellis, & Whishaw, 1992). In particular, the orbital

frontal cortex (OFC), a brain region reciprocally connected with the amygdala, appears to

be important for partner-dependent modulation of the pattern of play (Kolb, Pellis, &

Robinson, 2004). Interestingly, anatomical analyses that have examined the complexity

of cells in OFC, medial prefrontal cortex, and parietal cortex, show that there are between

strain differences in cell morphology for FAST and SLOW rats (Reinhart, McIntyre,

Pellis, & Kolb, 2004). Thus, it appears that selective breeding for amygdala excitability

renders differences between FAST and SLOW rats not only in terms of amygdala

function, but also in the patterns of cell organization in brain regions that likely work in

conjunction with the amygdala to regulate play behavior, making FAST and SLOW rats

ideally suited to examine questions about play fighting.

14

The objectives for this thesis

The primary objective of this thesis was to use two strains of selectively-bred rats (i.e., FAST and SLOW) to examine the development of play fighting, and thus ascertain whether there may be genetic underpinnings that control the components of play fighting. In particular, it was of interest to first examine the development of components of play fighting in the FAST and SLOW strains of rats. Same-strain pairs of FAST and SLOW rats were tested during the juvenile phase and in adulthood so that a 'play profile' for each strain could be created. It was thought that the characterization of both attack and defense during play fighting (in each strain) would help to answer two important preliminary questions regarding the potential for genetic control of play fighting. First, can developmental changes that lead to the retention of juvenile features produce more complex patterns of play? If so, we would expect that at all ages, the pattern of play in FAST rats would be more complex and juvenile-like than that observed in SLOW rats. Second, do all features associated with play (i.e., attack and defense) covary with changes in juvenility? Some comparative evidence suggests that changes in postnatal development can result in changes in different components of play fighting (Pellis & Iwaniuk, 2004). However, these comparisons have been made between different species of rodents, and not within the same species. The first experiment then, was designed to generate a basic understanding of how selective breeding may affect the entire suite of behaviors associated with play fighting, within a single species.

Once play fighting had been characterized in each strain, it seemed logical to examine the potential effects that gene-environment interactions may have on the development of play

fighting. Genes and environment both play important roles in the expression of behavior. Therefore, in the second experiment, cross-strain pairs of FAST and SLOW rats were reared and tested together. Because a play profile for each strain had already been established, it was possible to determine which components of play fighting changed or stayed the same in response to a change in the environment (i.e., a change in the environment, relative to the previous same-strain experiment), and whether environmentally-influenced changes in play behavior were age-dependent.

In retrospect, it seemed that there was an important limitation in the developmental studies of play fighting that needed to be addressed. In the previous two developmental experiments, the rats were housed as pairs from weaning onwards, and subsequently tested with their respective cage mate. Therefore, it could not be determined if changes in the pattern of play for one partner were due to the peculiarities in behavior of other partner (while they were in the play-testing situation), or if changes in play were representative of more general modifications in behavior that were a consequence of having been reared with a cage mate from a particular strain. For the third experiment, juvenile FAST rats were tested with an unfamiliar FAST and SLOW partner on subsequent occasions to determine whether or not their play behaviors were partner-specific. That is, if focal animals were able to identify unfamiliar partners as being from the same or different strain by responding to their partner's immediate behavior, then one would expect the focal animals to respond differently with unfamiliar FAST vs. SLOW partners. If the play behavior of the focal animals was partner-specific, it could be

suggested that the rats may have been responding to subtle behavioral cues from their play partners.

FAST and SLOW rats have provided some new insights into the organization and regulation of play fighting. This thesis not only reinforces previous suggestions that genetic variation contributes to differences in play, and that components of play fighting are dissociable, but also that the genetic variation may be due, in part, to changes in juvenility. This work also brings forth the idea that different components of a distinct behavioral entity (i.e., play fighting) may be controlled by different neural mechanisms, and that the components may differ with regard to their capacity for modification. The independent variation for each component of play fighting may account for the species-level diversity in the actual content and capacity of play fighting across species (Pellis & Iwaniuk, 1999, 2004). Furthermore, it seems that the respective efficacy that genetic and environmental factors may have on the expression of complex social behavior may differ depending on developmental stage at which they are experienced. That is, environmental influences may be more important at some ages than others (e.g., Arakawa, 2005; Workel et al., 2001). Interestingly, the present results suggest greater environmental modification in the adult rather than juvenile stage, revising the commonly held assumption that older animals are less susceptible to change (e.g., Hol, Van den Berg, Van Ree, & Spruijt, 1999; Van den Berg et al., 1999; Vanderschuren, Niesink, Spruijt, & Van Ree, 1995).

CHAPTER TWO[1]

Development of play fighting in kindling-prone (FAST) and kindling–resistant (SLOW) rats: How does the retention of phenotypic juvenility affect the complexity of play?

ABSTRACT

Rats selectively bred for susceptibility to amygdala kindling (FAST) have been shown to retain neural and behavioral features of the juvenile phase into adulthood. In contrast, rats selectively bred for resistance to amygdala-kindling (SLOW) are neurobehaviorally more typically adult. The development of play fighting in male and female rats of both strains was studied. Given the apparent association of juvenility and play often noted in the literature for mammals in general, it was predicted that the FAST rats should be more playful and be more likely to retain the juvenile tactics of play that lead to more prolonged and complex patterns of social contact. As expected, FAST rats initiated more playful attacks and were more likely to defend against attacks than SLOW rats as both juveniles and adults. Unexpectedly, however, both strains exhibited patterns of defense that reduced the likelihood of complex and prolonged social contact. Importantly, the two strains did so by very different means. FAST rats did so by avoiding contact, whereas SLOW rats did so by responding in an adult-typical manner that blocks contact. That is, FAST rats exaggerated the changes typically occurring at puberty, whereas SLOW rats, at all ages, responded in a more adult manner. These data suggest that the different components of play fighting do not change uniformly with changes in the neurobehavioral underpinnings of juvenility.

INTRODUCTION

Social play, especially play fighting, is the most common type of play observed in the juveniles of most mammalian species (Pellis & Pellis, 1998a), and despite the near universal agreement in recognizing instances of this behavior (Panksepp, Siviy, & Normansell, 1984), little is known about the neurobehavioral mechanisms that control these playful interactions. Most of what is known about the neurobiology of play has been derived from studies of rodents, especially rats. Play fighting in rats involves attack and defense of the nape of the neck (Pellis & Pellis, 1987; Siviy & Panksepp, 1987b). If contacted, the nape is gently nuzzled (Pellis, 1988). To defend against nape contact, the recipient of an attack can use a variety of defensive tactics, the frequency of which change in a relatively predictable manner during development (Pellis & Pellis, 1990, 1997).

The frequency of play peaks during the juvenile phase in most species, and this observation has led several investigators to hypothesize that species with relatively long periods of juvenile development should show more frequent and elaborate play (e.g., Fagen, 1981; Ortega & Bekoff, 1987; Pereira & Altmann, 1985). In support of this possibility, a comparative study by Pellis and Iwaniuk (2000) has shown that social play complexity in muroid rodents is significantly predicted by the degree of prenatal growth in body size. In other words, species that are born closer to their adult size, and, therefore, more mature at birth, show less complex play than species requiring a greater proportion of postnatal development to reach maturity (see Diamond & Bond, 2003, for similar findings for birds); however, this is a cross-species correlation, leaving two questions

19

unanswered. First, is the relationship between an increased juvenile phase and increased prevalence of play or play complexity a specific effect on play or a more general effect on a wide range of neurobehavioral systems? Second, do all features associated with play covary, and if so, is variance on this suite of characters present within a species?

Some comparative evidence suggests that all features of play fighting typical of juveniles are not exaggerated or contracted together. Rather, changes in postnatal development can result in changes in different aspects of play fighting, in different lineages of rodents (Pellis & Iwaniuk, 2004). Conversely, there is evidence that suggests that prolonged postnatal development affects a wide range of neurobehavioral systems, and not just play. For example, mice (*Mus domesticus*) have only a rudimentary form of social play compared to rats (Pellis & Pasztor, 1999), although the solitary, locomotor forms of play in mice seem to be as complex as that in rats (Laviola & Alleva, 1995; Pellis & Pellis, 1983; Terranova, Laviola, & Alleva, 1993). Not only do mice have a rudimentary pattern of social play but they also are simpler than rats for a wide range of social and non-social cognitive capacities (Whishaw, Metz, Kolb, & Pellis, 2001). With regard to development, mice have more of their neural growth occurring before birth than do rats (Pellis & Iwaniuk, 2000). Therefore, comparison of these two species suggests the possibility that changes in developmental timing could account for species differences in the complexity of play, which in turn reflects a general change in cognitive complexity. But again, these are broad species comparisons, which may not be informative about the pattern of relationship of these traits within a single species.

20

Some preliminary comparisons between rat strains suggest that there may be dissociated differences in the performance of the attack, defense, and counterattack components of play fighting (Pellis, Field, Smith, & Pellis, 1997; Siviy, Baliko, & Bowers, 1997). Indeed, a detailed comparison of play fighting in Lewis and Fischer-344 rats has shown that Lewis rats initiate play more frequently (attack) and respond (defend) in ways that leads to more prolonged bodily contact (Siviy, Love, DeCicco, Giordano, & Seifert, 2003). While such differences across strains support the idea that there is likely genetic variation that contributes to differences in various types of play (Siviy et al., 2003; Walker & Byers, 1991), it is not clear that these differences are associated with variation in juvenility.

In the present study, two strains of rats showing differences in the presence of behavioral and neural features of juvenility (McIntyre, Poulter, & Gilby, 2002; Racine, Steingart, & McIntyre, 1999) were contrasted with respect to their pattern of play. It was predicted that the strain that has retained the most juvenile-like pattern of neural organization and behavior in adulthood also should be more juvenile-like in its pattern of play fighting. Such a test is possible because rats continue to play after puberty, albeit at a reduced frequency (Pellis & Pellis, 1990, 1991a). It is also possible to differentiate between play fighting and serious fighting because in the rat, the two behaviors involve different targets of attack (Pellis & Pellis, 1987). In a play fight, the target of attack is the opponent's nape of the neck, whereas in a serious fight the attacker will direct bites to the opponent's lower flanks and rump. In rats, playful targets persist into adulthood, and there also are distinctive, age-related changes in the tactics of playful defense (Pellis &

Pellis, 1987, 1990). Therefore, clear behavioral markers can be used (discussed later) to assess whether adult rats are in fact play fighting, and whether they are playing like juveniles or like adults.

From a parental stock derived from breeding two strains, Long-Evans and Wistar, two lines of rats were selectively bred for 11 generations for their susceptibility to amygdala kindling (Racine et al., 1999). More specifically, the FAST (kindling-prone) line was generated by breeding the rats that required the fewest amygdala stimulations to elicit a Stage-5 convulsive seizure (Racine, 1972), whereas the SLOW (kindling-resistant) line was generated by breeding the rats that required the most amygdala stimulations. These strains have different amygdalar physiology as well as significant differences in behavior (McIntyre, Kelly, & Dufresne, 1999). Of particular interest for the present study is that FAST rats appear to be juvenile-like in many respects when adult. The expression of $GABA_A$ subunits in different areas of the limbic system, for example, shows interesting differences (Poulter et al., 1999). Poulter and coworkers (1999) show that in FAST rats, the $\alpha 2$, 3, and 5 subunits are highly up-regulated and the $\alpha 1$ subunit is significantly down-regulated compared to control rats and the SLOW line (which shows the opposite trend). This is an important finding because, normally, the $\alpha 2$, 3, and 5 subunits are highly expressed early in development and are diminished and replaced by the $\alpha 1$ subunit in adulthood. FAST rats are thus expressing a juvenile $GABA_A$ subunit pattern in adulthood.

Similarly, with regard to behavior, there is a tendency for hyperactivity/impulsivity in FAST rats. This is presumed to be associated with increased temporal lobe excitability (McIntyre, Poulter, et al., 2002). During tasks that measure exploratory behavior in an open field, FAST rats show persistent activity over days of testing, indicating poor habituation to the environment. In addition, FAST rats show vigorous struggling throughout the entire test period during physical restraint whereas SLOW rats show a relatively non-hyperactive, immobile posture in the same context. During learning procedures such as the Morris water maze, FAST rats are more easily distracted by irrelevant cues than are SLOW rats, although pretraining can ameliorate these performance deficits (Anisman & McIntyre, 2002). The impulsive nature of FAST rats also is evident in the context of tests for sexual behavior in that the males have difficulty withholding approach responses to non-estrous females (McIntyre, Poulter, et al., 2002). Mating behavior is normally highly dependent upon the male's response to various sensory cues emitted by a receptive female (Beach, 1976), but FAST males immediately attempt to mount females irrespective of those appropriate cues. The impulsivity of the FAST line is further exemplified by their lower stress-related responses to anxiety-producing tests (Mohapel & McIntyre, 1998).

Impulsivity, distractibility, and reduced fear are typical features of juvenile mammals (Spear, 2000). That is, as adults, the FAST strain retains more juvenile-like features. We therefore studied the development of play fighting and its constituent components, attack and defense, in these rats. We predicted that FAST rats would show a juvenile-like play profile in adulthood and that SLOW rats would show more adult-typical behavior. More

23

specifically, we predicted that FAST rats should initiate more play fights than SLOW rats both in the juvenile and adult phases, and retain the juvenile-typical pattern of defense into adulthood. If so, this would support the possibility that a relatively simple developmental change leading to the retention or prolongation of juvenile characteristics could produce more complex patterns of play. Since selective breeding can often result in the expression of comorbid features (Parmigiani, Palanza, Rodgers, & Ferrari, 1999), the relative levels of activity and anxiety of the animals also were measured. We therefore wanted to assess differences in the rate of play, independent of differences in overall activity. Because of the known differences in the response to stressors between these strains (Mohapel & McIntyre, 1998) and the inhibitory effect that stress is often thought to have on play behavior (Burghardt, 2004; Fagen, 1981), it also was relevant to assess differences in behaviors that are indicative of anxiety.

METHODS

Animals.

Kindling-prone (FAST) and kindling-resistant (SLOW) rats were obtained from Carleton University, and breeding pairs were established at the animal housing facility of the Department of Psychology & Neuroscience at the University of Lethbridge. A total of 71 animals derived from these breeding pairs were used in several experiments. Breeding pairs were housed in 46 x 25 x 20 cm polyethylene tubs with processed corn cobs for bedding. In all cases, the animals had *ad libitum* access to food and water. The animal colony was maintained at 21°C on a 12:12 hr light: dark cycle (lights on at 0730 hr). The care and use of animals was in accordance with local standards set by the Animal

24

Welfare Committee, as well as the national standards set by the Canadian Council for Animal Care.

Experiment 1. Social interaction tests

Subjects.

Twenty-four FAST (12 males, 12 females) and 24 SLOW (12 males, 12 females) rats from four litters of each were used. At weaning (Day 22), the subjects were removed from their mothers and placed in same-sex, same- strain pairs (6 pairs of males and 6 pairs of females per strain), in polyethylene tubs. All rats were weighed every 5 days beginning on the day of birth until Day 95.

Procedure.

On Days 29, 30, and 31, each pair was given 15-min habituation periods in the test enclosure. On Day 31, following habituation, each animal was isolated for 24 hr in a 46 x 25 x 20 cm polyethylene cage, as such isolation prior to testing has been shown to increase the frequency of play fighting (Panksepp & Beatty, 1980; Pellis & Pellis 1990). On Day 32, following isolation, each pair of rats (cage mates) was placed together in the test enclosure. Following testing on Day 32, the animals were once again housed as pairs. On Day 33, each pair again went through the 15-min habituation and 24-hr isolation procedures, and was tested on Day 34. The animals were then housed as pairs until the adulthood, at which time the procedure was repeated. On Days 87 and 88, the animals were habituated for 15 min. On Days 89 and 91, the animals were both habituated and isolated. On Days 90 and 92, the animals were tested and then again housed in pairs.

25

Unfortunately, it is not possible to control the amount of social experience each individual subject will have between testing phases when using a group-housing paradigm. Some researchers have tried to control for the amount of social experience by using prolonged individual housing (Ikemoto & Panksepp 1992; Panksepp, 1981), but several studies have shown that social isolation is not a neutral manipulation. Even social isolation lasting days rather than weeks can lead to severe social deficits (for review, see Pellis, Pellis, & Foroud, 2005). Controls isolated for the 2 months necessary to cover the age changes from the juvenile phase to the early adult phase would likely result in adults with profound deficits in social behavior. For example, Hol, Van den Berg, Van Ree, and Spruijt (1999) showed that during postnatal Weeks 4 and 5, social isolation in the rat causes reduced social activity in adulthood. Von Frijtag, Schot, van den Bos, and Spruijt (2002) showed that as adults, rats reared individually during the juvenile period display less ability to use stress-reducing behaviors when confronted by stressful social situations and less success in averting aggression directed at them. Finally, it has also been shown that rats exhibit individual differences in their style of play behavior (Pellis & McKenna, 1992; Reinhart & Pellis, unpublished observations); this emphasizes the importance of testing the same individuals during both the juvenile and adult phases of development. Thus, although the uncontrolled level of play an individual might experience in a group-housed rearing condition may attenuate our ability to draw causal conclusions regarding the effects of that experience, it would seem that using a paradigm where individuals are not exposed to social interactions for prolonged periods of time would be worse. Studies using the same paradigm as that used here have shown the same pattern of developmental change (Pellis, 2002). The small differences in social experience among rats when reared

26

in social groups may account for the differences in magnitude in those developmental patterns seen across studies; however, this issue was beyond the scope of the present study.

Habituation and testing commenced each day between 0800 to 0900 hr. On the first day of habituation in both the juvenile and adult phases of testing, the black and white pelage markings for each animal were drawn so that the animals could be identified during video analysis. A movable mirror located at the back of the enclosure facilitated observation of otherwise hidden parts of the rats' bodies. The dimensions of the enclosure during the juvenile testing were 50 x 50 x 32 cm; during the adult testing, they were 50 x 50 x 50 cm to accommodate the larger body size of the adults. Test periods lasted 10 min and were videotaped in the dark using the "night shot" function on a Sony 8-mm camcorder. The camera was placed in front of the enclosure at an oblique angle at a distance that could capture the entire volume of the testing area.

Behavioral Analyses.

After collecting the behavioral data, each 10-min test period was scored for various components of play and other behaviors. The 8-mm tapes were converted to VHS format with the addition of a time-code (30th of a s), which allowed for slow-motion and frame-by-frame inspection. Each of the following measures of play fighting were quantified for all animals (for more detailed description, see Pellis, Pellis, & Whishaw, 1992):

(I)   Frequency of attack: the number of playful initiations (i.e., nape contacts) per 10-min period

(II)   Probability of defense: the number of times an animal elicits a defense

(withdrawal of the nape area from the snout of an approaching partner) relative to

the number of attacks received per 10-min period

(III)   Type of defense:

   (i)   Probability of evasion – the number of evasive defenses (withdrawal of

         the nape by leaping, running, or turning away from the partner) relative to

         number of total defenses

   (ii)   Probability of complete rotation – the number of times the defender rotates

         around its longitudinal axis to lie supine (facing the attacker to block nape

         access) relative to number of total defenses

   (iii) Probability of partial rotation – number of facing defenses whereby the

         defender turns to face the attacker by rearing on the hind legs relative to

         number of total defenses

In addition, two behaviors were scored for each individual rat in the 10-min tests that

provide a measure of anxiety and stress. These measures were of body shakes (which

could involve the whole body or just the head) and hind leg scratches of the anterior of

the body. Both measures have been shown to be more frequent in stressful contexts in

rodents and other mammals (Redmond & Huang, 1979; van Hooff & Aureli, 1994; van

den Bos, 1998). Although freezing is a more common measure of fear and anxiety in

rodents (Fendt & Fanselow, 1999), it rarely occurred during the play sessions. Shakes and

scratches were combined to provide an anxiety index. Therefore, if these measures do

reflect anxiety, then a high score on this index would represent a higher level of stress.

28

Experiment 2. Circadian Activity

Subjects.

Twelve FAST (6 males, 6 females) and 11 SLOW (5 males, 6 females) rats, each from two different litters, were used. At weaning, subjects were removed from their mothers and placed into same-sex, same-strain groups.

Procedure.

Animals from each of the four types (strain, sex) were randomly chosen to form three groups: two groups of 8 animals and one group of 7 animals. The use of groups was necessary to accommodate the limits of the testing apparatus. Animals from each of the three groups were housed singly in computer-monitored circadian activity cages for 48 hr so that baseline activity levels could be analyzed (Gibb, 2001). These cages are fitted with infrared light beams and detectors so as to assess activity by monitoring motion. Each time the animal disrupts the light beam, the computer records the side of the cage at which the activity occurred, and a combined activity (for activity occurring across both left and right sides of the cage) is computed. During activity monitoring, the animals continued to have *ad-lib* access to food and water and were maintained on a 12:12 hr light: dark cycle. The animals were tested during the juvenile period (Days 36-41), and again in adulthood (Days 88-93). The information on movement or activity for each individual animal was used to calculate group means $\pm$ SEM.

Statistical Analyses

For all datasets collected, an ANOVA with strain (FAST or SLOW), sex (male or female) and age at testing (juvenile or adult) as independent factors was used for analysis. For graphical purposes, the group means are shown.

RESULTS

Play fighting involves attack by one animal and defense by the other. Therefore, the overall frequency of play can be influenced by both the frequency of launching attacks and the probability of defending against attack. In this study, there were age and strain effects on these measures of play. For attacks, there was a significant age effect [$F(1, 88)$ $= 154.90, p < 0.0001$], and strain effect [$F(1, 88) = 9.42, p < 0.01$]. Juveniles attacked more frequently than adults, and FAST rats attacked more frequently than SLOW rats (Figure 2.1A). Probability of defense showed a significant age effect [$F(1, 88) = 9.13, p <$ 0.01], and strain effect [$F(1, 88) = 27.32, p < 0.0001$]. Juveniles defended against attacks more frequently than adults, and FAST rats defended against attacks more frequently than SLOW rats (Figure 2.1B). While there was a decrease in the probability of defense during development for all groups, the magnitude of the decrease appeared to be especially large for SLOW males; however, there was no significant sex difference between SLOW males and females ($p > 0.05$) for the probability of defense.

30

A. Playful attack

B. Playful defense
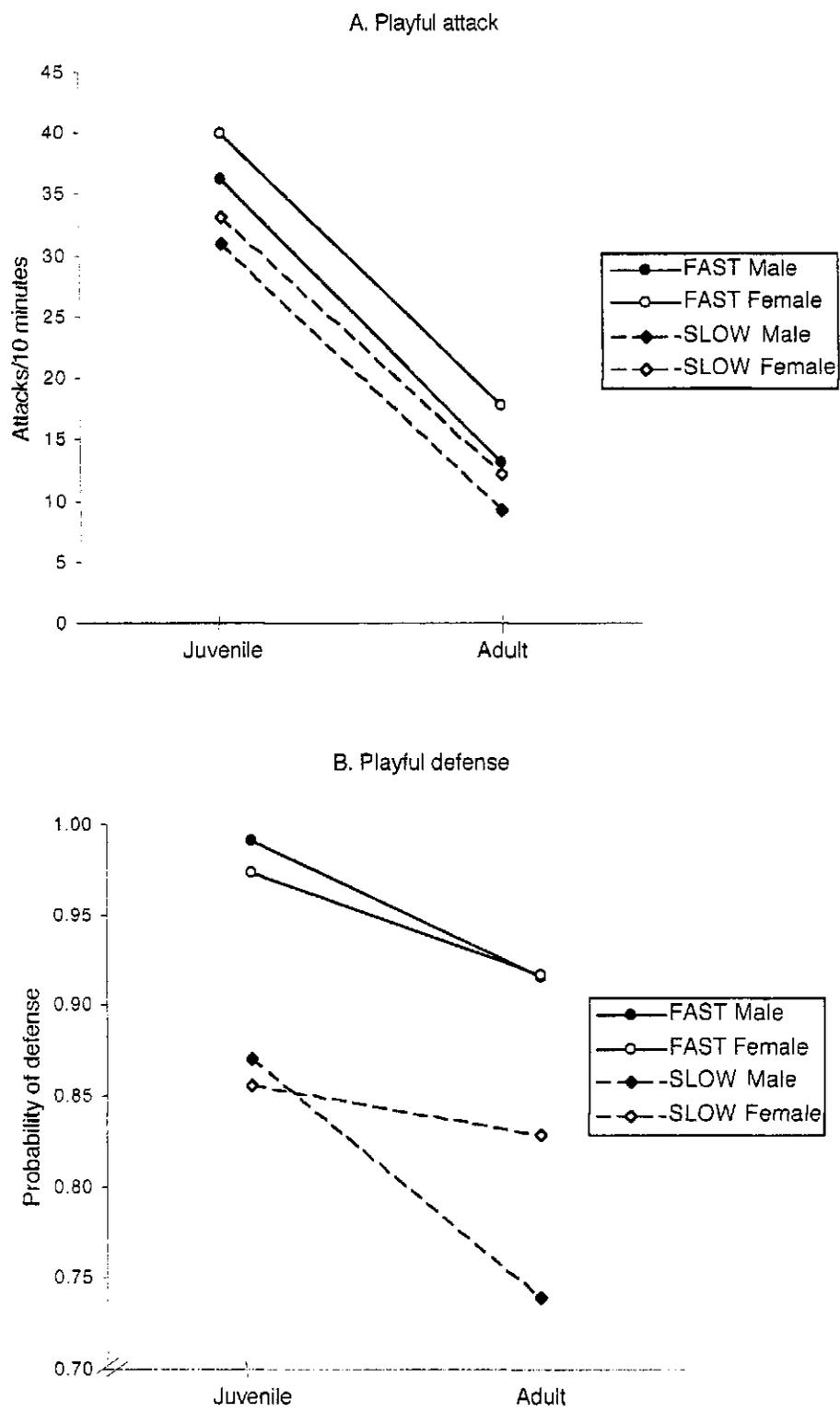
Figure 2.1 Developmental changes in the mean frequency of attack (A) and the mean probability of defending against attack (B) for FAST and SLOW rats.

31

When defending, rats can either move away (i.e., evade), or turn to face to protect the nape. For probability of evasion, there was a significant Age x Strain interaction [$F_{(1, 88)}$ = 11.92, $p < 0.001$], with FAST rats using this defensive tactic more often in adulthood than SLOW rats. With age, FAST rats increased the use of evasion whereas SLOW rats decreased their use of this tactic (Figure 2.2A). With regard to turning to face defensive tactics, there was a significant age effect [$F_{(1, 88)}$ = 8.46, $p < 0.01$], and Age x Strain interaction [$F_{(1, 88)}$ = 5.15, $p < 0.05$], for complete rotation. Typically, the complete rotation tactic is used more frequently in the juvenile phase, but then declines with puberty when it is replaced by the partial rotation tactic, the more frequent tactic in adulthood (Pellis & Pellis, 1990). Not only did FAST rats perform the complete rotation more often than SLOW rats as juveniles but also FAST rats, unlike SLOW rats, showed the typical age-related decline of use of the complete rotation tactic (Figure 2.2B). Although the decrease was strongest in male FAST rats, the decline was not significantly different to that of the female FAST rats. Similarly, there was a significant age effect [$F_{(1, 88)}$ = 6.75, $p < 0.05$], Age x Strain interaction [$F_{(1, 88)}$ = 8.19, $p < 0.05$], and Strain x Sex interaction [$F_{(1, 88)}$ = 4.09, $p < 0.05$], for probability of partial rotation. That is, there was an age-related increase in partial rotation, complementing the decrease in complete rotation, with this increase being more marked in SLOW rats than in FAST rats. Furthermore, SLOW females increased their use of partial rotation with age, whereas FAST females showed a slight reduction in the frequency with which partial rotation was used in adulthood.
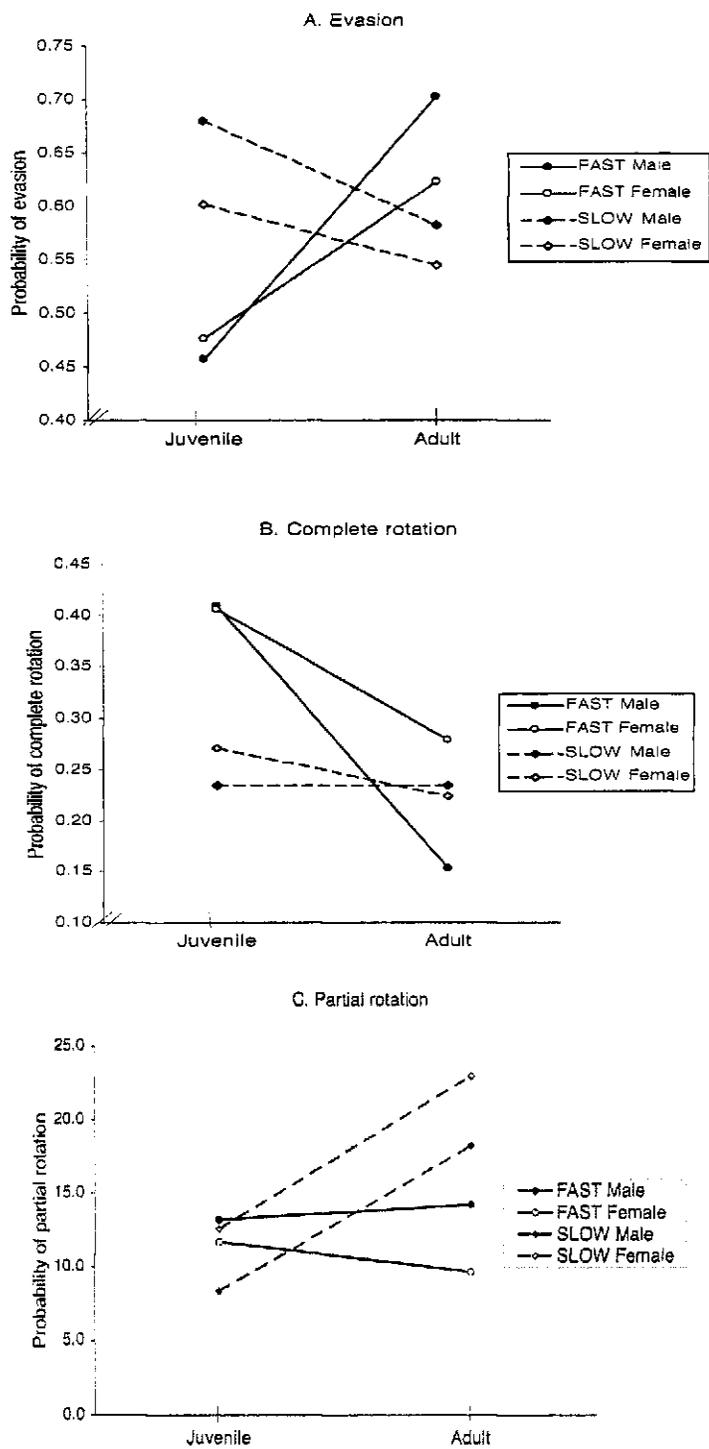
Figure 2.2 Developmental changes in the mean probability of evasion (A), the mean

probability of complete rotation (B), and the mean probability of partial rotation (C) for

FAST and SLOW rats.

33

For the anxiety index score, there was a significant strain effect [$F_{(1, 88)} = 8.70$, $p < 0.01$], sex effect [$F_{(1, 88)} = 21.87$, $p < 0.0001$], and Age x Sex interaction [$F_{(1, 88)} = 7.35$, $p < 0.01$]. Females had higher anxiety index scores than males, which increased with age, and SLOW rats had higher scores than FAST rats (Figure 2.3). There were no significant effects for the circadian activity data ($p > 0.05$). That is, both strains were active for comparable amounts of time during the 48-hr test period. With regard to body weight, there was a significant age effect [$F_{(14, 660)} = 563.30$, $p < 0.0001$], strain effect [$F_{(1, 660)} = 120.79$, $p < 0.0001$], sex effect [$F_{(1, 660)} = 1592.95$, $p < 0.0001$], and Age x Sex interaction [$F_{(14, 660)} = 46.68$, $p < 0.0001$]. Body weight increased as the animals aged, with the males outweighing the females, and FAST rats outweighing SLOW rats (Figure 2.4). With regard to strain differences, the growth curve shows that FAST rats grew faster than SLOW rats.
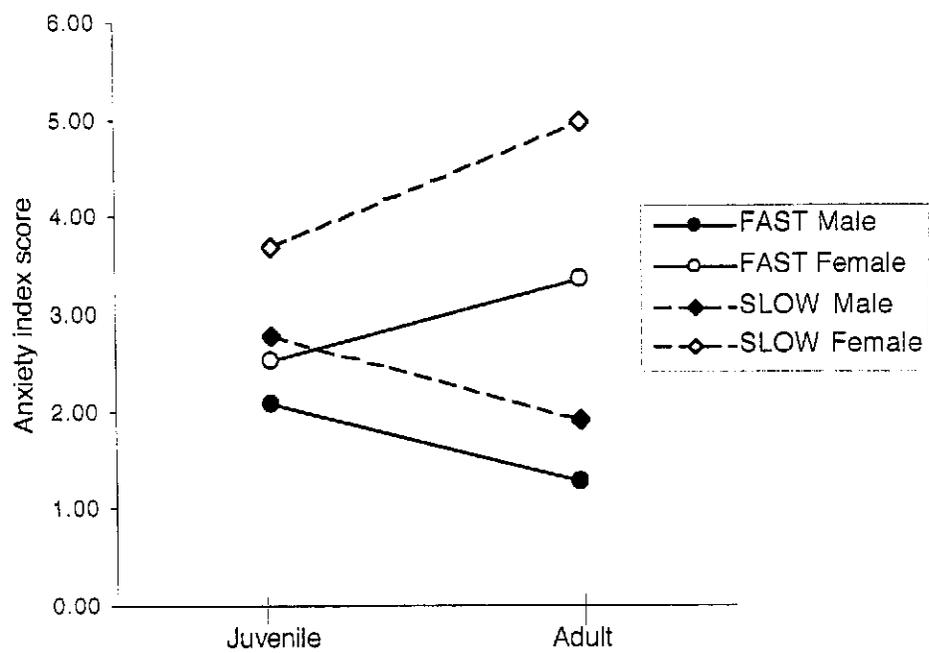
Figure 2.3 Developmental changes in mean levels of anxiety scores for FAST and SLOW rats.
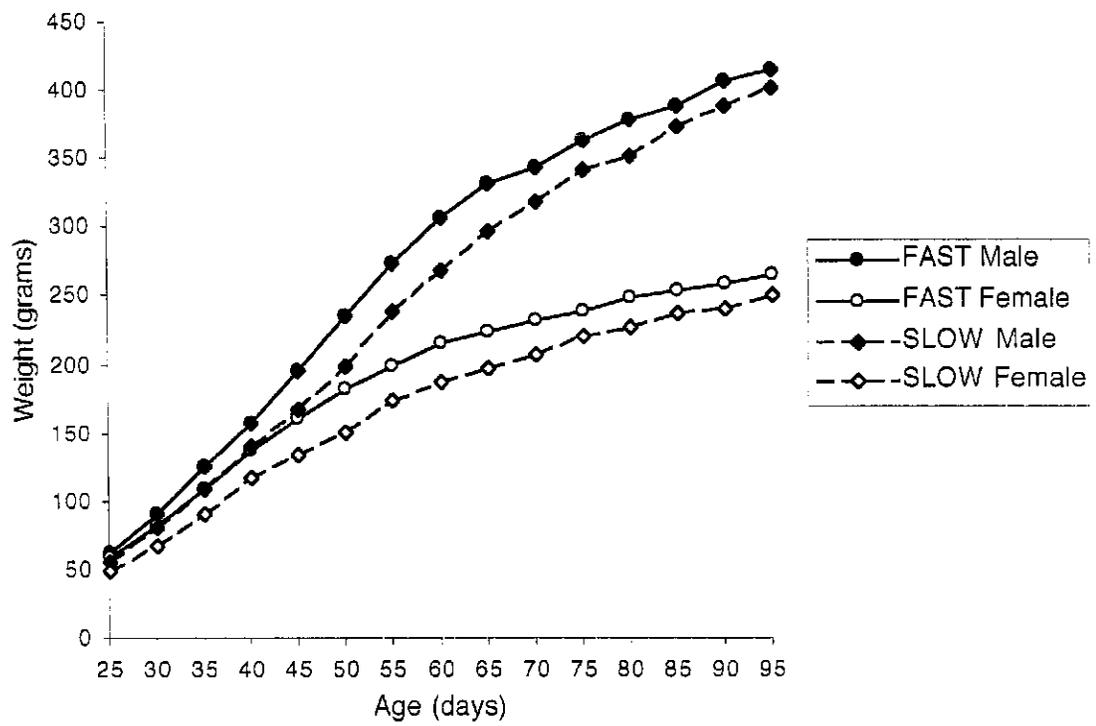
Figure 2.4 Developmental changes in mean body weights for FAST and SLOW rats.

36

DISCUSSION

In part, the results were as predicted. FAST rats were more playful and remained more
playful into adulthood than SLOW rats. However, some results were not as predicted,
suggesting that the components of play fighting are a mosaic which can change
independently of one another[2]. Previous studies have shown that the decline in the
frequency of play fighting at puberty results from a decrease in the frequency of initiating
playful attacks, whereas the probability of defense remains relatively unchanged
throughout development (Pellis & Pellis, 1990, 1997). In the present study, FAST rats
were more playful than SLOW rats throughout development in that they launched playful
attacks more frequently and were more likely to defend themselves when attacked (Table
1). Even though both strains exhibited a decline in playfulness with age, the reduction
observed in FAST rats was less than that observed in SLOW rats. Unexpectedly, there
was a significant decrease in the probability of defense with age, although the decrease in
FAST rats was small and overall within the typical range found in other studies (Pellis et
al., 1997). Conversely, the decline in probability of defense for SLOW rats, especially for
the males, was large and well outside the range of previous studies (Table 1).

The coincident, age-related decreases in both frequency of attack and probability of
defense suggest that the SLOW strain, being selected for a more extreme adult
phenotype, may have a predisposition for being less playful. Furthermore, it appears that
the play behaviors of SLOW rats in the juvenile phase of development are indeed
representative of adult-typical behavior (Table 1). The FAST strain, being selected for the
retention of a juvenile-like phenotype, seems to retain a higher level of playfulness, but

37

Table 1. Levels of play behavior observed in same-strain pairs of FAST and SLOW rats, expressed in comparison to previously quantified levels of the same behaviors in Long Evans rats (e.g., Pellis & Pellis, 1990, 1992; Pellis et al., 1992; Smith et al., 1998).

Play Behavior	EXPECTED CONTROL		OBSERVED FAST		OBSERVED SLOW	
	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult
Attack	+ + + +	+ +	+ + + +	+ +	+ + +	+
Defense	+ + + +	+ + + +	+ + + +	+ + + +	+ + +	+ +
Evasion	+ +	+ + +	+ + +	+ + + +	+ + + +	+ + +
Complete	+ + + +	+ +	+ + +	+ +	+ +	+ +
Partial	+ +	+ + + +	+ +	+ +	+ +	+ + +

38

one that is still within the typical range of playfulness that has been reported in several studies using Long-Evans rats (Table 1). The pattern of age-related change in the use of defense tactics is only partially consistent with this interpretation.

With the onset of puberty, two changes are typical for the type of defensive tactics most often used. The likelihood that an attack will be defended against by moving away from the partner (evasion) tends to increase, and when the defender defends itself by turning to face the attacker, it is more likely to do so by only partially rotating around the longitudinal axis of the body rather than by fully rotating to the supine position (Pellis & Pellis, 1987) (Table 1). These changes are more pronounced in males than in females (Pellis, 2002; Pellis et al., 1997). The two strains studied here differed from this typical pattern, but in different ways. FAST rats exhibited an atypically large age-related increase in evasion whereas SLOW rats had a modest decrease (Table 1). With regard to complete rotation, FAST rats exhibited the age-related decrease, with the change in males being greater than the change in females. In contrast, SLOW rats did not change with regard to complete rotation; it remained at a low likelihood at both the juvenile and adult stages.

The large increase in evasion by FAST rats is unexpected because evasion reduces the physical contact so characteristic of complex and exuberant play fighting (Iwaniuk, Nelson, & Pellis, 2001; Pellis & Iwaniuk, 2000). This seems counter to this strain's retention of a higher frequency of play fighting in adulthood. The lack of change in the likelihood of using the complete rotation by SLOW rats is unexpected because it shows

39

that at all ages they are more likely to use not only evasion, but also partial rotation, a tactic with a stronger agonistic component and one that more effectively blocks the partner from gaining the advantage; thus curtailing prolonged bouts of play fighting (Pellis & Pellis, 1987, 1998b).

Previous studies in the Pellis lab suggest that the relationship between rotatory tactics of defense (i.e., complete rotation and partial rotation) is reciprocal. If so, then it would be expected that changes in complete rotation would be accompanied by changes in partial rotation, in the opposite direction. However, the present study suggests that rotatory tactics of defense may not be reciprocal, and that independent changes are possible for complete rotation and partial rotation. FAST rats show the expected age-related decrease in complete rotation and little change in partial rotation, whereas SLOW rats show the expected age-related increase in partial rotation and little change in complete rotation (Figure 2.2B, 2.2C). In this regard, SLOW rats resemble rats that have been decorticated at birth. Decorticates do not show the age-related switch in complete to partial rotation, but rather, from the outset, perform complete rotations at a lower frequency than partial rotations (Pellis, Pellis, & Whishaw, 1992). That is, they exhibit the adult-typical pattern of playful defense at all ages.

One way of interpreting the differences between the strains is that in terms of overall levels of play fighting (frequency of attacks plus probability of defense), the more juvenilized animals (i.e., FAST strain) are the more playful. However, regarding the content of the play, which is determined by the actions taken during the play fight such as

40

the defensive tactics employed (Foroud & Pellis, 2003; Pellis & Pellis, 1987), the overall

juvenility of the subjects seems less of a determining factor. FAST rats are more likely to

avoid physical contact and SLOW rats are more likely to use adult patterns of defense at

all ages. These subtle, but significant, differences in the content of play fighting between

these strains suggest that this activity is a composite, with different components being

regulated by different mechanisms. If that is the case, it is likely that all differences in the

complexity of play across species can be accounted for only in part, by differences in the

duration of the juvenile phase. It is likely that overall playfulness may be sensitive to

changes in life history patterns, but also that specific components of play have arisen due

to particular functional needs within different lineages (Pellis & Iwaniuk, 2004).

Moreover, the differences in play between the two strains are not a by-product of more

general differences.

Given that there were no differences in activity level, this cannot account for the higher

frequency of play in the FAST strain. Even if the activity levels for the strains were

different, Walker and Byers (1991) showed that differences in the activity rates of inbred

BALB/c and DBA/2 strains of house mice do not vary predictably with the mean rate of

locomotor play. That is, changes in the rate of activity that may result from selective

breeding do not necessarily facilitate similar changes in the rate of play. Similarly, while

there were significant differences in body weight changes between FAST and SLOW

rats, these differences were unlikely to have produced the differences in play. The reason

for this conclusion is that the sexes showed the patterns of play typical of the strain, even

though there were sex differences in body weight that were larger than the differences

41

between the strains. Furthermore, the strains followed a similar trajectory in their rate of growth (i.e., the body weight changes that occur during development). Indeed, it appeared that FAST rats physically matured faster than SLOW rats (see Fig. 4), which, if prolonged juvenility were to account for more play, should have led to less play and faster decline in play by FAST rats. The same logic that applied to the strain differences in weight also applies to the differences between the strains in response to stress. Consistent with earlier findings (Mohapel & McIntyre, 1998), SLOW rats were found to exhibit the behavioral markers of stress more often than FAST rats. It has been frequently noted that play is suppressed in stressful situations (Fagen, 1981). But again, the stress effects are larger for the sexes than for the strains, therefore differences in stress between the two strains are unlikely to account for the differences in play.

The differences between FAST and SLOW rats suggest that play fighting does not change as a whole, but that the subcomponents of play are dissociable and are able to be modified independently. The two strains do not change in the same way for both the frequency and content of play. That juvenile features of play can be almost completely lost in some subcomponents in SLOW rats, suggests that not all traits in play fighting change concurrently. Indeed, this conclusion is consistent with developmental and experimental studies showing that different components of play fighting are dissociable (e.g., Pellis & Pellis, 1990, 1997; Pellis, Pellis, & Whishaw, 1992; Siviy, 1998; Smith, Forgie, & Pellis, 1998).

42

Unlike the findings which have shown that male rats play fight more frequently than females (e.g., Meaney & Stewart, 1979, 1981; Pellis & Pellis, 1990; Poole & Fish, 1976), and, more specifically, that males initiate play (i.e., attack) more frequently than do females (Pellis & Pellis, 1990; Thor & Holloway, 1983), the present results showed an absence of a sex difference in play. Indeed, if anything, there was a trend for females to launch playful attacks more frequently; however, note that sex differences in the frequency of play have not been reported for all paradigms in which play is tested (Panksepp, 1981; Panksepp et al., 1984; Thor & Holloway, 1984). Nonetheless, the paradigm used for testing play in the present study has repeatedly revealed sex differences in the frequency of play (e.g. Pellis & Pellis, 1990, 1997; Pellis, Pellis, & McKenna, 1994; Smith et al., 1998). Relative to previous studies that have employed this method for the analysis of play behavior, the absence of a sex difference in the frequency of play in the present study was unexpected, although the results are consistent with earlier findings that the amygdala is critically involved in the sexual differentiation of play in rats. Meaney, Dodge and Beatty (1981) found that bilateral amygdala lesions in weanling rats decreased social play in male rats, but had no effect on the social play of female rats, indicating that the sex differences in the anatomy and physiology of the amygdala, arising from the effects of testosterone (Meaney & McEwen, 1986), partly accounts for the sex differences in social play. That FAST and SLOW rats have been selectively bred for their susceptibility to amygdala kindling suggests that the selective breeding of these strains may have altered some of the neural mechanisms (particularly those involving the amygdala) that control the behavioral responses necessary to produce the sex difference in the frequency of play.

43

Various lines of research, when considered in combination, favor the amygdala as a likely candidate for being an important neural substrate that modulates social behavior. For example, it has been shown that (a) the amygdala is crucial for regulating fear (Fendt & Fanselow, 1999), (b) damage to the amygdala alters social behavior in non-human primates (Emery et al., 2001; Meunier, Bachevalier, Murray, Malkova, & Mishkin, 1999; Prather et al., 2001), and (c) there are signs of amygdala dysfunction in people with autism (Baron-Cohen et al., 2000). Not unexpectedly, then, genetically induced functional changes to the amygdala, as seen in FAST and SLOW rats, lead to marked changes in the organization of a complex social behavior such as play fighting.

Because of the behavioral and anxiety differences that have been observed in the strains, it would be of interest to investigate how individuals from different strains would behave when paired together in our paradigm for testing play. How would the play interaction proceed? Would the FAST individual dominate during the interaction because of its impulsive, juvenile-like nature, or would it be the SLOW individual with the more adult-typical phenotype that would regulate the content of play sessions? Future studies could test these competing alternatives, and so characterize more fully the behavioral profiles of the FAST and SLOW strains of rats, yielding potentially new insights into the function of the amygdala.

NOTES

1. This chapter was modified from the following publication: Reinhart, C.J., Pellis, S.M., & McIntyre, D.C. (2004). Development of play fighting in kindling-prone (FAST) and kindling-resistant (SLOW) rats: How does the retention of phenotypic juvenility affect the complexity of play? *Developmental Psychobiology*, *45*, 83-92. © 2004 John Wiley & Sons, Inc. Reprinted with permission of John Wiley & Sons, Inc.

2. A concern may arise given that some measures used are absolute measures and some are proportions. This mixture of measures reflects the fact that not all developmental changes in play behavior can be measured using the same units. This, however, is no different to physical development, where weight, size and shape need to be measured using different units. It is the pattern of change and how these different measures correlate with one another over development that is the issue. Based on numerous previous studies on the development of play fighting in rats, we have shown that there are consistent age-related changes in the frequency of initiating play (i.e. attack) and in how rats respond to being attacked (i.e. defense) (Pellis, 2002).

CHAPTER THREE[1]

Play fighting between kindling-prone (FAST) and kindling-resistant (SLOW) rats.

ABSTRACT

Differences in the play behavior of two strains of rats suggest that (1) different components of play fighting (PF) can be modified independently, and (2) there are genetic contributions to the expression of play behaviors. The development of PF in cross-strain pairs was examined to determine whether the typical PF in each strain may be altered by interacting with a non-congruent pairmate. Changes in PF were observed in both strains throughout development, however, environmental context was more influential during adulthood. These data suggest that components of play may be highly stable, highly labile, or contingently labile. That is, some components of play may be more resistant to environmental context than others.

46

INTRODUCTION

Play fighting is one of the most common forms of play observed (Aldis, 1975; Bekoff &

Byers, 1981; Fagen, 1981; Pellis & Pellis, 1998b), and it has been documented in

mammalian and avian species, as well as in some reptiles (Burghardt, 1999). Despite the

frequency with which play fighting is observed, and the diversity of species that engage

in this behavior, most of what is known about the neurobiology of play has come from

studies of the laboratory rat (Panksepp, 1998; Siviy, 1998; Vanderschuren, Niesink, &

Van Ree, 1997). The characteristic behaviors that distinguish play fighting from serious

fighting, such as target of attack (Pellis & Pellis, 1987) and the specific components of

play fighting (i.e., attack, defense), have been well characterized in the rat (e.g., Pellis,

Field, Smith, & Pellis, 1997; Pellis & Pellis, 1997), but little is known about the

neurobehavioral mechanisms that control different aspects of play fighting.

Play fighting in rats involves playful attack by one individual, and subsequent defense by

the other, with successful contact involving gentle nuzzling of the partner's nape (Pellis,

1988). The frequency of launching playful attacks peaks during the juvenile phase,

between 30-40 days (Thor & Holloway, 1984). The recipient of a playful attack can use a

variety of defensive tactics to prevent nape contact (Pellis & Pellis, 1990); the frequency

of which changes in a distinctive, age-related manner (Pellis & Pellis, 1990, 1997).

Because rats continue to play into adulthood, albeit at a reduced frequency (Pellis &

Pellis, 1990, 1991a), it has been possible to identify how attack and defense components

change during development. Attack and defense components of play fighting are clearly

47

dissociable at the behavioral level, and previous studies suggest that these components are also motivationally independent (Pellis & Pellis, 1991a; Siviy, Love, DeCicco, Giordano, & Seifert, 2003). That is, frequency of attack depends on the animals' motivational state (e.g., social isolation prior to testing increases the frequency of playful solicitations) (see Panksepp & Beatty, 1980; Pellis & Pellis, 1990; Siviy et al., 2003), whereas the probability of defending against attacks remains between 80-90% throughout development (Pellis et al., 1997), regardless of pre-testing social experience (Siviy et al., 2003).

Animals selectively bred on the basis of differences in behavior have been increasingly used to investigate the neurobiological mechanisms underlying behavioral differences (Ellenbroek & Cools, 2002). Strains of rats with consistent, quantifiable differences in play behavior have been identified, suggesting that there are genetic controls over the components of play behavior (e.g., Ferguson & Cada, 2004; Reinhart, Pellis, & McIntyre, 2004; Siviy, Baliko, & Bowers, 1997; Siviy et al., 2003), but how stable are these genetically-based neurobehavioral traits? Both genetic and environmental factors play an important role in determining the phenotypic expression of behaviors (Ellenbroek & Cools, 2002). By using selectively bred rat strains with unique play profiles, we can ask (a) which components of play fighting change or stay the same when the testing environment changes, and (b) whether environmentally-influenced changes in play behavior are age-dependent?

Reinhart, Pellis and McIntyre (2004) examined the development of play fighting in

kindling-prone (FAST) and kindling-resistant (SLOW) rats; two lines of rats selectively

bred for their susceptibility to amygdala kindling (for more detailed description, see

Racine, Steingart, & McIntyre, 1999). The FAST (kindling-prone) strain was generated

by breeding the animals that required the fewest electrical stimulations of the amygdala

to elicit a Stage-5 convulsive seizure (Racine, 1972), and the SLOW (kindling-resistant)

strain was generated by breeding the animals that required the most amygdala

stimulations. These strains show differences in amygdalar physiology (i.e., miniature

GABA inhibitory postsynaptic potentials and $GABA_A$ subunit expression) (McIntyre,

Hutcheon, Schwabe, & Poulter, 2002; Poulter et al., 1999) and in various behavioral

characteristics such as Morris water maze performance (Anisman & McIntyre, 2002),

measures of impulsivity (McIntyre, Poulter, & Gilby, 2002), fear-related responses

(Mohapel & McIntyre, 1998), and stress-related responses (McIntyre, Kent, Hayley,

Merali, & Anisman, 1999). When considered in combination, the physiological and

behavioral data suggest that in many respects, FAST rats express a juvenile-like

phenotype, whereas SLOW rats express an adult-typical phenotype.

In the play study (Reinhart et al., 2004), same-strain pairs were tested as juveniles and

adults, and it was found that (a) FAST rats were more playful than SLOW rats throughout

development, (b) FAST and SLOW rats used different patterns of defense in adulthood to

reduce the likelihood of prolonged social contact, (c) FAST rats exaggerated the

defensive changes typically occurring at puberty, and (d) SLOW rats behaved in a more

adult-typical manner at all ages.

In the present study, the development of play behavior in cross-strain pairs of FAST and SLOW rats was examined. Because specific differences in the play profiles of the two strains have already been identified for same-strain pairs, it was possible to examine how components of play fighting changed in each strain, in response to changes in the rearing and play-testing environments. If genetic differences are independent of environmental factors, it was predicted that juvenile-like FAST rats would be more playful than adult-like SLOW rats. More specifically, FAST rats should show a relatively higher frequency of playful solicitations (i.e., playful attacks), a higher probability of defending against attacks, and should use defensive tactics that signal a willingness to continue playful encounters more so than SLOW rats. If these proved to be true then, this would suggest that providing a FAST rat with a less nurturing play environment (i.e., less playful SLOW partner) does not overcome the genetic predisposition to be more playful. Similarly, we predicted that SLOW rats would be less playful, and more frequently use adult-typical defensive tactics that reduce the physical contact necessary during play fighting, thus suggesting that providing an environment that is more conducive to playful interaction (i.e., more playful FAST partner) does not overcome the genetic predisposition for a low level of playfulness.

In addition to the development of play fighting between FAST and SLOW rats, the development of social dominance between the strains was examined. It has been shown that in adulthood, male pairmates exhibit dominance-subordinance relationships (Lore & Stipo-Flaherty, 1984), and that these dominance relationships appear to be reflected in the play fighting of male pairmates (Pellis & Pellis, 1991b; Pellis, Pellis, & McKenna, 1993).

50

Subordinate pairmates in adult pairs of male rats initiate more playful attacks than the dominant, and when attacked, defend themselves in a more juvenile manner (Pellis & Pellis, 1992). In the present study, we wanted to assess which strain would be dominant in observational (i.e., play fighting), competitive (i.e., food competition, water competition), and physiological (i.e., plasma corticosterone levels, body weight) measures of social status. That is, when paired together, would the more juvenile-like (i.e., FAST) or the more adult-like (i.e., SLOW) pairmate become socially dominant?

It was predicted that SLOW rats would be dominant in all measures of social status because of their generally adult-typical behavioral phenotype. More specifically, SLOW rats should playfully attack less frequently than FAST rats during play fighting and should use adult-typical tactics of defense more than FAST rats. If SLOW rats are dominant, they should also weigh more than FAST rats, have priority of access to limited resources in competitive tests of social dominance, and exhibit lower levels of stress hormones (see later). If one strain was shown to be dominant, this would support the possibility that the systems that have been selected in the creation of the two strains, also affect traits necessary for the establishment of social status. In turn, a strain bias in developing dominance relationships could account for any observed changes in the strain-typical play profile of adult rats.

51

METHODS

Animals.

FAST (kindling-prone), and SLOW (kindling-resistant) weanling rats were obtained from Carleton University and cross- strain pairs (one FAST and one SLOW animal per cage) were established at the animal housing facility of the Department of Psychology & Neuroscience at the University of Lethbridge. Pairs were housed in 46 x 25 x 20 cm polyethylene tubs with processed corncobs for bedding. The animals had *ad libitum* access to food and water, and the animal colony was maintained at 21°C on a 12:12 hr light: dark cycle (lights on at 0730 hr). The care and use of animals was in accordance with local standards set by the Animal Welfare Committee, and the national standards set by the Canadian Council for Animal Care.

Subjects

Twelve FAST and 12 SLOW male rats were used. On postnatal Day 26, when the animals arrived to the University of Lethbridge facility, they were housed as cross-strain pairs.

Part 1. Play fighting

Procedure

On postnatal Days 28, 29, and 30, each pair was given 15-min habituation periods in the testing apparatus. On Day 30, following habituation, each animal was isolated for 24 hr in a 46 x 25 x 20 cm polyethylene cage, as such isolation prior to testing has been shown to increase the frequency of play fighting (Panksepp & Beatty, 1980; Pellis & Pellis,

52

1990). On Day 31, following isolation, each pair of rats (cage mates) was placed together in the testing apparatus. Following testing on Day 31, the animals were once again housed as pairs. On Day 32, each pair again went through the 15-min habituation and 24 hr isolation procedures, and was tested on Day 33. The animals were then housed as pairs until adulthood, at which time the procedure was repeated. On Days 87 and 88, the animals were habituated for 15 min. On Days 89 and 91, the animals were both habituated and isolated. On Days 90 and 92, the animals were tested and then again housed in pairs.

Habituation and testing commenced each day between 1430-1500 hr. On the first day of habituation in both the juvenile and adult phases of testing, the black and white pelage markings for each animal were drawn so that the animals could later be identified during video analysis. The testing apparatus had a Plexiglas floor lined with processed corncobs, two wooden walls, an anterior Plexiglas wall, and a mirrored posterior wall. The movable mirror located at the back of the testing apparatus facilitated observation of otherwise hidden parts of the rats' bodies. The dimensions of the apparatus during the juvenile testing were 50 x 50 x 32 cm; during the adult testing, they were 50 x 50 x 50 cm to accommodate the larger body size of the adults. Test periods lasted 10 min and were videotaped in the dark using the "night shot" function on a Sony 8-mm camcorder. The camera was placed in front of the apparatus at an oblique angle at a distance that could capture the entire volume of the testing area.

53

Behavioral Analyses

After collecting the behavioral data, each 10-min test period was scored for various

components of play. The 8-mm tapes were converted to VHS format and a time code

(30th of a s) was added using an Horita TRG-50 time encoder (Horita, Mission Viejo,

CA). Videos were viewed in slow motion and analyzed frame-by-frame. Each of the

following measures of play fighting were quantified for all animals (for more detailed

description, see Pellis, Pellis, & Whishaw, 1992):

(I)   Frequency of attack – the number of playful initiations (i.e., nape contacts) per

10 min period

(II)   Probability of defense – the number of times an animal elicits a defense

(withdrawal of the nape area from the snout of an approaching partner),

relative to the number of attacks received per 10 min period

(III)   Type of defense:

(i)   Probability of evasion – the number of evasive defenses (withdrawal of

the nape by leaping, running, or turning away from the partner) relative to

number of total defenses

(ii)   Probability of complete rotation – the number of times the defender rotates

around its longitudinal axis to lie supine (facing the attacker to block nape

access) relative to number of total defenses

(iii)   Probability of partial rotation – number of facing defenses whereby the

defender turns to face the attacker by rearing on the hind legs, relative to

number of total defenses

54

Statistical Analyses

For the play data collected, an ANOVA with strain (FAST or SLOW) and age at testing (juvenile or adult) as independent factors was used for analysis. For graphical purposes, the group means are shown.

It should be noted that when animals are group-housed it is not possible to control the amount of social experience each individual subject will have between testing phases. Indeed, the uncontrolled level of play an individual may experience in a group-housed rearing condition may result in small differences in the pattern of behavioral development observed in each animal. Individual housing has been a method used by some researchers (e.g., Ikemoto & Panksepp 1992; Panksepp, 1981) to control the amount of social experience an individual will have. However, social isolation lasting even less than one week can lead to severe social deficits (see review in Pellis, Pellis, & Foroud, 2005). The play-testing paradigm used in the present study has been used for several experiments and study after study has shown the same pattern of developmental change (Pellis, 2002; Reinhart et al., 2004).

Part 2. Social dominance

Procedure.

A. Play measures

In part 1 of the present study, various components of play fighting were analyzed and the data were collated in terms of age and strain. For part 2 of the experiment, the adult play scores for each strain were examined more closely. Lore and Stipo-Flaherty (1984) have

shown that male pairmates exhibit a dominance-subordinate relationship in adulthood, thus affecting the social interactions that take place between adult male rats. Previous studies on play fighting and dominance (Pellis & McKenna, 1992; Pellis & Pellis, 1991b, 1992; Pellis, Pellis, & Kolb 1992; Smith, Fantella, & Pellis, 1999) have shown that as adults, dominants launch fewer playful attacks than subordinates, and that their defensive responses to attacks received from subordinates are more adult-typical (i.e., partial rotations). In the present study, the relative playful attack rates of FAST and SLOW rats at the adult stage were used to identify the more frequently and the less frequently attacking strain, with the less frequent attackers being "dominant," and the more frequent attackers being "subordinate." Similarly, the probability of using the partial rotation defense in adulthood was compared between strains. It was predicted that the less frequently attacking, or dominant strain, would also be the strain that was more likely to use partial rotation in adulthood.

B. Competitive measures

In addition to the observational measures of social dominance that were analyzed as part of play fighting between pairmates, competitive measures of dominance were examined. Most competitive tests may be classified into two main categories (Syme, 1974).

The first competitive test involved the animals in an all-or-none food competition where pairmates had to compete for a single food item. Habituation and testing commenced each day at 1700 h. On Days 110, 111 and 112, four food items (almonds) were placed in the home cage of each pair of cage mates to habituate the animals to the novel food item.

56

The pairs were observed on all three days to ensure that each individual consumed at least one food item per day. On Days 113 and 114, the animals were individually placed in the testing apparatus and given two food items so that they would become more comfortable eating the now familiar food item in the novel environment. Animals were returned to their home cages immediately after they had finished eating the food items.

The testing apparatus was a thin Plexiglas cylinder, 40 cm in diameter and 45 cm high, that was positioned on a table with a clear glass surface. On pre-test and test days, the rats were filmed with a Sony 8-mm camcorder, from a ventral view, off a mirror that was mounted under the glass table top at 45 degrees (see Figure 1, Field, Whishaw, & Pellis, 1997). Two 150-W spotlights provided additional light. Pre-testing took place on Days 115, 116, 117 and 118 to habituate pairmates to being in the cylinder together. Pairmates were placed in the testing apparatus together and filmed until all food items (four items for pre-tests 1 and 2, five items for pre-tests 3 and 4) were consumed. For test days 1-4 (Days 119, 120, 121 and 122), one food item was placed in the apparatus and pairmates were then placed inside to compete for the item. Once the item was fully consumed, the animals were placed back in their home cage and the trial was repeated four more times (for a total of five trials per day per pair).

Field, Whishaw, Forgie, & Pellis (2004), have shown that food deprivation is not necessary when highly palatable food items such as almonds are used in a competitive context. Thus, the animals in the present experiment were not food deprived prior to testing. For all filming days, one rat from each pair was marked on the ventral surface for

57

identification purposes. The total amount of food items consumed by each rat was quantified during video analysis, and the scores from each competition were cumulated. The number of items consumed by each rat over the four testing days was converted to a percentage (number of items consumed/ total possible items x 100%), and mean strain comparisons were made - the strain that consumed more food items being "dominant," and the strain that consumed fewer food items being "subordinate."

The second competitive test was a limited access situation in which pairmates had to compete for control of a drinking tube. In this competition, the reward source (i.e., drinking tube) was restricted to a point location. Therefore, only one animal could drink from the tube at a time and pairmates had to compete for control of the water source during the test period. Pre-testing and testing for the water competition took place at 0900 hr. The testing apparatus was a 64 x 57 x 62 cm Plexiglas aquarium lined with processed corncobs. A 27 x 33 cm section of one wall of the apparatus was removed and it was replaced with a section of wire mesh. A water bottle was attached to the outside of the apparatus, such that only the end of the drinking tube entered the inside of the testing apparatus through the wire mesh at a height of 17 cm from the floor. Water bottles were removed from the home cages at 1700 hr so that the animals were water deprived for 16 hr prior to pre-testing days 1, 2 and 3 (Days 129, 130 and 131). On pre-testing days 1-3, each individual was placed inside the testing apparatus for 3 min to habituate to the novel environment and allow them to drink from the mounted drinking tube. Two water bottles were placed on the home cage after pre-testing to prevent pairmates from fighting for water, and both bottles were removed later in the day to ensure the appropriate length of

water deprivation preceded the next testing session. Pre-testing days 4, 5 and 6 (Days 132, 133 and 134) were the same as the previous days except that water bottles were taken from the home cages at 1500 hr (for 18 hr of water deprivation), and individuals were placed in the testing apparatus for only 2 min. On testing days 1 and 2 (Days 135 and 136), pairmates were simultaneously placed in the testing apparatus after 18 hr of water deprivation, and allowed to compete for access to the drinking tube for 2 min.

Pairmates were weighed immediately before and after the competition period so that changes in the subjects' weights could be used as an index for the amount of water consumed. Following the competition sessions, the animals were returned to their home cages, which were equipped with two water bottles per cage. Individual scores (i.e., water volume consumed) from each competition were summed to provide a composite score and strain comparisons were made - the strain that consumed the most water being "dominant," and the strain that consumed less water being "subordinate."

The water competition paradigm used in the present study was a modified version of that used by Drews and Dickey (1977). To reduce the water deprivation to as little as possible, an intermediate period between the 23.5 hr used by Drews and Dickey (1977), and the 14 hr used by Baenninger (1970) was selected.

C. Plasma corticosterone

In addition to evaluating behavioral markers of social dominance, we also assessed plasma corticosterone (CORT), which can be used as a physiological marker of social

59

status. Previous studies have shown that plasma CORT levels are higher in subordinate

rats (Blanchard, Sakai, McEwen, Weiss, & Blanchard, 1993; Raab et al., 1986), and mice

(Veenema, Meijer, de Kloet, & Koolhaas, 2003), relative to dominants. Therefore,

relative levels of plasma CORT were compared between strains. When all behavioral

testing was completed, blood samples were collected on Day 144 to determine the level

of plasma CORT for each rat. All rats were weighed 30 min prior to sacrifice and cage

mates were transported to the tissue processing room one pair at a time. The rats were

killed by decapitation between 0930 to 1030 hr. Trunk blood was collected in 10mL

polyethylene tubes, and tubes were kept on ice until centrifugation. Plasma samples were

centrifuged for 10 min at 2500 rpm and serum aliquots were frozen at -20°C.

Corticosterone was measured using solid-phase radioimmunoassay kits (Coat-A-Count,

Diagnostic Products Corp., Los Angeles, CA).

D. Body weight

It has been shown that weight asymmetries develop when male rats mature in stable

groups, with the heaviest male being dominant (Lore & Stipo-Flaherty, 1984). Therefore,

body weight was measured for each pairmate throughout the experiment (i.e., Days 30,

40, 90, 100, 110 and120).

Statistical Analyses

Unpaired t-tests were used for the behavioral and physiological measures of social

dominance and a repeated measures ANOVA was used to analyze the body weight

measures.

RESULTS

Part 1. Play fighting

There was a significant age effect [F (1,44) = 48.26, $p < 0.0001$], but no significant strain effect ($p > 0.05$) for frequency of attacks (Fig. 3.1A). Conversely, for probability of defense, there was a significant strain effect [F (1,44) = 49.38, $p < 0.0001$], but no significant age effect ($p > 0.05$) (Fig. 3.1B). Juveniles attacked more frequently than adults for both strains, and there was a trend, although not significant, for FAST rats to attack more frequently than SLOW rats. Unlike the case for the attack data, FAST rats defended against attacks more frequently than SLOW rats as both juveniles and adults.

With regard to types of defense, there was a significant age effect for evasion [F (1,44) = 6.34, $p < 0.05$], with adults using this type of defense more frequently than juveniles (Fig. 3.2A). There was also a significant age effect for probability of complete rotation [F (1,44) = 15.54, $p < 0.001$], with juveniles using this defensive tactic more frequently than adults (Fig. 3.2B). Finally, for probability of partial rotation, there was a significant age effect [F (1,44) = 10.56, $p < 0.01$], and strain effect [F (1,44) = 5.96, $p < 0.05$]

A. Playful attack

B. Playful defense
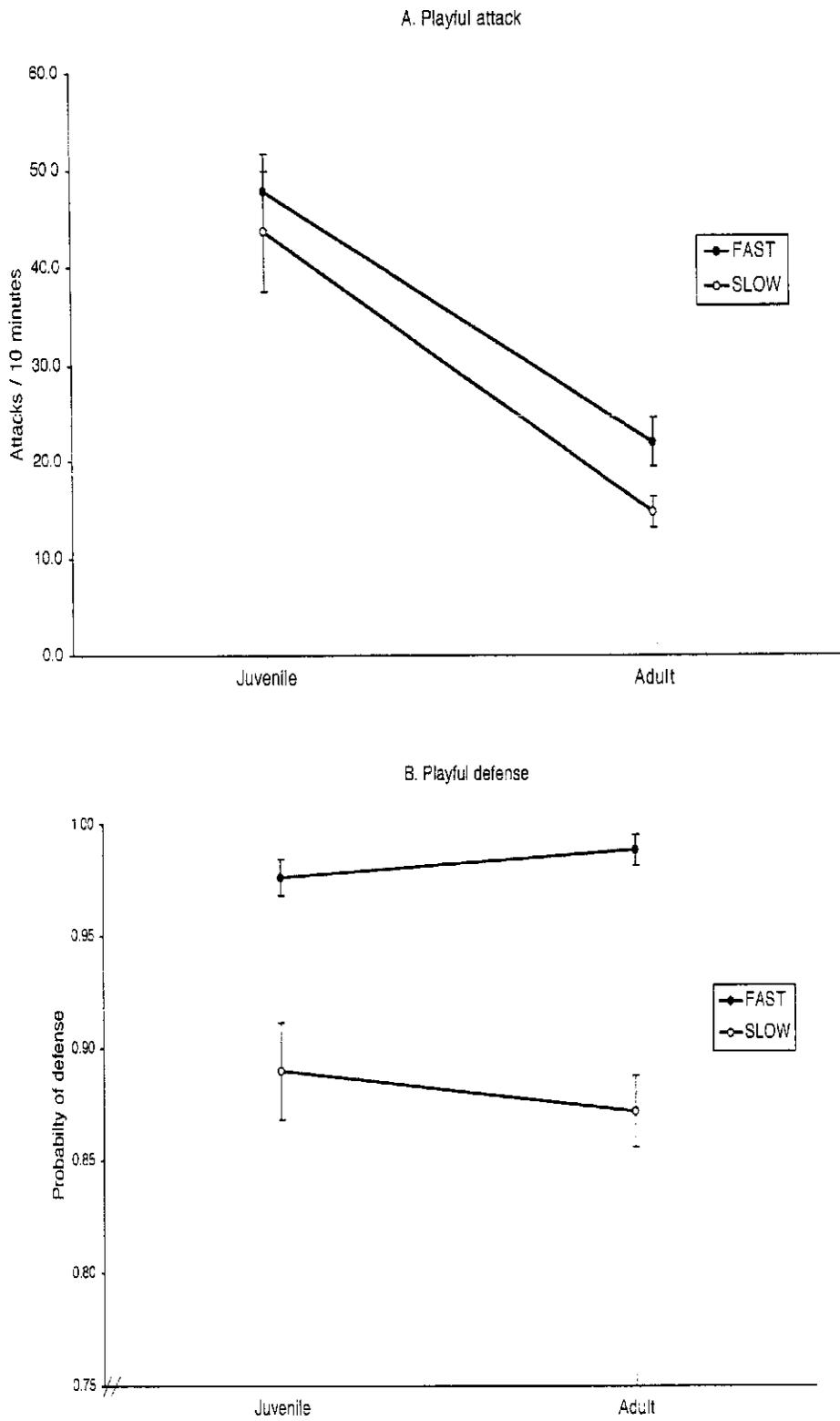
Figure 3.1 Developmental changes in the mean (± SE) frequency of attack (A) and the mean (± SE) probability of defending against attack (B) for FAST and SLOW rats.

62

A. Evasion

B. Complete rotation
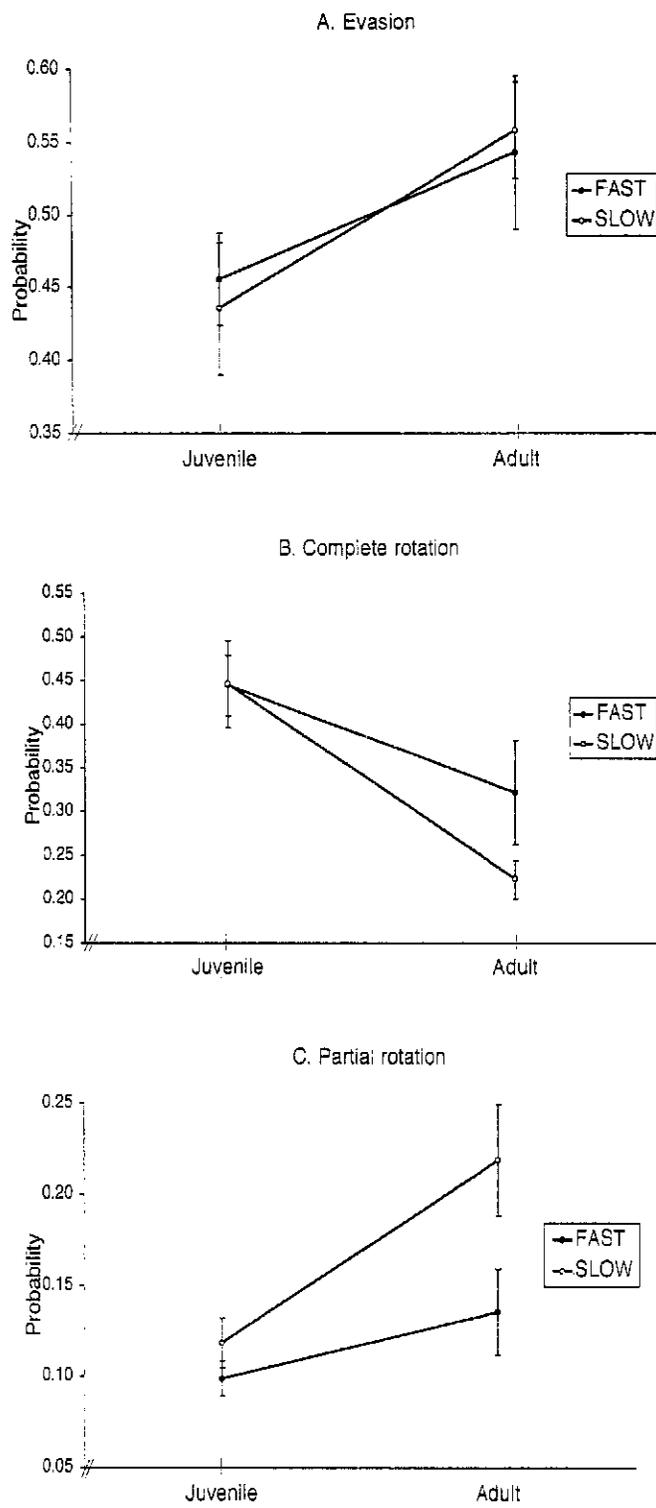
C. Partial rotation

Figure 3.2 Developmental changes in the mean ($\pm$ SE) probability of evasion (A), the mean ($\pm$ SE) probability of complete rotation (B), and the mean ($\pm$ SE) probability of partial rotation (C) for FAST and SLOW rats.

63

(Fig. 3.2C). While not significantly different, the trend was for FAST rats to decrease

their use of complete rotations more slowly with age than SLOW rats; this is consistent

with the differential increase in the use of partial rotations. Overall, the data show that

both strains undergo age-related changes in the components of play fighting that are

typically reported for Long Evans hooded rats (Pellis, 2002).

Part 2. Social dominance

A. Play Measures

The mean rate of playful attacks launched during adult play fighting was significantly

lower for SLOW rats as compared to FAST rats (Mean $\pm$ SE: 14.8 $\pm$ 1.6 vs. 22.0 $\pm$ 2.5; t

= 2.39, df = 22, $p < 0.05$), and the percentage of partial rotations used in adulthood was

significantly greater for SLOW rats as compared to FAST rats (Mean $\pm$ SE%: 21.9 $\pm$

3.1% vs. 13.6 $\pm$ 2.4%; t = -2.15, df = 22, $p < 0.05$). While not significant, SLOW rats

tended to use complete rotations less often (Fig. 3.2B) and evasive defense more often

(Fig.3.2A). These patterns of difference in play are consistent with studies showing

dominance-subordinance among pairmates (Pellis & Pellis, 1991b; Smith et al., 1999). In

this case, the SLOW rats behaved in a manner consistent with them being dominant

members of the pairs.

B. Competitive measures

There was no significant strain difference for the mean percentage of food items

consumed during the food competitions ($p > 0.05$), although the tendency was for SLOW

rats to express greater food consumption than FAST rats (Mean $\pm$ SE%: 54.9 $\pm$ 8.7% vs.

45.1 $\pm$ 8.7%). In the water competition, SLOW rats consumed significantly less water than FAST rats (Mean $\pm$ SEg: 3.8 $\pm$ 0.5g vs. 5.5 $\pm$ 0.5g; t = 2.45, df = 22, p < 0.05). The competitive tests thus yield inconsistent results with regard to determining dominance. It should be borne in mind, that limited access paradigms, may produce measures of skill or competitive performance at a specific task (e.g., individual reaction times to take a reward), rather than providing an index that is predictive of social dominance (Drews & Dickey, 1977).

C. Plasma corticosterone

There was no significant strain difference in plasma CORT concentrations (p > 0.05), although the present results, showing that SLOW rats had slightly higher plasma CORT concentrations than FAST rats (Mean $\pm$ SE ng/mL: 93.3 $\pm$ 7.8 ng/mL vs. 91.8 $\pm$ 11.3 ng/mL), are consistent with previous literature for these strains (Anisman et al., 1997).

D. Body weight

There was a significant strain effect [F (1,22) = 10.13, p < 0.01], and Age x Strain interaction [F (1,5) = 11.82, p < 0.0001] for body weight (Fig. 3.3). Unpaired t-tests at all ages showed that there were no significant strain differences (p > 0.05) on Days 30 and 40, and that SLOW rats were significantly heavier (p < 0.05) than FAST rats from Day 90 onwards. The heavier body weight of SLOW rats is consistent with them being the dominant pairmates (Lore & Stipo-Flaherty, 1984).

65
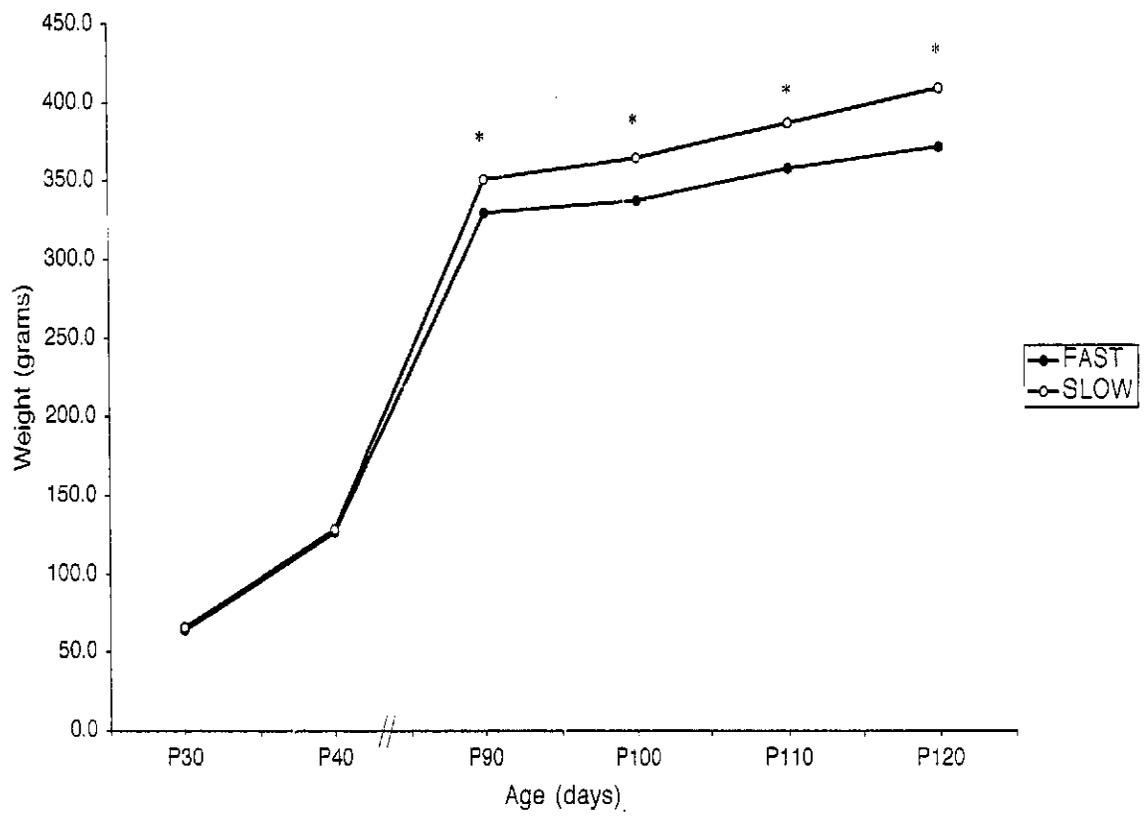
Figure 3.3 Developmental changes in mean body weights for FAST and SLOW rats.
($* p < 0.05$)

DISCUSSION

Play fighting involves both attack and defense, and changes in the frequency of play

bouts may vary due to changes in either or both (Pellis et al., 1997). In this study, when

tested in cross-strain pairs, probability of defense was significantly higher in FAST rats

during the juvenile phase and in adulthood, as was previously shown for play between

same-strain pairs (Reinhart, et al., 2004). Similarly, Siviy and coworkers (2003) found

that Fischer-344 and Lewis rats also differed significantly along this dimension of play

fighting; Fischer-344 rats were less likely than Lewis rats to respond playfully when

approached and contacted by another rat. Given that playful defense does not change as a

function of age (Pellis et al., 1997) or the level of social isolation prior to testing (Siviy,

et al., 2003), it may be the case that SLOW rats and Fischer-344 rats may have a

predisposition for lower levels of response to playful solicitations. As playful defense

remains stable, irrespective of the partner involved (Table 1), it may represent a relatively

stable, genetically determined, neurobehavioral trait.

In the present study, while FAST rats showed a tendency for higher frequency of attack,

there was no significant strain difference as was the case when they played with partners

of the same strain (Reinhart et al., 2004). Surprisingly, both strains attacked more

frequently than expected both as juveniles and adults (Table 1). Ferguson and Cada

(2004) have shown similar results for juvenile Sprague-Dawley rats. That is, Sprague-

Dawley rats were more playful when paired with a different strain partner (e.g.,

Spontaneously Hypertensive rats or Wistar-Kyoto rats) compared to a same strain

partner. One possible explanation for these results is that there is a contagion effect

Table 2. Levels of play behavior observed in cross-strain pairs of FAST and SLOW rats, expressed in comparison to previously quantified levels of the same behaviors in same-strain pairs.

Play Behavior	FAST/SLOW Juvenile	FAST/SLOW Adult
Attack	higher/higher	higher/higher
Defense	same/same	same/same
Evasion	same/less	less/same
Complete rotation	higher/higher	higher/same
Partial rotation	less/higher	same/higher

* higher, less, or same as, seen in same-strain pairs (Reinhart et al., 2004)

between play partners. Previous studies have shown that a more playful partner can stimulate more play in the other rat (e.g., Hole & Einon, 1984; Pellis & McKenna, 1992; Pellis, Pellis, & Kolb, 1992; Reinhart & Pellis, unpublished observations; Varlinskaya, Spear, & Spear, 1999). In the present case, FAST rats could have stimulated more play in their SLOW partners.

The defensive tactics used by SLOW rats during juvenile play fighting provide further support for the possibility that there was a contagion effect. As juveniles, SLOW rats played very much like FAST rats in that they attacked more, evaded less, and used complete rotations more frequently than was expected (Table 1). Although juvenile FAST rats were slightly more playful than expected in the present study (i.e., higher frequency of attack and less partial rotations), the overall changes in play behavior, when pairs of different strain partners were examined, were much greater in SLOW rats. One way of interpreting the changes in juvenile play behavior for the strains is that for SLOW rats, exposure to highly playful FAST partners is an environmental influence sufficient to overcome the SLOW rats' genetic predisposition to play less.

Environmental context seems to be more influential in adult play fighting compared to play fighting during the juvenile phase (Table 1). The levels of play fighting and tactics of defense used by each strain during the juvenile phase were very similar. However, the use of particular defensive tactics diverged between the strains in adulthood (see Fig. 3.2B and 3.2C). That is, FAST rats switched to a more juvenile-like defensive strategy by evading less and using complete rotations more frequently than was expected, based on

69

their same-strain behavior. In turn, SLOW rats switched to a more adult-like defensive strategy in adulthood by increasing their use of partial rotations.

It is possible that some of the changes in play fighting that were observed in adulthood result from the adoption of a dominant or subordinate status within the pairs. Indeed, the results for the observational measures of social status in play fighting and body weight measures suggest that SLOW rats were dominant. The prediction that the adult-typical phenotype expressed by SLOW rats would manifest into social dominance was supported by several results; (1) SLOW rats playfully attacked less frequently than FAST rats during adulthood, (2) SLOW rats used partial rotation (an adult-typical tactic of defense) more frequently than FAST rats, and (3) SLOW rats were significantly heavier than FAST rats in adulthood. These behavioral indices of dominance in play fighting have been reported multiple times in previous studies (see method section for specific references). In the present study, the body weight results were especially convincing. In the same-strain play study (Reinhart et al., 2004), rats were reared with a cage mate of the same strain and the developmental changes in mean body weight for each strain were examined. It was shown that FAST rats grew at a faster rate than SLOW rats, but there were no significant strain differences in body weight from Day 85 onwards. In contrast, in the present study, SLOW rats were significantly heavier than FAST rats from Day 90 onwards, supporting the argument for SLOW selected-line dominance (see Lore & Stipo-Flaherty, 1984; Pellis & Pellis, 1991b).

Given the observed changes in the tactics of defense used by each strain in adulthood and the observed body weight asymmetry, it appears that providing FAST rats with socially dominant SLOW partners can overcome the genetic predisposition for FAST rats to use their (previously identified) highly evasive style of defense, and to mature physically at a faster rate. Instead, their rate of growth is decreased and they switch to using the more juvenile-typical patterns of playful defense (i.e., complete rotation) characteristic of subordinates. In contrast, SLOW rats increase their rate of growth and exaggerate the use of the more forceful patterns of playful defense typical of dominant adult males (i.e., partial rotation).

Unlike the findings for the observational measures of social dominance, the results for the competitive measures did not suggest that the SLOW strain was dominant. We expected that if dominant, then SLOW rats would obtain more food items and consume more water than FAST rats in the competitive tests of dominance. However, there were no strain differences in the food competition and FAST rats consumed significantly more water in the water competition. Because FAST rats have been shown to be hyperactive and impulsive (McIntyre, Poulter, et al., 2002), easily distracted by irrelevant cues (Anisman & McIntyre, 2002), and to habituate poorly to novel environments (Mohapel & McIntyre, 1998), it would have seemed likely that these behavioral characteristics would hinder their performance in competitive tasks. However, our competitive tasks were relatively simple and the testing environments were uncomplicated, thus strain differences in learning ability may have been minimized.

Even if SLOW rats were dominant in the food competition, as indicated by their greater, but not significant, consumption of food, there are several reasons why this may not have been clearly indicated by the results. When pairmates were placed inside the relatively confined area of the cylinder that was used for the food competition, it appeared that the physically smaller FAST rats were able to maneuver more easily than SLOW rats to get to the food items. McIntyre, McLeod and Anisman (2004) have previously reported that FAST rats are much faster in a T-maze (i.e., from the start box to the choice point) for food reinforcement as compared to SLOW rats. In addition, and although the following measure was not quantified, FAST rats appeared to steal food items from their SLOW partners more frequently than vice versa. FAST rats have been shown to respond inappropriately in other social contexts (e.g., McIntyre, Poulter, et al., 2002), and so perhaps in the present study, the FAST rats' impulsiveness (as indicated by their speed and aggressiveness during competition), may have led them to steal food items regardless of their "subordinate" status. That being said, one should expect that if FAST rats reach the limited resource (i.e., food or water) first, then they should acquire more of the resource than SLOW rats regardless of their dominance status.

The FAST rats' greater water consumption in the water competition test may be attributed to a differential physiological requirement for water between strains. When water intake has been measured throughout development and early adulthood in FAST and SLOW rats reared in same-strain pairs, in all instances, it has been the case that FAST rats drink more water than SLOW rats (McIntyre, unpublished observations). Thus, FAST rats may actually "need" more water than SLOW rats. For the water

competition in particular, the duration of drinking during the two-minute testing period may have been greater for FAST rats as compared to SLOW rats, or perhaps FAST rats were drinking more efficiently (i.e., faster) when they had access to the drinking tube. There were neither apparent dominance postures observed for SLOW rats, nor aggressive interactions at the spout during competitions. Therefore, even if SLOW rats were dominant in the water competition, their lesser need for water consumption may have led them to be tolerant of FAST rats (i.e., allowing their FAST pairmates access to the drinking tube).

In order to regard a competitive measure as a dominance index, you need proof that it is indeed an adequate measure of a socially mediated event, in that one animal has priority of access over another (Syme, 1974). It is possible that our conflicting results reflect differences in the animals' abilities to perform in these specific competitive tasks, rather than indicating the respective social status of each strain. The discrepancy between the two competitive measures of social dominance, and more generally, between the observational and competitive measures of social dominance in the present study is not uncommon. Previous studies, in both rats and mice, that have used more than one competitive measure, often fail to find a high correlation between competitive measures (e.g., Baenninger, 1970; Benton, 1982; Benton, Dalrymple-Alford, & Brain, 1980; Drews & Wulczyn, 1975). The lack of consistency between different tests of dominance lend support to the idea that dominance is not a unidimensional trait in the rat (Baenninger, 1970). Also, dyadic relationships may be multifaceted (Drews, 1993), with pairmates exhibiting different relationships in different conflict contexts.

73

Social conflict can result in long-lasting behavioral and physiological changes that are detectable long after the termination of the stressful encounter (Meerlo, Sgoifo, De Boer, & Koolhaas, 1999), which is why, in addition to the behavioral analyses, a physiological marker of stress that has been shown to change in response to subordination stress was examined in the present study. Blanchard and coworkers (1993) have shown that subordinate male rats have higher levels of plasma CORT, a stress hormone, than dominant male rats. Based on the view that subordination involves chronic social stress for male rats, and that social defeat can result in prolonged increases in plasma CORT levels (Blanchard et al., 1993), it was predicted that the subordinate strain in the present study would show higher levels of plasma CORT. However, no significant difference between FAST and SLOW rats was found. Even though our expectation, that (presumably subordinate) FAST rats would have higher CORT levels than SLOW rats, was not met, our results indeed replicate previous work by Anisman and coworkers (1997). That is, SLOW rats had slightly higher levels of plasma CORT than FAST rats. While inconsistent with the presumed pattern of dominance, the CORT levels may be consistent with the higher scores on behavioral measures of stress and anxiety in SLOW rats (Mohapel & McIntyre, 1998; Reinhart et al., 2004). That is, SLOW rats may have a higher baseline level of stress.

It is clear that the observational and competitive analyses of social dominance behavior were not congruent; perhaps the level of dominance asymmetry between FAST and SLOW pairmates was not sufficient to cause chronic social stress and the associated physiological change that was expected. A greater difference between strains may have

been found had the samples been collected immediately following a session of play, during which time the SLOW strain appeared to be behaviorally dominant, rather than eight days after the final behavioral tests were completed. Because the strength of dominance relationships (i.e., level of dominance asymmetry), and the resulting stress on individual rats can be varied (Blanchard et al., 1993), it appears that the intensity of subordination stress can be manipulated and thus account for some differences between behavioral and physiological markers of social status.

Irrespective of whether SLOW rats become dominant over FAST pairmates, what is striking is that there are large changes in some aspects of play fighting when interacting with a same or opposite strain partner (Table 1). Intriguingly, some components of play appear to be more resistant to context-dependent change than others. Indeed, it would seem that different components of play fighting fall into one of three categories: (1) highly stable, irrespective of environment (e.g., defense), (2) highly labile, with the absolute level varying markedly with environment (e.g., attack), or (3) contingently labile, with modulation of the absolute level being constrained within a narrow boundary (e.g., rotatory defense changes, but remains low compared to Long Evans hooded rats).

NOTES

1. This chapter is modified from a paper that has been submitted for publication.

CHAPTER FOUR[1]

Play fighting between unfamiliar pairs of kindling-prone (FAST) and kindling-resistant (SLOW) juvenile rats.

ABSTRACT

Previous studies of play fighting in two strains of rats suggest that selective breeding does not change all components of play uniformly, and that some components of play are more resistant to context-dependent change than others. Play fighting between unfamiliar pairs of juvenile rats of opposite strains was examined to determine whether behavior during play fighting is partner-specific at the time of testing, or reflective of prior behavioral modifications that are contingent on cage-mate identity. Focal animals behaved differently with unfamiliar partners of each strain. These data suggest that juvenile rats may be capable of identifying differences between unfamiliar conspecifics and that they are able to subsequently alter their play behaviors in response to subtle behavioral cues from their play partners.

INTRODUCTION

One of the most common forms of social behavior observed in the juveniles of many mammalian, avian, and even some reptilian species is play fighting (PF) (Aldis, 1975; Bekoff & Byers, 1981; Burghardt, 1999; Fagen, 1981; Pellis & Pellis, 1998a). Even though PF is frequently observed in young animals and has been documented in an assortment of species, little is known about the neurobiology of play. Most of what is known about the neurobehavioral mechanisms that control different components of PF has come from studies of the laboratory rat (Panksepp, 1998; Siviy, 1998; Vanderschuren, Niesink, & Van Ree, 1997).

Play fighting in the rat can be partitioned into attack and defense components (Pellis, Field, Smith, & Pellis, 1997; Pellis & Pellis, 1997), the latter of which can be further subdivided (e.g., different defensive tactics can be used). During a play fight, the attacker tries to make contact with and gently nuzzle the nape of the defender's neck (Pellis, 1988). Simultaneously, the defender tries to prevent nape contact (Pellis & Pellis, 1990). To facilitate PF, play partners must alternate the roles of attacker and defender to prevent the interaction from escalating into a serious fight (Pellis & Pellis, 1990, 1991b). Not only are attack and defense dissociable behaviorally, but it has also been suggested that they are motivationally distinct (Pellis & Pellis, 1991a; Siviy, Love, DeCicco, Giordano, & Seifert, 2003), being mediated by different neural systems (Field & Pellis, 1994). For example, while the frequency of playful interactions increases when animals are socially isolated prior to testing (Panksepp & Beatty, 1980; Pellis & Pellis, 1990; Siviy et al., 2003), playful defense remains unchanged when pre-testing social experiences have been

78

manipulated (Siviy et al., 2003). Furthermore, amphetamine (Field & Pellis, 1994) and haloperidol (Marshall, Pellis, Pellis, & Teitelbaum, 1989) reduce attack at lower doses than defense, suggesting that pharmacological agents do not have a uniform effect on all components of play behavior. Thus, it appears that attack and defense components of PF may involve separate neural control systems.

Studies that have used different strains of rats also suggest that the components of PF are dissociable and can be independently modified (Ferguson & Cada, 2004; Reinhart, McIntyre, Metz, & Pellis, 2005; Reinhart, Pellis, & McIntyre, 2004; Siviy, Baliko, & Bowers, 1997; Siviy et al., 2003). In a developmental study of PF, Reinhart and coworkers (2004) characterized the play profiles of two selectively-bred lines of rats based on differential amygdala excitability (kindling-prone or FAST and kindling-resistant or SLOW) (see Racine, Steingart, & McIntyre, 1999, for a detailed description of the selective breeding procedures). They reported that the strains employed different tactics of defense, that FAST rats were more playful than SLOW rats throughout development, and that at all ages SLOW rats played in a more adult-typical manner. These data suggest that selective breeding can lead to changes in PF that are distinct to each component, rather than being generalized across all components.

In a subsequent study (Reinhart, McIntyre, Metz, et al., 2005), FAST and SLOW pairs of rats were crossed-reared (post-weaning) to evaluate the stability of the neurobehavioral traits of PF. That is, Reinhart and coworkers (2005) examined whether all aspects of PF, during the juvenile phase and in adulthood, were equally influenced by changes to rearing

79

and testing environments. Based on the results of that study, they proposed that some components of PF were more resistant to context-dependent change than others, and that PF behaviors may be categorized as (1) highly stable (e.g., defense against playful attacks), (2) highly labile (e.g., frequency of playful attacks), or (3) contingently labile (e.g., use of particular defensive tactics). To summarize, it appears that different aspects of PF between FAST and SLOW rats are differentially affected by gene-environment interactions, and that environmental influences on play behavior are stronger during adulthood, as compared to the juvenile phase of development.

During PF between pairs of FAST and SLOW rats, the respective strain of each play partner appears to influence the expression of PF behaviors. However, a limitation of the developmental studies of PF in same (Reinhart et al., 2004) and different (Reinhart et al., 2005) strain pairs of rats is that pairs were housed and tested with the same cage mate from weaning onwards. Therefore, it cannot be determined if changes in patterns of play in one partner were due to the incongruent pattern of play of the other partner at the time of testing, or due to an adaptation process that preceded the testing. That is, is the play being modified because of the known idiosyncrasies of the partner with whom each animal has been reared, or because of the immediate behavior of the present play partner?

The present study assessed the play behavior of FAST juvenile females that were tested with unfamiliar same and different strain partners. It was hypothesized that, if the focal animals were (1) identifying unfamiliar play partners as being from the same or different strain, and (2) responding to that partner's immediate behavior, then focal animals should

behave differently with FAST vs. SLOW unfamiliar partners. That is, focal animals

should modify their play behaviors at the time of testing, so as to complement the unique

play behaviors being expressed by their particular partners.

METHODS

Subjects.

Eighteen FAST and 6 SLOW female rats that were obtained from Carleton University

were used for the present study. When the animals arrived at the University of Lethbridge

facility on postnatal Day 26, they were housed as same-strain pairs (6 pairs of FAST

females) and same-strain triads (2 triads of FAST females, 2 triads of SLOW females).

Animals were housed in 46 x 25 x 20 cm polyethylene tubs with processed corncobs for

bedding. The animals had *ad libitum* access to food and water, and the animal colony was

maintained at 21°C on a 12:12 hr light: dark cycle (lights on at 0730 hr). The care and use

of animals was in accordance with local standards set by the Animal Welfare Committee,

and the national standards set by the Canadian Council for Animal Care.

It should be noted that dominance asymmetry in PF has been documented in both

juvenile and adult male rats, and that behavior during PF can be modified depending on

an individual's status (Pellis & Pellis, 1991b, 1992; Pellis, Pellis, & Kolb, 1992; Pellis,

Pellis, & McKenna, 1993). In addition, Reinhart and coworkers (2005) have shown that

dominance asymmetries are evident during PF between pairs of FAST and SLOW adult

male rats (i.e., SLOW rats become dominant over FAST pair mates during PF). However,

females neither form the same types of dominance-subordinance relationships as males

(Barnett, 1975; Ziporyn & McClintock, 1991), nor do they have the PF asymmetries that are observed in males (Pellis & Pellis, 1990, 1992). To avoid any possibility of a dominance effect, and because it has been shown that there are no within strain sex differences in the frequency of play in FAST and SLOW rats (Reinhart et al., 2004), juvenile females were tested in the present study.

Procedure.

One individual from each of the FAST pairs was designated as the focal animal, and the other individual was designated (and will be referred to as) the "familiar partner." On postnatal Days 27, 28, and 29, each of the six FAST pairs was given 30-min habituation periods in the testing apparatus. On Day 29, following habituation, each animal was isolated for 24 hr in a 46 x 25 x 20 cm polyethylene cage, as such isolation prior to testing has been shown to increase the frequency of PF (Panksepp & Beatty, 1980; Pellis & Pellis, 1990). On Day 30, following isolation, each pair of FAST rats was placed together in the testing apparatus for 10 min. Following testing on Day 30, the animals were once again housed as pairs. On Days 31, 33, 35, 37, 39, 41, 43, and 45, each pair again went through the 30-min habituation and 24 hr isolation procedures, and were then tested on Days 32, 34, 36, 38, 42, and 44.

On Days 40 and 46, the focal animals from each FAST pair were not tested with their familiar partner, but with an unfamiliar, age-matched partner of either the same or different strain. The unfamiliar partners were similarly habituated with their cage mates (see triads above) on Days 37, 38, 39, 43, 44, and 45. Following habituation on Days 39

82

and 45, the unfamiliar partners were isolated for 24 hr prior to testing. Pairing of the focal animals with unfamiliar same or different strain partners on Days 40 and 46 was counterbalanced (i.e., half of the focal animals were paired with a FAST partner and half with a SLOW partner on Day 40, followed by pairing with the opposite strain on Day 46).

Habituation and testing commenced each day at 0730 hr. On the first day of habituation, the black and white pelage markings for each animal were drawn so that the animals could later be identified during video analysis. The testing apparatus had a Plexiglas floor lined with processed corncobs, two wooden walls, an anterior Plexiglas wall, and a mirrored posterior wall. The mirror located at the back of the testing apparatus facilitated observation of otherwise hidden parts of the rats' bodies. The dimensions of the apparatus during testing were 50 x 50 x 32 cm. Test periods lasted 10 min and were videotaped in the dark using the "night shot" function on a Sony 8-mm camcorder. The camera was placed in front of the apparatus at an oblique angle at a distance that could capture the entire volume of the testing area.

Behavioral Analyses.

After collecting the behavioral data, each 10-min test period with an unfamiliar partner was scored for various components of play. The 8-mm tapes were converted to VHS format and a time code (30th of a s) was added using an Horita TRG-50 time encoder (Horita, Mission Viejo, CA). Videos were viewed in slow motion and analyzed frame-by-

83

frame. Each of the following measures of play fighting were quantified for all animals (for more detailed description, see Pellis, Pellis, & Whishaw, 1992):

(I)   Frequency of attack – the number of playful initiations (i.e., nape contacts) per 10 min period

(II) Probability of defense – the number of times an animal elicits a defense (withdrawal of the nape area from the snout of an approaching partner), relative to the number of attacks received per 10 min period

(III) Type of defense:

   (i)   Probability of evasion – the number of evasive defenses (withdrawal of the nape by leaping, running, or turning away from the partner) relative to number of total defenses

   (ii) Probability of complete rotation – the number of times the defender rotates around its longitudinal axis to lie supine (facing the attacker to block nape access) relative to number of total defenses

   (iii) Probability of partial rotation – number of facing defenses whereby the defender turns to face the attacker by rearing on the hind legs, relative to number of total defenses

Statistical Analyses

Matched pairs t-tests were used for all measures of play.

84

RESULTS

There were no significant partner effects for frequency of playful attack and probability of playful defense ($p > 0.05$) (Table 2). Focal animals did not differ in the frequency with which they attacked FAST and SLOW unfamiliar partners, and the probability of defense against attacks from unfamiliar partners was comparable to levels that have been reported in previous studies on the PF of FAST rats (Reinhart et al., 2004; Reinhart et al., 2005).

With regard to the defensive tactics used by focal animals during PF, there were significant partner effects for probability of evasion [$t(5) = 3.78, p < 0.05$], and probability of complete rotation [$t(5) = 3.67, p < 0.05$] (Table 2). Focal animals were more likely to use evasion and less likely to use complete rotation when playing with unfamiliar FAST partners. The opposite tendency was observed when focal animals played with unfamiliar SLOW partners. That is, the focal animals were less likely to use evasion and more likely to use the complete rotation tactic of defense. For probability of partial rotation, there was no significant partner effect ($p > 0.05$).

Table 3. Levels of play behavior for focal animals with unfamiliar play partners.

Play Behavior	FAST Partner	SLOW Partner	t-value
Attack	69 ± 10	60 ± 4	NS
Defense	$97.7 \pm 1.5\%$	$96.6 \pm 1.8\%$	NS
Evasion	$53.8 \pm 6.8\%$	$34.2 \pm 7.0\%$	3.78*
Complete rotation	$41.3 \pm 6.8\%$	$61.2 \pm 6.5\%$	3.67*
Partial rotation	$5.0 \pm 2.1\%$	$4.4 \pm 1.1\%$	NS

(Mean $\pm$ SE, * $p < 0.05$)

DISCUSSION

Previous studies of PF in FAST and SLOW rats have shown that each strain has a unique play profile (Reinhart et al., 2004), and that the genetically-typical pattern of each component of PF may be differentially modified in response to changes in partner identity (Reinhart et al., 2005). That is, FAST and SLOW rats appear to modify their play behaviors in a manner that is dependent on the age at testing and the strain of the partner with whom they are playing. When FAST rats were paired with an unfamiliar conspecific from the FAST and SLOW strains in the present study, again, the animals behaved in a partner-dependent manner, specifically for the components of PF that have been proposed to be relatively labile (e.g., frequency of playful attack and tactics of defense).

There was no significant difference in the frequency of playful attacks directed at the unfamiliar partners. However, the absolute number of attacks directed toward unfamiliar partners of both strains was higher than has been observed previously for FAST rats with familiar partners (Table 3), and for studies of PF in Long Evans rats (Pellis et al., 1997). Reinhart and coworkers (2005) proposed that frequency of attack is a highly labile neurobehavioral trait that can vary markedly with the environment, and indeed, the present results seem to support this idea. One possible explanation for the atypically high levels of playful attack is that there is a novelty effect between unfamiliar play partners. Previous studies have reported higher levels of PF between unfamiliar vs. familiar play partners (Cirulli, Terranova, & Laviola, 1996), and that partner novelty increases social interactions between pairs of juvenile rats (Barefoot, Aspey, & Olson,

Table 4. Mean levels of play behavior for FAST rats with familiar and unfamiliar play partners.

Play Behavior	Familiar*/Unfamiliar FAST + FAST	Familiar**/Unfamiliar FAST + SLOW
Attack	40 / 69	48 / 60
Defense	97% / 98%	97% / 94%
Evasion	47% / 54%	45% / 34%
Complete rotation	41% / 41%	44% / 61%
Partial rotation	12% / 5%	11% / 4%

* juvenile females (Reinhart et al., 2004); ** juvenile males (Reinhart et al., 2005)

1975; Monroe & Milner, 1977). It should be noted however, that the suggested

motivating role of partner novelty has not been supported in all studies (e.g., Kahana,

Rozin, & Weller, 1997; Latané, Schneider, Waring, & Zweigenhaft, 1971; Terranova,

Cirulli, & Laviola, 1999). What does appear to be consistent across most studies,

including the present one, is that juvenile rats seem to be capable of identifying partners

as being unfamiliar and that some of their social behaviors are subsequently altered.

The probability of defending against playful attacks does not change as a function of age

at testing (Pellis et al., 1997), or the level of social isolation prior to testing (Siviy et al.,

2003). In FAST and SLOW rats, the probability of defending against playful attacks

remains stable irrespective of the strains of the play partners (Reinhart et al., 2005) (Table

3). In the present study, the level of playful defense for focal animals remained high

when they were paired with either an unfamiliar FAST or SLOW partner, further

supporting the idea that playful defense is a relatively stable neurobehavioral component

of PF. Because the probability of defense did not change, we can further conclude that

only some aspects of PF are subject to environmentally-based modification of the

genetically-typical pattern, as was proposed previously by Reinhart and coworkers

(2005).

In the present study, focal animals used different tactics of defense when playing with

partners from each strain. The use of evasive and complete rotation tactics of defense

differed for focal animals in a partner-dependent manner (Table 2). Furthermore, the

focal animals' mean probability of evasion with unfamiliar FAST partners, and mean

89

probability of complete rotation with unfamiliar SLOW partners, exceeds the mean levels observed previously in FAST rats PF with familiar FAST and SLOW partners (Table 3). Coincident with the relatively high level of complete rotation observed in focal animals during PF with unfamiliar SLOW partners, was a relatively low level of evasion (Table 3).

Indeed, the FAST females behaved differently with unfamiliar partners of each strain, suggesting that they may be responding to some subtle behavioral cue(s) from the play partner at the time of testing. One way of interpreting the partner-specific defensive responses that were observed (i.e., high levels of evasion and complete rotation with unfamiliar FAST and SLOW partners respectively), is that they were an exaggeration of the FAST rats' typical pattern of defense, in response to unfamiliarity. That being said, Reinhart and coworkers (2004) reported an atypically high level of evasion during play fights between familiar FAST adult rats. Similarly, as juveniles and in adulthood, FAST rats have been shown to use complete rotation more frequently with familiar SLOW partners than would have been predicted based on their behavior with familiar partners of the same strain. Perhaps, familiarity with a conspecific may allow for some behavioral flexibility between partners during social interactions (i.e., you know how far you can "push your limits" with a known partner). However, in the absence of familiarity, as in the present study, to behave in a manner that is typical of the strain during a complex social interaction such as PF, may be a default response.

NOTES
1. This chapter is modified from a paper that has been submitted for publication.

CHAPTER FIVE

General Discussion

Social play has long been recognized in a variety of species (Groos, 1898), being especially prevalent among the juveniles of most mammalian species (Fagen, 1981). Furthermore, descriptive studies have shown that in rats, social play occurs mostly as play fighting (e.g., Meaney & Stewart, 1981; Poole & Fish, 1976), the frequency of which peaks during the juvenile phase of development (Thor & Holloway, 1984). While it has been suggested that there is an association between juvenility and play complexity in muroid rodents (Pellis & Iwaniuk, 2000), and that components of play fighting are behaviorally and motivationally dissociable (e.g., Pellis & Pellis 1987, 1990, 1991a; Siviy, Love, DeCicco, Giordano, & Seifert, 2003), it has been unclear as to whether all components of play fighting are equally modified in response to changes in juvenility within a single species (e.g., rats). That is, do all components of play fighting become more complex when the period of juvenile development has been prolonged? Moreover, if there are genetic controls over the components of play that may be differentially expressed with changes in the retention of juvenility, then is it possible that the potential for modification of each component of play may also be differentially regulated? Stated otherwise, could components of play behavior be differentially labile?

The main objective of this thesis was to use two strains of selectively-bred rats (FAST and SLOW) with known differences in their phenotypic expressions of juvenility (i.e., differences in behavior and patterns of neural organization), to gain a better understanding about potential genetic controls over the components of play fighting (i.e.,

92

playful attack, playful defense). However, before discussing the implications of the findings regarding play fighting, one limitation of using selectively-bred animals must be considered. In any species, the alleles present in a population are co-adapted (i.e., linked) with each other because of past natural selection (Papini, 2002), implying that a 1:1, gene: behavior relationship is improbable. Consequently, because selectively-bred animals were at one time subject to artificial selection, they can differ for a relatively wide range of characteristics (i.e., they often differ for more than just the trait of interest) (Parmigiani, Palanza, Rodgers, & Ferrari, 1999). That being said, and given that FAST and SLOW rats have been shown to differ for a variety of behaviors (McIntyre, Poulter, & Gilby, 2002), it was also relevant to examine various developmental and behavioral factors associated with play fighting (e.g., development of body weight, circadian activity, anxiety, social dominance, stress hormones), for FAST and SLOW rats. The implications for some of the "non-play" findings will be discussed in conjunction with the data for play fighting.

Genetic controls over play fighting

In terms of play fighting, the findings in chapter two supported previous suggestions that there are genetic contributions to the expression of play behaviors (Ferguson & Cada, 2004; Siviy, Baliko, & Bowers, 1997; Siviy et al., 2003). FAST rats were more playful than SLOW rats throughout development (i.e., FAST rats initiated more playful attacks and were more likely to respond to playful attacks), and SLOW rats behaved in an adult-typical manner at all ages (e.g., low probability of defending against playful attacks, adult typical defensive tactics used as juveniles). Interestingly, the play profile for SLOW rats,

93

particularly during the juvenile stage, was similar to the play profile reported for Fischer-344 (F-344) rats. Comparative studies of play behavior for F-344 rats with Buffalo and Lewis rats (Siviy et al., 1997; Siviy et al., 2003) have shown that like SLOW rats, F-344 rats, when tested as juveniles, also exhibit fewer playful attacks, are less likely to use complete rotation, and are more likely to use evasive tactics of defense during play fighting (relative to Buffalo and Lewis rats). In addition, when tested in cross-strain pairs (i.e., F-344 and Buffalo), F-344 rats exhibited levels of playful defense that were well below the range reported for other strains (Siviy et al., 1997), suggesting a genetic predisposition for low levels of playfulness. It may be possible that both SLOW and F-344 strains of rats possess certain neurobehavioral characteristics that make them unique in how they respond to playful solicitations from conspecifics. To strengthen the comparison between SLOW and F-344 rats, it would be of benefit to examine play fighting for F-344 rats in adulthood to see if the developmental trajectory for play fighting would be fully congruent with that observed in the SLOW strain.

The play profiles for FAST and SLOW rats indicate that even though the overall level of playfulness differed between the two strains, the typical age-related decline in playfulness was present in both strains. More importantly, FAST and SLOW rats used different tactics of defense in adulthood to reduce the likelihood of prolonged social contact, which is a fundamental part of play fighting. Rather than exhibiting juvenile-like patterns of both playful attack and playful defense in adulthood (as was predicted), FAST rats exhibited an atypically high level of evasions to avoid physical contact. In contrast, SLOW rats blocked physical contact by using a combination of adult-typical defensive

94

tactics (i.e., evasion and partial rotation). Because the components of play changed for both strains of rats but in different ways, it can be suggested that components of play fighting do not change in a uniform manner with changes in the neurobehavioral underpinnings of juvenility.

It appears that there are consistent, quantifiable, between-strain differences in levels of play, and the findings from chapter two emphasize the importance for future studies to evaluate individually the development of each component of play fighting. Subsequent to the establishment of play profiles for inbred or selectively-bred lines of rats, it becomes feasible to ask questions about play fighting that extend beyond the level of behavior. That is, the genetic homogeneity of these animal models makes them more amenable to comparative analyses at various levels. Between-strain differences in behavior can provide clues as to which neurotransmitter systems, brain circuits, or specific genes may modulate components of play fighting. For instance, between-strain comparisons of neurotransmitter levels in the amygdala have been made for FAST and SLOW rats (e.g., noradrenaline, dopamine, serotonin, glutamate, aspartate, GABA), indicating differences between the strains for some systems and not others (Shin, Anisman, Merali, & McIntyre, 2004). Similarly, F-344 and Buffalo rats have been shown to differ in noradrenergic and dopaminergic receptors (Siviy et al., 1997). Knowing that there are strain differences in various transmitter systems can help us to understand the extent to which these neurotransmitters are involved in modulating or regulating play behavior - also bearing in mind that their respective influences may be specific for a particular component of play.

Differential influence of genetic controls

While the observed differences between FAST and SLOW rats from chapter two

supported the idea that genetic variation likely contributes to differences in play behavior,

the strength of potential genetic influences on behavior was still unclear. In conjunction

with the role that genes play in modulating the expression of behavioral phenotypes,

environmental factors that influence behavior also need to be considered. Thus, in the

second experiment (chapter three), the effects that gene-environment interactions had on

the development of play fighting in FAST and SLOW rats were examined.

It has been suggested that there are chronological periods throughout development during

which specific types of stimulation have their most robust effects (i.e., critical period

hypothesis) (Hol, Van den Berg, Van Ree, & Spruijt, 1999). As such, some research has

shown that rats are unusually susceptible to environmental influences during the juvenile

phase of development (Hol et al., 1999). If so, then one would predict that the changes in

rearing and testing environments (chapter 3) would have a greater effect on play fighting

between juvenile rats as opposed to adults. However, other studies have found that

environmental effects on behavior are age-dependent (e.g., Arakawa, 2005). That is,

some factors have differential effects on behavior when they are experienced during

different periods of development, leaving open the possibility that adult behavior may be

more readily altered in response to environmental modifications.

Similar to the patterns of play observed between same-strain pairs of rats, when tested in

cross- strain pairs, FAST rats were more likely than SLOW rats to defend against playful

attacks as juveniles and in adulthood. Previous work has shown that playful defense does not change as a function of age (Pellis, Field, Smith, & Pellis, 1997) or the amount of time spent in social isolation prior to testing (Siviy et al., 2003). Thus, the cross-strain experiment with FAST and SLOW rats support those data, in that the likelihood of defending against playful attacks remained stable for both strains irrespective of the partner involved. Considering that the within-strain proclivity for defending against playful attacks appeared to be relatively invariable, it could be suggested that the high stability of this component of play fighting may be attributed to the fact that is it subject to strong genetic control.

In contrast, the between-strain variability for levels of playful attack would suggest that the attack component of play fighting may be more readily influenced by manipulations of the environment. That is, playful attack may be relatively labile, as compared to playful defense. Case in point: FAST and SLOW rats differed significantly for level of attack when tested in same-strain pairs, but did not differ when tested in cross-strain pairs. In fact, as juveniles and adults, both strains of rats attacked more frequently when in cross-strain pairs than would have been predicted based on their same-strain play profiles. Sprague-Dawley rats have also been shown to exhibit more playful solicitations when paired with a partner of different strain than with a play partner of the same strain (Ferguson & Cada, 2004). One explanation for these results is that more playful partners can stimulate more play in the other rat (i.e., a contagion effect) (e.g., Hole & Einon, 1984; Pellis & McKenna, 1992; Pellis, Pellis, & Kolb, 1992; Varlinskaya, Spear, & Spear, 1999). Similarly, levels of playful attack have been shown to vary when the

97

motivation to play has been manipulated via social isolation prior to testing. That is, the frequency of playful attack increases as a function of the amount of social isolation (Panksepp & Beatty, 1980; Pellis & Pellis, 1990; Siviy et al., 2003). To reiterate, because levels of playful attack have been shown to vary indefinitely in response to changes in environmental factors (e.g., play partner, isolation), it could be suggested that the attack component of play fighting may be a neurobehavioral trait that is less genetically-determined, and consequently, less resistant to context-dependent change than playful defense.

Conceivably, because there were known differences in the behavioral phenotypes for each strain (including their play profiles), it was predicted that playful interactions between FAST and SLOW pairmates would be qualitatively different in some way, relative to the interactions that took place between same strain pairmates. If so, then the resulting cross-strain play profiles for each strain would likely be different than those previously observed in same-strain pairs. As mentioned, playful defense remained stable and playful attack was highly labile irrespective of age, but how were more qualitative aspects of play fighting (i.e., tactics of defense) affected by gene-environment interactions? Additionally, were the effects age-dependent?

Even though the overall playfulness for FAST and SLOW rats in cross-strain pairs differed from their respective same-strain levels of play, during the juvenile phase, the levels of play fighting and tactics of defense used by both strains were almost indistinguishable. That is, when tested in cross-strain pairs, the patterns of play fighting

98

for juvenile FAST and SLOW rats were highly similar. However, in adulthood, the particular tactics of defense used by each strain diverged. FAST rats evaded less and used complete rotations more frequently, whereas SLOW rats increased their use of partial rotations. Thus, it appears that the environmental manipulation made in the second experiment (chapter 3) had greater implications for adult behavior, as compared to juvenile behavior.

The differences in tactics of defense used by FAST and SLOW rats in adulthood can be interpreted with two considerations in mind: (1) the significance of the age-dependent effect, and (2) the direction and magnitude of change observed in each strain, for each tactic. Notably, environmental context was manipulated not in a physical way (e.g., light intensity, ambient temperature, novel environment), but in a manner that was more socially-relevant. One possible explanation for the modulation of defensive tactics in the FAST and SLOW strains stems from the complexities associated with adult social interactions in rats. That is, adult social behavior is characterized by the ability of individuals to respond with appropriate, well-timed, context-dependent behaviors, and the development of such a repertoire of social behaviors can be influenced by factors such as genetic constitution, sex, and social rank (Hol et al., 1999). As juveniles, rats do not yet have a fully developed set of social skills, making it plausible that cross-strain pairs of FAST and SLOW rats behaved similarly during play fighting at the juvenile stage. In contrast, adult rats have an acquired set of social behaviors; this perhaps explains why the change in play partner had a greater influence on play fighting in adulthood. Moreover, because juveniles are relatively "socially immature", the effects on juvenile behavior may

99

have been more detrimental had the social manipulation been more extreme (e.g., long-term social isolation or deprivation). Socially relevant environmental manipulations that are relatively less extreme (e.g., interacting with a genetically-different conspecific) seem to hold greater salience in adulthood, having an effect on adult social exchanges.

The importance that social variables have for adult interactions (e.g., play fighting) is further exemplified by the dominance-subordinance relationships that form between male rats in adulthood (Lore & Stipo-Flaherty, 1984). Previous studies have examined the nature of dominance relationships, and the respective effects that these relationships have on the patterns of play fighting in dominant and subordinate pairmates (e.g., Pellis & Pellis, 1991b, 1992; Pellis, Pellis, & McKenna, 1993). Dominance relationships are reflected in the play fighting of male pairmates as asymmetries in particular components of play. That is, subordinates initiate more playful contacts than dominants, and use juvenile-like defensive tactics (complete rotation) when playing with dominant males. Conversely, dominant males attack subordinates less frequently and they use adult-typical (i.e., partial rotation) tactics of defense. Correspondingly, these exact patterns of play behavior were observed in cross-strain pairs of rats, with FAST rats adopting the behaviors associated with being subordinate, and SLOW rats adopting tactics that are typical of being dominant.

The direction of change for the defensive behaviors can be explained, at least in part, by the establishment of dominance relationships between FAST and SLOW pairmates in adulthood. In terms of the magnitude of change for each tactic, it appears that the relative

100

potential for modification of defensive tactics lies somewhere in between the levels

suggested for playful attack and probability of defending against playful attack. That is,

the tactics of defense in play fighting may be contingently labile. The tactics seem to

change in a context-dependent manner. However, the degree of change seems to be

confined to a strain-typical range (e.g., changes in rotatory defense are exhibited in FAST

and SLOW rats, but remain low compared to Long Evans hooded rats). Tactics of

defense neither appear to be as stable as the quantitative measure of playful defense (i.e.,

probability of defending against playful attacks), nor does their capacity for modification

appear to be as pliable as that observed for playful attack; this suggests that there may be

some intermediate level of genetic control over this aspect of play fighting.

Reverting to the issue of strain differences in social dominance, SLOW rats were not only

behaviorally dominant during adult play fighting, but they also exhibited a

physiologically relevant characteristic of dominant male mammals - greater body weight

(Lore & Stipo-Flaherty, 1984). In chapter two, it was shown that FAST rats grew at a

faster rate than SLOW rats, but when they were reared in cross-strain pairs (chapter

three), SLOW rats were significantly heavier throughout adulthood. In contrast to these

affirmative indices of social dominance in the SLOW strain, competitive measures of

social dominance and measures of stress hormones revealed either an absence of strain

differences, or differences in the opposite direction (i.e., FAST rats being dominant to

SLOW rats). Putative explanations for the inconsistencies reflected in the battery of

social dominance tests were provided in chapter three, some relating to between-strain

physiological differences (e.g., body size, water requirements), and others to previously

documented behavioral idiosyncrasies associated with a particular strain (e.g., impulsivity of FAST rats).

Additionally, it is possible that selective breeding has led to the presence of comorbities in FAST and SLOW rats that have relatively global (i.e., widespread) effects on social behavior; this would provide a more parsimonious explanation for not only the documented discrepancies in social dominance measures (chapter 3), but also for the between-strain differences that have been reported for various social behaviors. One possibility is that FAST and SLOW rats differ for neurobehavioral traits associated with coping style (i.e., the way individuals perceive and cope with challenges). It has been suggested that the consequences of social conflict are determined not by the intensity of social interactions between pairmates, but by the coping style of the individuals (Meerlo, Sgoifo, De Boer, & Koolhaas, 1999). For example, rats that have experienced repeated social defeat show changes in food intake, body weight, and social activities. Because these changes vary greatly between individuals, it is hypothesized that there are variations in coping style (Meerlo et al., 1999). Similarly, following repeated social defeat, idiosyncratic patterns of stress symptoms have been observed in mice that were selectively bred for an active coping style vs. a passive coping style (Veenema, Meijer, de Kloet, & Koolhaas, 2003), indicating between-strain differences in coping style. We know that FAST and SLOW rats respond differently behaviorally and physiologically in social, and stress-related situations (e.g., Anisman et al., 1997; McIntyre, Poulter, et al., 2002; Mohapel & McIntyre, 1998; Reinhart, Pellis, & McIntyre, 2004). Thus, it is possible that differences between FAST and SLOW rats for coping style may explain, in

102

part, the often contrasting responses that FAST and SLOW rats exhibit while engaged in different social experiences (e.g., play fighting, social dominance competitions, sexual contexts).

It is clear that FAST and SLOW rats differ fundamentally not only in behavior, but also in the brain as well. Physiological and anatomical studies have shown that selective breeding for amygdala excitability affects not only the amygdala, but also the activity and morphology of cells in various brain regions (e.g., Flynn et al., 2004; McIntyre, Hutcheon, Schwabe, & Poulter, 2002; Poulter et al., 1999; Reinhart, McIntyre, Pellis, & Kolb, 2004; Xu, McIntyre, Fahnestock, & Racine, 2004). Interestingly, the morphology of cells found in cortical regions of the brain that are connected to the amygdala, and that are thought to be important for social interactions (i.e., prefrontal cortex, parietal cortex), have been shown to differ between the strains (Reinhart, McIntyre, et al., 2004). As yet, it is unclear as to what the specific implications of the anatomical findings may be for social behaviors in general. However, it should be acknowledged: (1) that selective breeding appears to have widespread effects throughout the brain, and (2) that the tendency to be more juvenile-like (i.e., FAST) or adult-like (i.e., SLOW) because of selective breeding may cause changes in the brain systems that influence an assortment of social behaviors.

The role of familiarity?

When considered in combination, the findings from chapters two and three support the existing argument for potential genetic contributions to the expression of play behaviors,

103

and suggest that components of play fighting may be subject to varying levels of genetic influence, making each component differentially labile. To strengthen the potential validity of the previous interpretations, it was necessary to address an important limitation that existed for the experimental design employed in chapters two and three. During the developmental studies of play fighting in the FAST and SLOW strains, rats were housed as pairs immediately after weaning, and were then tested as juveniles and adults with their respective cage mate. However, the potential cause for differences in play behavior that were observed in same vs. cross strain pairs was questionable. It was unclear as to whether changes in play behavior in one partner were due to the peculiarities in behavior of the other partner during play-testing, or if the changes in play behavior were representative of more general behavioral modifications that developed from having been reared with a genetically similar or different cage mate.

To address this uncertainty, juvenile FAST rats were tested on subsequent occasions with unfamiliar FAST and SLOW partners. By pairing unfamiliar juveniles that had neither previous histories together, nor the burden of social complexities that are associated with adult social interactions, it was possible to eliminate some of the confounding factors that hindered the previous experiments, and examine the immediate behaviors of the unfamiliar focal animals. More importantly, it could be determined whether the unfamiliar focal animals exhibited partner-specific play behaviors, as would be predicted if there are indeed qualitative differences between FAST and SLOW rats for the components of play fighting. As shown in chapter four, focal animals behaved differently with partners from each strain, specifically for those components of play fighting that

104

were proposed in chapter three, to be potentially modifiable (i.e., playful attack, tactics of defense). Given that the focal animals appeared to behave in a partner-specific (and strain specific) manner, it could be suggested that they may have been able to identify differences between unfamiliar conspecifics, and that they were subsequently capable of modifying their own behaviors while in the respective play situations. That is not to say that focal animals were able to identify specific individuals, but rather, that they may have perceived differences in some qualitative feature(s) of play behavior when interacting with a same vs. different strain partner. If this is so, then to what cues were they responding?

It has been shown that during social interactions, rats rely heavily on olfactory and tactile cues to guide their behaviors, which makes it difficult to identify behaviorally the ways in which play fighting may differ between FAST and SLOW rats. Conceivably, focal animals may have modified their own behaviors in response to pheromonal differences that were detected between strains, or perhaps there were between-strain differences in terms of how playful behaviors were executed. For example, previous work has shown that qualitative differences in the performance of playful attack by one partner can affect the corresponding defense of the other partner. Nape contacts that are more vigorous or that occur in rapid succession may be more likely to result in rotatory defensive tactics by the defending partner, whereas less vigorous nape contacts may be less likely to result in a facing style of defense (Pellis et al., 1997; Siviy et al., 2003). Although it was not reported in chapter four, there was no difference in the mean frequency of playful attacks launched by the unfamiliar FAST and SLOW partners towards the focal animals. In

addition, when a subset of FAST and SLOW rats was examined to determine whether there may be strain differences for "style of attack" (i.e., angle of approach for the attacker, speed of attack), no significant differences ($p > 0.05$) were detected between FAST and SLOW rats. For example, for both strains, a perpendicular angle of approach was the most common angle of attack observed (70-80% of all attacks), and approximately 80% of the attacks involved a combination of either walking toward the potential defender or bending down from a rearing position to attack the defender. It is possible that there are other, more subtle qualitative differences in how FAST and SLOW rats execute their respective attacks. However, there do not seem to be any distinct strain differences in attack style that can account for the focal animals' use of partner-specific tactics of defense.

It would also be of interest to examine the behavior of the unfamiliar partners in greater detail, so as to determine how differential experiences and partner novelty may affect behavior. Prior to being tested with the unfamiliar partners, focal animals had more play fighting experiences (i.e., with their cage mates), and, consequently, greater exposure to the play-testing environment than their unfamiliar partners. It may be possible that because of the focal animals' relatively extensive experience with play fighting in the testing environment, the novelty effect of a new partner played a facilitating role in the expression of play behaviors (e.g., unusually high level of playful attacks). But how did partner novelty affect the behavior of the unfamiliar animals? Knowing that fear and curiosity about novel environments can compete with social attraction (Terranova, Cirulli, & Laviola, 1999), the presence of a new partner in an environment with which the

106

unfamiliar animals had relatively little experience (i.e., compared to the focal animals) may have been overwhelmingly novel. If this is so, then one would predict that play fighting behaviors in the unfamiliar partners may have been somewhat repressed or inhibited.

Furthermore, the tactics of defense in the unfamiliar partners of each strain would presumably differ, particularly because of the known behavioral and anxiety differences that that have been observed in the selected-lines (e.g. Mohapel & McIntyre, 1998; Reinhart, Pellis, et al., 2004; Reinhart, McIntyre, Metz, & Pellis, 2005). Would the impulsive, juvenile-like FAST rats behave in a manner that encourages playful contact (e.g., high level of complete rotations), irrespective of the situational uncertainty? Conversely, would the highly anxious, adult-typical SLOW rats behave in a way that is not conducive to play (e.g., high level of evasions)? A closer look at the data collected in chapter four could test these hypotheses.

Is there more to learn from FAST and SLOW rats?

It has been suggested that social play is important for behavioral development in mammals (Vanderschuren, Niesink, & Van Ree, 1997), and more specifically, for the development of social skills (Panksepp, Siviy, & Normansell, 1984; Thor & Holloway, 1984). Bearing that in mind, if social play behaviors were to share similar characteristics in all mammalian species, then it is possible that information relevant to human play may be derived from studying social play in a rat model. Play fighting is the most common form of social play for the rat, and even though most of what is known about the

neurobiology of play fighting has come from studying the laboratory rat, there are still many outstanding questions regarding the neurobehavioral mechanisms underlying playful behaviors. As mentioned in chapter one, previous analyses of play fighting in the rat have been limited or lacking in a variety of ways. Consequently, we have yet to discover and fully understand which brain system(s), neurotransmitters, or specific proteins may play a role in modulating playful behaviors.

Because selectively-bred animals often exhibit little within-strain variation in gene expression (often for a number of genes) (Koch & Britton, 2005), they are particularly valuable for examining the genetic underpinnings of behavior. For example, in FAST and SLOW rats, 15 genes of interest have been isolated and show no within-strain variation in their expression, whereas relatively more variation in baseline gene expression has been found in control animals (McIntyre, Poulter, et al., 2002). Thus, FAST and SLOW rats serve as applicable tools for investigating molecular mechanisms underlying complex behaviors such as social play.

Using FAST and SLOW rats to examine neurobiological aspects of social play may also be of interest for investigating human disorders involving disturbances in social (play) behavior (i.e., juvenile autism, ADHD) (Vanderschuren et al., 1997). The causes of many neurological disorders involve multiple gene alterations that interact to create the disorder (McIntyre, Poulter, et al., 2002). However, most animal models used to study genetic contributions to disorders use mice, the functional implications of which are drawn from changes in the expression of one gene (McIntyre, Poulter, et al., 2002). The selective

breeding of FAST and SLOW rats is relatively more natural and representative of the human disorders. Therefore, it appears that the comorbidities that were initially considered a limitation in FAST and SLOW rats may be of some benefit. Interestingly, selective breeding of FAST and SLOW rats has produced a variety of physiological and behavioral differences between the strains that appear relevant to human disorders such as epilepsy and ADHD (McIntyre, Poulter, et al., 2002); again emphasizing the value of these strains of rats to investigate molecular mechanisms underlying biological disorders and the genetic underpinnings of social behaviors.

While it appears that there are genetic controls that differentially influence the components of play fighting, it remains to be understood why the neurobehavioral traits of play fighting may be more or less susceptible to context-dependent change. Why is playful defense so rigid? Conversely, why is the capacity for modification so great for playful attack? Future experiments using selectively-bred animals may provide insight into the proximate mechanisms controlling playful behaviors.

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