

**STRESS AND THE POWER OF PLAY**

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## **ABSTRACT**

### **Stress and the Power of Play**

Stress is thought to be antithetical to play. However, this thesis shows that mild stress (e.g., social deprivation) enhances rough-and-tumble play, as opposed to other social behaviors, in adolescent rats. Social deprivation results in both higher levels of corticosterone (a stress hormone) and higher levels of play. When non-socially deprived rats were injected with ACTH (a precursor to corticosterone), the frequency of play was elevated to levels comparable to that seen when juveniles were socially deprived. Moreover, corticosterone was reduced by the opportunity to play, but not when given social contact (but no play) or solitary exercise (i.e. a running wheel). Therefore, this thesis provides evidence that play is not only enhanced by mild stress, but that it is used by animals to reduce stress.

## ACKNOWLEDGEMENTS

Most people reading a thesis skip over this boring proclamation of thanks to the author's supervisors, family, and co-workers. I would like, however, to take a different tactic. My thanks go primarily to the little guys who sacrificed their lives in the name of science: rats. I delighted in getting to know these intelligent, affectionate, and all together cute critters!

That said, I will now continue on to give thanks to my supervisor, Sergio M. Pellis. His open-door policy made communication much easier, even when the topic had nothing whatsoever to do with my studies. Which brings me to another point: the man is incredibly patient! I am a (ahem) "mature" student who has not been involved in academia for almost a quarter of a century, and long-suffering is a requisite trait for a supervisor in such a position! I thank-you, Sergio, for constantly bringing me back on track after letting me ramble off on a tangent until I became completely lost.

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## CHAPTER 1

### INTRODUCTION

Newspapers are full of ominous predictions about the latest potential pandemic. We are inundated with news of recent death tolls from the “bird ‘flu” virus. Worries about vaccine shortages plague healthcare workers around the world. Given the devastation that accompanied the Spanish ‘flu a century ago, it makes sense to take every precaution to avoid succumbing to the latest killer strain of influenza. Other life-threatening maladies like Ebola, West Nile virus, and SARS strike fear in our collective hearts. Scientists work overtime trying to find the latest vaccine or cure. It is no wonder our society is becoming more and more phobic as each new environmental health threat arises.

But what about something that comes not from our surroundings, but from within us, something that we are born with, that has been proven to kill off brain cells, disrupt memory, exacerbate the debilitating effects of stroke or seizure, causes impotence, decreased resistance to disease and cancer, increased risk of heart and digestive problems, and cause many other afflictions? No vaccine or cure exists to prevent these incapacitating effects. How can we possibly protect ourselves? What is this chemical, and if it is so lethal, why is it in our bodies? The frightening answer is that this substance is a result of stress. We have all experienced it. How can it be that there are so many negative effects associated with such a common state, yet most of us survive relatively unscathed?

Well, stress isn't all bad. According to Sapolsky (2002), stress is "anything that disrupts physiological balance". This "physiological balance" has been referred to as homeostasis, whereby energy is expended in an effort to maintain or restore the original steady state. This can involve cellular maintenance, immune function, reproduction, and thermogenesis (Nelson, 2000). However, Sapolsky (2004) points out that the initial idea of homeostasis doesn't go quite far enough to account for this "physiological balance", and refers to a more modern concept called "**allostasis**". With homeostasis, there is a single optimal state for any given measure in the body, but what is ideal under basal conditions is different than during stress. Sapolsky refers to this as "constancy through change". Another difference between homeostasis and allostasis is that, rather than achieving an ideal set point through a local regulatory mechanism, any given set point can be achieved through many different means. Thus, he characterizes allostasis as the *brain coordinating changes throughout the body, and can include changes in behavior*. The stress response is an attempt to return to this optimal state, and is considered to be adaptive in terms of helping individuals cope with emergency situations; however, over the long term, stress can cause many disorders that may jeopardize health and survival. But, the stress response actually comes in rather handy when one needs to escape from a predator. The primary function of this response is to prepare the body for a sudden burst of energy demands. In the animal world, we often think of this in terms of evading a predator, or at the very least, minimizing the effects of injury so that the individual who runs away today can live to run away another day! Thus, it makes sense that there is a shift away from energy storage to energy use, increased cardiovascular tone, inhibited

digestion, growth and reproduction (those can wait until the immediate emergency has abated), compromised immune and inflammatory responses, and finally, enhanced cognition and analgesia (Sapolsky, 2002). Of course, the same response occurs when a university student must defend a thesis, or when the rent cheque is due and there is no money in the bank (here we have the non-specificity of the stress response). Although these reactions are ideal to cope with the threat of being disemboweled by some very scary creature with really big teeth and claws, they can expose us to the negative effects of the very response that was designed to save us. Sapolsky (1992) includes fatigue, hypertension, peptic ulcers, impotence, impaired disease resistance and cancer, and accelerated neural degeneration during aging as some of the pathological states associated with chronic stress. These negative effects of stress occur when an individual is exposed to prolonged psychosocial stress as well. Hence, stress exposure has both good and bad consequences (Table 1).

How does stress cause these reactions? Glucocorticoids (GCs; corticosterone in most rodents, birds and reptiles; cortisol in most primates and carnivores (Stratakis & Chrousos, 1995)) are both the hero and the villain. On one hand, GCs are required to mobilize energy; on the other, too much cortisol or corticosterone results in the disruption of learning, memory and plasticity (McEwen & Sapolsky, 1995), inhibition of neurogenesis (Gould, 1994; Reagan & McEwen, 1997), atrophy of neuronal processes (Reagan & McEwen), endangerment of hippocampal neurons (Sapolsky, 1996a), neurotoxicity (McEwen, 1992; Sapolsky, 1996a), as well as the other maladaptive stress responses previously noted.



Table 1. Adaptive and maladaptive responses to stress.

<ul style="list-style-type: none"><li>• <b>Adaptive Stress Response</b><ul style="list-style-type: none"><li>– Mobilization of energy (glucose)</li><li>– Increased cardiovascular tone</li><li>– Suppression of digestion</li><li>– Suppression of growth</li><li>– Suppression of immune system</li><li>– Sharpening of cognition</li></ul></li></ul>	<ul style="list-style-type: none"><li>• <b>Stress Related Disorder</b><ul style="list-style-type: none"><li>– Myopathy, fatigue, diabetes</li><li>– Stress-induced hypertension</li><li>– Ulceration, colitis</li><li>– Psychogenic dwarfism</li><li>– Amenorrhea, impotency, loss of libido</li><li>– Increased disease risk</li></ul></li></ul>
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(Robert Sapolsky, personal communication, May 2004)

So what does this have to do with play?

Play behavior is a common occurrence in the young of many animals, including humans (Fagen, 1981). A frequently reported observation is that play only occurs in a *relaxed* field, when the organism is *free from stress*, healthy, well fed, and when all the basic needs are met (Burghardt, 2001; Poole & Fish, 1975; Vanderschuren, Niesink, Spruijt, & van Ree, 1995). Indeed, cortisol levels have been found to be inversely related to the amount of play in squirrel monkeys (Biben & Champoux, 1999). Similarly, it has been reported that if playing youngsters are disturbed by unpleasant events, the play ceases. For example, two juvenile baboons playing together will immediately cease when two neighboring adults engage in a serious altercation (Fagen, 1981). Arnold and Siviy (2002) claim that the most commonly reported consequence associated with stressors is a reduction in playfulness. When rats were exposed to a predatory odor (e.g. a cat), play was abolished and remained suppressed for a number of days (Sivy, Harrison, & McGregor, 2006). Biben and Champoux (1999) suggest that playfulness can be an indicator of psychosocial well-being in captive animals. In a more naturalistic setting, the presence of play has been interpreted as a means of signaling vigor as well as the health status in the organism. Courtship is one of the more reliable places to find playful behavior in adult humans and animals (Fagen; Pellis & Iwaniuk, 1999). From such observations, a general consensus has emerged among researchers that stress is antithetical to play. That is, animals do not play when stressed.

There are hints, however, that low to moderate levels of stress may in fact promote the occurrence of play. Biben and Champoux (1999) claim that it is not uncommon to encounter youngsters who persist in playing even under the most impoverished and stressful conditions. Reports of rhesus monkeys continuing to play despite 6 of 69 study animals starving to death indicate that play is very difficult to extinguish completely (Loy, 1970), although play behavior was reduced when obtaining food became difficult (Baldwin and Baldwin, 1976). Brief stressors may have a stimulating effect on the occurrence of play, perhaps by introducing novelty (Moodie & Chamove, 1990). There are a variety of instances whereby novelty initially suppressed play, but subsequently enhanced this behavior. When polecats were introduced to a new situation or presented with a novel object, play initially subsided while the situation or object was carefully investigated, then rebounded in terms of aggressive play directed towards one another (Poole, 1966). Redican and Mitchell (1974) observed that environmental changes, such as noise, and brief within-dyad aggression in rhesus monkeys can terminate an on-going play session, but these stimuli often preceded play. When black-tailed deer were presented with a novel object such as a piece of newspaper lying on the ground, they approached it cautiously, sniffed it, and then jumped away. This was followed by playful activity (Müller-Schwartz, 1968). It has even been proposed that some behaviors such as play may reduce stress or act as a coping mechanism, serving to reduce physiological distress and leading to a more rapid return to physiological equilibrium (Coe, Franklin, Smith, & Levine, 1987). Von Frijtag, Schot, van den Bos, and Spruijt (2002) found that socially reared rats living with an aggressive partner demonstrated increased social grooming and play behavior. This implies that the rats may well be using some kind of

play-related coping mechanism. Conversely, Crepeau (1989) concluded that circulating CORT does not exert specific effects on levels of play.

There are many incidences in the animal world in which play ceases when stress is present; however, a number of studies indicate that a moderately stressful state and play can, at times, co-occur. It is not clear why this would happen, and many would consider it counter-intuitive. This situation is unmistakably in opposition to classical play theories. Some have proposed that playing may actually serve as a type coping mechanism. It is clear that the relationship between stress and play is largely ambiguous. This thesis will explore the well-known but poorly understood phenomena of play, and how it relates to stress.

A recognized feature of play is called, “play rebound”, whereby after rats are socially isolated, there is a large increase in play fighting (Neisink & van Ree, 1982; Panksepp & Beatty, 1980; Panksepp, 1981; Panksepp, Siviy & Normansell, 1984). This is true of many other species including humans (Smith & Hagan, 1980). When social partners are reintroduced after periods of separation, the amount of their play increases in direct relation to the number of hours they had been separated, reaching a peak following twenty-four hours isolation (Pellis, Field, Smith & Pellis, 1997). Longer isolation periods may result in some of the previously mentioned stress-related adult disorders. It has been found that this consequence of short-term separation indicates that there is something special about play experience itself that causes the increase in play behavior (Hole, 1991). Varlinskaya, Spear, and Spear (1999) suggest that play and the motivation

for social contact may have different underlying mechanisms. There is support for the notion that social behaviors related and unrelated to play are differentially regulated (Vanderschuren, Niesink, & van Ree, 1997). If juvenile rats are housed with an adult partner or a peer that has been made unresponsive to play-initiation, there is an increase in play similar to that which occurs when there is complete isolation (Panksepp et al., 1984). When deprived of whole body contact (the predominant feature of rough and tumble play), the rats behaved as though they had been in total isolation; that is, there was a play rebound effect (Hole, 1991). It was suggested that this effect was due to the lack of some factor obtainable only in social play, and not in non-playful social interaction. In a similar experiment, Holloway and Suter (2004) compared two groups of adolescent rats: one group consisted of pairs which were housed together; the second group was treated in the identical manner but had wire mesh separating them, which allowed for social contact but denied play (PD). When the PD pairs were reunited, there was a significant increase in play compared to the non-play deprived group. Another factor that could account for the increase in play after social isolation is the fact that motor activity is also reduced (Holloway & Suter, 2004). Pellis and McKenna (1995) demonstrated that reciprocity is an important component in playful activity. When a play partner was treated with scopolamine (a drug known to cause a decrease in play fighting), both playful attack (e.g., nape contact) and playful defense (e.g., avoidance of nape contact) decreased. Not only did the scopolamine-treated rats not respond to playful attacks, they tended to actively avoid social contact. It was suggested that, in order to find playful contact rewarding, the recipient needs to defend itself. This supports the notion that a lack of play coincides with lower motor activity levels. Conversely, many studies

indicate that isolation-reared rats show hyperactivity in a novel environment (see Arakawa, 2003; see Harkin, Andrew, Connor, O'Donnell, & Kelly, 2002; Parker & Morinan, 1986). Other results, however, indicate that isolated rats become less active than socially reared rats (File, 1978; Holson, 1986). Arakawa (2003) suggests that these discrepancies may be due to the rat's developmental stage. For example, rats which were isolated only during the juvenile stage were found to exhibit decreased locomotor activity in a novel environment (File, 1978; Parker and Morinan, 1986). When juvenile squirrel monkeys were separated for a 30-min period, exposure to a novel room resulted in decreased locomotor activity as well as increased cortisol (Hennessy, Mendoza, & Kaplan, 1982). Weiss, Pryce, Jongen-Relo, Nanz-Bahr, and Feldon (2004) found that when both males and females were tested in the open field after social isolation, there was no effect on locomotor activity. Therefore, it cannot be concluded with any certainty that the resultant increase in play following social isolation is due to a generalized "hyperactivity". Again, this points to the notion that play after isolation is a unique phenomenon that is most likely not a result of a need for locomotor activity, nor social contact.

A "play rebound" effect seems counterintuitive as many consider social isolation to be stressful for rats (File & Vellucci, 1979; Greco, Gambardella, Sticchi, D'Aponte, & De Franciscis, 1990; Hennessey & Weinberg, 1990; Lovely, Pagano, & Paolino, 1972; Niesink & van Ree, 1983a; Niesink & van Ree, 1983b; Parker & Morinan, 1986; Wilson, 2001). Arakawa (2003) claims that isolation rearing is considered ecologically unnatural for rats, and hence this provides an animal model of chronic stress. Other studies indicate

that separation has no effect on stress (Amario, Luna, & Balasch, 1983; Cirulli, Terranova, & Laviola, 1996; File & Peet, 1980; Giralt & Amario, 1989; Holson, Scallet, Ali, & Turner, 1991; Terranova, Cirulli, & Laviola, 1999). However, the majority of studies on the neuroendocrine effects of social isolation have been done on mature rats (Hall, 1998). It can be stated safely that under some circumstances, adult rats show stress responses resulting from social isolation. Adolescent rats have proven to have behavioral anomalies, hyperactivity, and unusual pharmacological responses (Thor & Holloway, 1984a), which may account for these apparent discrepancies in the literature regarding separation stress responses. Spear (2000) claims that adolescent rats may differ behaviorally and physiologically in the way they respond to stressors when compared to animals at other ages. It is therefore possible that the immature rat responds to stress in a unique way. It is known that play frequency peaks between the ages of 30-40 days postnatally; that is, the period following weaning and preceding sexual maturity, exhibiting an inverted-U-shaped pattern of play across age (Panksepp, 1981; Thor & Holloway, 1984b). As previously stated, twenty-four hours social isolation induces a reliable increase in the incidence of social play (Panksepp & Beatty, 1980; Varlinskaya, et al., 1999). According to current play theories, if adolescent rats are truly stressed during this time frame, they should not play. If indeed the juveniles *are* stressed, it is posited that the increase in stress either causes the elevation of play behavior or, more radically, that play is may be used to reduce stress.

## CHAPTER 2

### EMPIRICAL STUDIES

#### Experiment 1

##### Social Isolation and Corticosterone Levels

#### INTRODUCTION

An often used play paradigm used to reliably increase play fighting in juvenile rats is to socially isolate pairs for twenty-four hours, and then re-unite the play partners (Pellis & Pellis, 1990; Smith, Forgie, & Pellis, 1998; Reinhart, Pellis, & McIntyre, 2004). Given the amount of research indicating that isolation is stressful, an increase in play is not a predictable response given current play theories' stance that play occurs only when there is an *absence of stress*. Juvenile rats differ markedly from slightly younger and older animals behaviorally and pharmacologically (Cirulli, et al., 1996; Spear, 2000). The processes that underlie these differences have yet to be clarified. It is possible that juvenile rats play more post-isolation because they do not have the same stress response as adults. This possibility needs further investigation.

The secretion of glucocorticoids (GCs) is a classic endocrine response to stress (Nelson, 2000; Selye, 1950; Sapolsky, 2002; Sapolsky, 2000; Vermetten, 2002). GCs are



represented in most rodents, birds and reptiles as corticosterone, and cortisol in most primates and carnivores (Stratakis & Chrousos, 1995). Measuring plasma corticosterone concentrations as a method of evaluating the stress response is well-documented (Cirulli et al., 1996; File & Vellucci, 1978; Gentsch, Lichtsteiner, & Feer, 1981; Hennessey & Levine, 1978; Holson, et al., 1990; Lovely et al., 1972; Weiss et al., 2004; Wilson, 2001). File and Peet (1980) determined that maximal corticosterone responses are easily reached and that a graded response will only be found with relatively mild stressors such as novelty, bright light, and the presence or absence of conspecifics. It was therefore determined that, for this experiment, plasma corticosterone levels will be utilized as a measure of stress. If indeed juvenile rats have a similar stress response to that of adult rats, we would predict that serum corticosterone levels would be higher in socially isolated rats compared to socially housed individuals.

## MATERIALS AND METHODS

### Subjects

The subjects were 24 Long-Evans male rats that were born and raised in the Canadian Centre for Behavioral Neuroscience (CCBN) rat colony at the University of Lethbridge. All animals were maintained on a 12h/12h light/dark cycle (light on at 0730 hrs), and kept at a constant room temperature of 21-23C. Water and food (Purina Rodent Chow) were available ad libitum. They were housed in 46 x 25 x 20 cm high standard

polyethylene tubs with corn cob bedding, until weaning at 20 days. Experimentation conformed to Canadian Council of Animal Care (CCAC) guidelines.

### Procedure

At 26-29 days of age (depending on day of birth), 24 Long-Evans male rats from four litters were removed to a separate room and placed in pairs of similar weight in 46 x 25 x 20 cm polyethylene tubs. A radio turned to low volume was used for background noise. One of each pair was handled daily and taken to the testing room where they were exposed to the open field for five minutes. The habituation was repeated for four consecutive days.

On the fifth day, one rat from each of the six pairs was removed leaving six habituated, isolated rats, along with the six remaining pairs of control rats. According to the classic play paradigm which is used to reliably increase play fighting in juvenile rats, pairs are socially isolated for twenty-four hours, and then re-united (Pellis & Pellis, 1990). Rather than re-pairing the rats after twenty four hours elapsed, one rat from each pair of control rats and each of the isolates was tested in an open field apparatus to expose them to an environment similar to that of a play box in keeping with the traditional play paradigm, as well as to measure locomotor activity. It has been suggested that exposure to a novel environment is mildly stressful, which may result in either no effect or decreased locomotor activity. As the animals had been habituated prior to testing, the open field apparatus can no longer be considered a “novel environment” (File & Peet, 1980);

however, the social isolation prior to the testing could be stressful. If the separation of the animals results in higher CORT levels and no change in locomotor activity, this would further support the theory that play is a unique entity not related to a need for physical activity. Testing occurred in a separate room in the presence of the experimenter. Each animal was placed individually in a Digiscan animal activity monitor (42 x 42 x 31 cm high) for 10 minutes. Infrared motion detectors located along the sides of the box recorded the distance traveled (cm), number of vertical movements, time spent in the center, and time spent in the margins, and time resting. Movements were recorded in two, five minute intervals.

Immediately upon cessation of each trial, individual rats were decapitated in an adjacent room. Blood was collected in 1 ml tubes, and plasma was obtained by centrifugation at 5000 rpm for 5 minutes. The sample was stored at -20C. Plasma CORT concentrations were determined by radioimmunoassay using commercial kits (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA).

## STATISTICAL ANALYSIS

The data were analyzed using the non-parametric one-tailed Mann-Whitney *U* test because homogeneity of variance and normality of the distribution were occasionally violated for some behavioral measures in later experiments. Hence, for consistency of comparison, nonparametric methods were used in all subsequent statistical analyses.

To determine if individual open-field behavior was related to individual levels of CORT, the control and experimental animals were combined for evaluation using Pearson's  $r$  correlation.

## RESULTS AND DISCUSSION

There were no significant differences between socially isolated and paired rats in the open field test for any of the parameters measured in the test apparatus ( $p > 0.05$ ), but there was a significantly higher serum corticosterone level in the isolated compared to the paired group ( $U = 0$  (6,6),  $p < 0.005$ ) (Figure 2.1). There were no significant correlations between any of the activity measures and CORT level ( $p > 0.05$ ) (Table 2).

The elevated CORT found in the isolate group is consistent with the rats having undergone stress in the preceding time frame, thus indicating that the stress response systems for juveniles and adults do not differ. It is known that rats play more after deprivation, and we now know that juvenile rats are stressed after social isolation. These events suggest that the rats are playing when they are stressed, which is inconsistent with current play theories. There are two obvious hypotheses which can account for this occurrence: one is that increased stress induces play; the second is that social isolation independently induces stress and play (Figure 2.2).

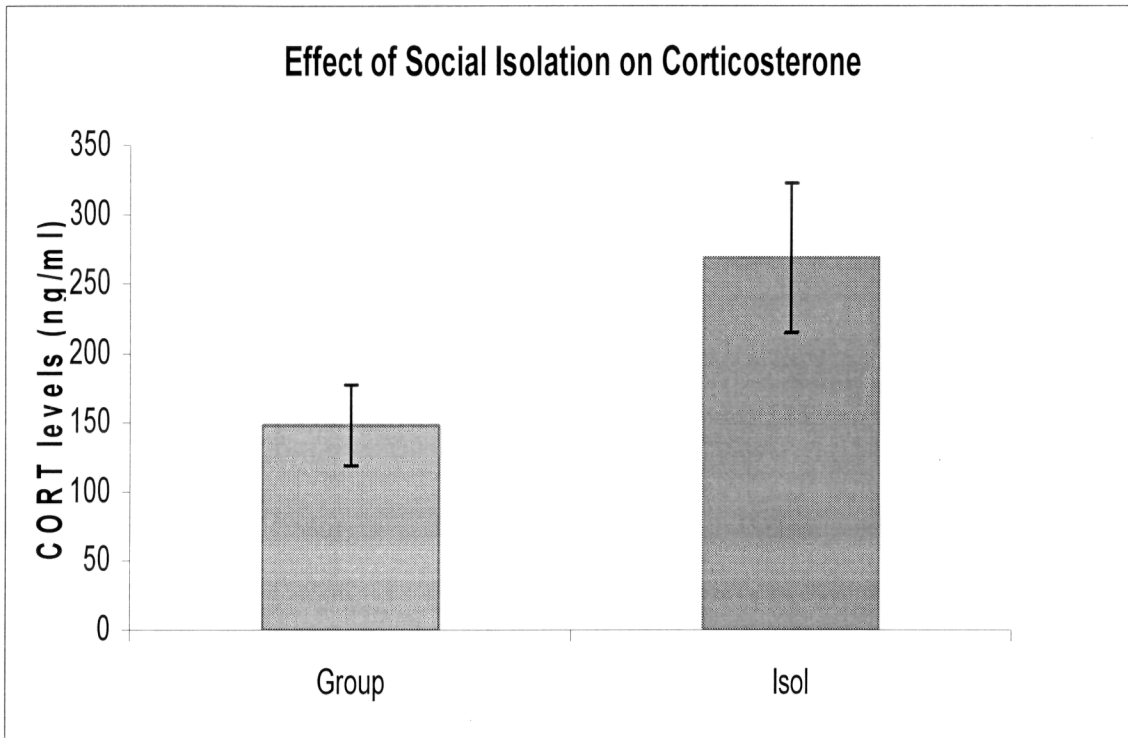
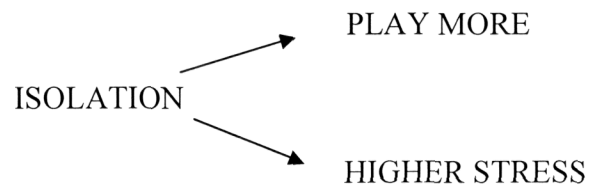


Figure 2.1. Male juvenile rats which are socially isolated for twenty-four hours have significantly higher levels of plasma CORT than rats which remained with a social partner, which indicates higher levels of stress in the isolates (\* $U = 0(6,6)$ ,  $p < 0.005$ ).

Table 2. There were no significant correlations between CORT levels and locomotor activity (\*2-tailed).

<b>FACTOR</b>	<b>r</b>	<b>Sig*</b>
Distance	-0.314	0.296
Rest	0.147	0.630
Vertical	-0.190	0.534
Margin	0.075	0.808
Center	-0.083	0.788



OR:



Figure 2.2. There are two possible hypotheses that could explain why play and stress co-occur.

The second instance in Figure 2.2 infers there is a causal relationship between stress and play. It would be prudent to determine whether or not it is the absence of play per se that is causing the increase in that behavior (Hole, 1991; Holloway and Suter, 2004) as opposed to the stress inducing play.

## **Experiment 2**

### The Effect of ACTH on Play

#### INTRODUCTION

As mentioned in the previous section, there is more than one possibility to explain the co-occurrence of stress and play. It has been suggested that play is motivationally distinct, and when the opportunity to play is removed, there is a need to restore the balance by playing until satiated (Panksepp & Beatty, 1980). It is therefore possible that play and stress can co-occur via differing neurobiological substrates. This experiment will determine if there is a causal relationship between increased stress and play.

To understand the logic of this experiment, it is necessary to consider what happens during the stress response. This is often characterized as a cascade of events, and is surprisingly complex. A simplified version is presented here.



There are basically two co-occurring series of happenings that begin during the onset of perceived stress: the sympathetic nervous system stimulates the adrenal medulla to secrete norepinephrine; within minutes, the adrenal cortex secretes glucocorticoids. Other hormones are secreted from various endocrine organs. These include prolactin, corticotropic releasing hormone (CRH; also known as corticotropic releasing factor, or CRF), adrenocorticotrophic hormone (ACTH) and vasopressin (Nelson, 2000). Because the hypothalamus, pituitary, and adrenal glands are the primary organs activated, this system is often referred to as the HPA axis. A simplified version of this scheme is illustrated in Figure 2.3. Perceived stress results in the activation of the sympathetic nervous system, releasing norepinephrine from most of its nerve endings, and epinephrine from the adrenal medulla. At the same time, the hypothalamus releases CRH which stimulates the release of ACTH from the anterior pituitary. ACTH then causes the release of the glucocorticoids from the adrenal cortex. The pituitary also discharges vasopressin and prolactin. There are different types of corticosteroid receptors in various sites in the brain, most notably in the hippocampus. When the amygdala has perceived danger, a resultant increase in steroid hormones (CORT) occurs via CRH and ACTH. The CORT moves freely through the blood and binds to specific receptors in areas of the hippocampus and amygdala, as well as other regions. Jacobson and Sapolsky (1991) suggest the most potential feedback sites include the hypothalamus and pituitary. After this “binding” has occurred, the hippocampus sends messages to the pituitary and adrenal glands, via the hypothalamus, to slow down the release of the glucocorticoids. As long as the amygdala still perceives danger, it keeps promoting the release of the steroids. Thus, the concentration of the stress hormone in the blood is regulated through this “negative

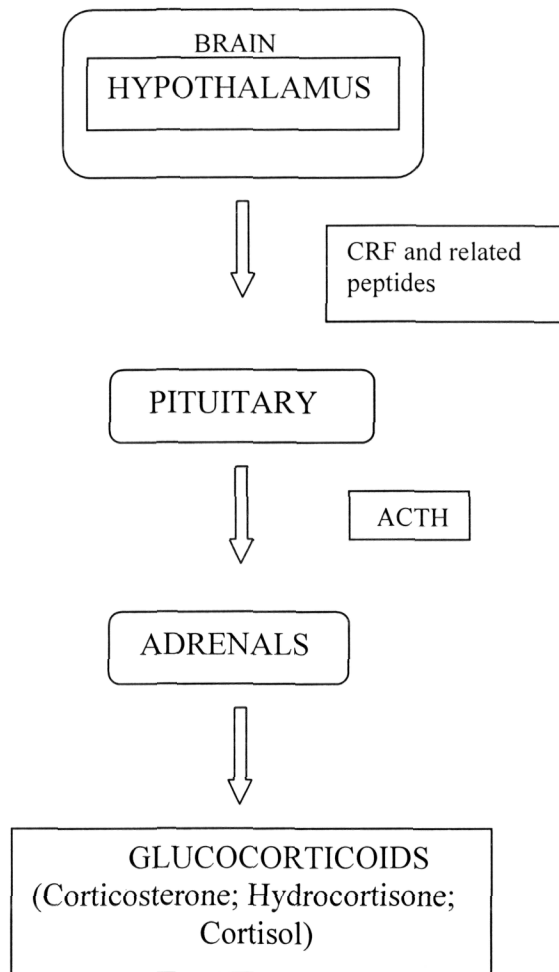


Figure 2.3. The brain perceives stress, which stimulates the hypothalamus to release CRF and other hormones, which then promote the release of ACTH from the pituitary gland. ACTH acts on the adrenals to stimulate the release of glucocorticoids.

(Robert. Sapolsky, personal communication, May 2004)

feedback system”. This is critical for understanding how chronic stress negatively affects our brain, as it is the exposure to GCs that causes all the stress-related maladies in our nervous system.

Most stress-related research has focused on administering CORT to mimic the stress response, and hence, related behaviors. However, Meaney and Stewart (1983) showed that male pups injected daily with CORT did not differ in their play-fighting from controls. Similarly, Gregus, Wintink, Davis, and Kalynchuk (2005) state that the behavioral effects of repeated high levels of CORT injections are not clear. As ACTH stimulates the release of CORT (see Figure 2.3), it is logical to examine its effects on behavior. File and Vellucci (1978) explored the possibility that physiological doses of ACTH might have an anxiogenic effect. They used the animal model of anxiety which was developed by File and Hyde (1977), whereby the time spent by pairs of male rats in active social interaction (such as sniffing, following, or grooming the partner) is measured under various test conditions. An increase in social interaction, without a concomitant increase in motor activity, is indicative of an anxiolytic effect, whereas a specific decrease in social interaction indicates an anxiogenic effect (File & Seth, 2003). It was found that ACTH, when administered to mimic the plasma corticosterone concentration that would be present during moderate to severe stress (Hodges & Mitchley, 1970), had clear behavioral effects. ACTH significantly reduces social interaction in pairs of male rats, but there was no evidence of a change in level of motor activity or that ACTH had a sedative action (File and Vellucci, 1978). The testing indicated that there would be higher CORT concentrations 15-25 minutes after injection,

but at that time the effect of the social interaction was not significant. The reduction in social interaction was most marked 3 minutes after ACTH injection, and was still significant 15 min after injection but no longer significant at 30 min. The results were similar when using a higher dose of ACTH, but not as consistent.

If the stress that is induced by social isolation is having a direct effect on play, then increased stress without social isolation should also increase play. It is therefore predicted that rats injected with ACTH should play more.

## MATERIALS AND METHODS

### Subjects

The subjects were 24 Long-Evans male rats that were born and raised in the Canadian Centre for Behavioral Neuroscience (CCBN) rat colony at the University of Lethbridge. The same protocol was used as in Experiment 1.

### Procedure

At 25-26 days of age (depending on day of birth), 24 Long-Evans male rats from three litters were removed to a separate room and placed in pairs of similar weight in 46 x 25 x

20 cm polyethylene tubs. A radio turned to low volume was used for background noise. The position of the cages in the rack was changed daily to equate experience of different levels of illumination.

Each pair was given 30-min habituation periods in the test enclosure (50 x 50 x 32 cm), commencing between 0900 and 1000 hr., for four days. 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.

On Day 5 (PND 30-31), all 12 pairs were weighed. ACTH (Tetracosactide, Sigma-Aldrich) was dissolved in 0.9% saline to a concentration of 0.025 mg/ml. The doses of ACTH were chosen to mimic the plasma corticosterone concentration that would be present during moderate to severe stress (Hodges & Mitchley, 1970). Six pairs received interperitoneal injections of 5 µg/100 g ACTH per rat. Controls received saline injections the same volume as the ACTH solution. Testing occurred in a random order between 0900 and 1200 h, during which time there is minimal fluctuation in endogenous corticosterone.

As noted above, File & Vellucci (1978) demonstrated that the behavioral effects of ACTH were maximal if the test was conducted in the period 3-13 min after the injection, hence test periods commenced within 3 min of injection and continued for 12 minutes. Social interaction is highest when rats are tested in a familiar arena lit by low light (File

& Seth, 2003); therefore, the sessions 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 Analysis

After collecting the behavioral data, each 12-min test period was scored for various components of play. The 8-mm tapes were converted to VHS format with the addition of a time code (30<sup>th</sup> of a s), which allowed for slow-motion and frame-by-frame inspection. Each of the following measures of play fighting was quantified for all animals (for more detailed description, see Pellis, Pellis, & Whishaw, 1992):

1. *Frequency of attack*: the number of playful initiations (i.e., nape contacts) per 12-min period
2. *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 12-minute period
3. *Type of defense*: (a) *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, (b) *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, and (c) *Probability of partial rotation*-

number of facing defences whereby the defender turns to face the attacker by rearing on the hindlegs relative to number of total defenses.

## RESULTS AND DISCUSSION

There was significantly more play occurring in the ACTH group, but this increased playfulness was not accompanied by any significant differences in the patterns of defense used in play (Figure 2.4 and Table 3).

Pellis and Pellis (1990, 1997) have demonstrated that rat play increases approximately 2 – 2 ½ times that of base levels after 24 hr social isolation. After ACTH injection and NO isolation, the magnitude of play increased similarly to that seen with social isolation. Therefore, the inference is that stress has a direct causal relationship with play. This conflicts unequivocally with the notion that play occurs only in a relaxed field.

Previous discussions have implicated social contact as being part of the experience of play, and that said deprivation may cause some of the stress associated with isolation preceding the play experience. This possibility needs to be further assessed.

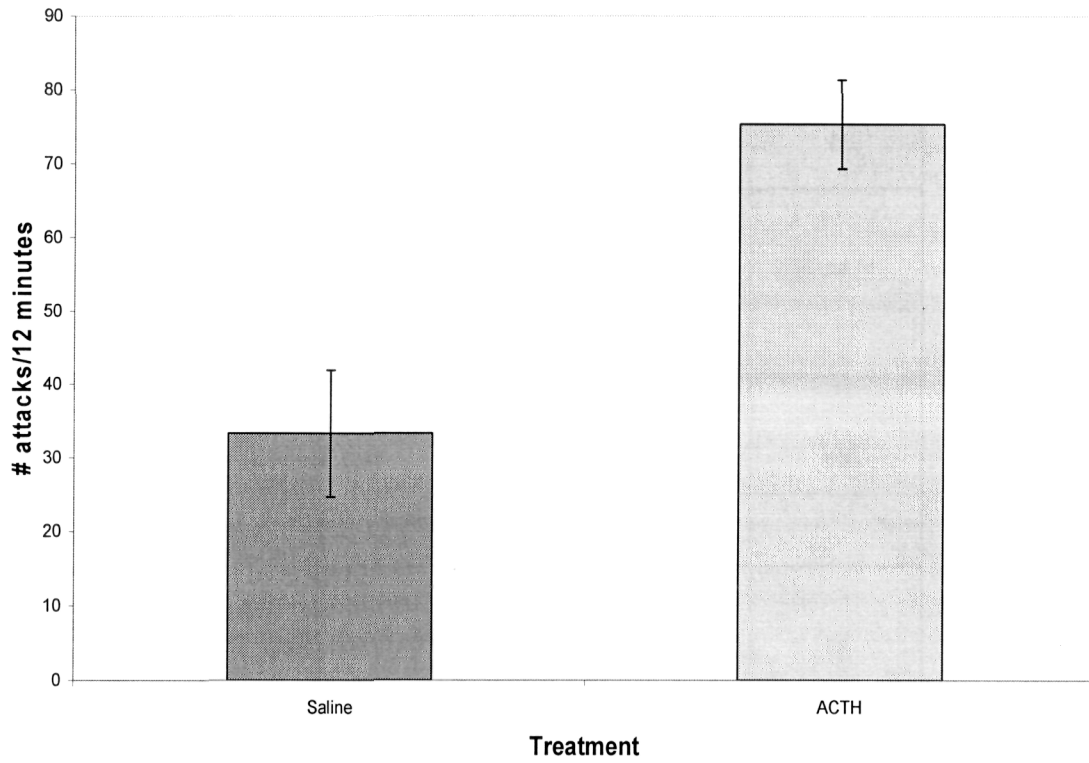


Figure 2.4. There was significantly more play fighting in the group that received ACTH than in the control group (\* $U = 0(6,6)$ ,  $p < 0.005$ ).



Table 3. There were no differences in any of the play content in the ACTH group compared with the control group.

	MEAN ± STANDARD		<i>U</i>
	ERROR		
	<b>SALINE</b>	<b>ACTH</b>	
Probability of defense	89.54±5.26	94.62±1.53	ns
Evasion (%)	8.30±3.44	10.21±2.42	ns
Complete rotation (%)	60.28±10.42	56.75±5.67	ns
Partial rotation (%)	18.34±4.74	10.95±1.76	ns
Other (%)	13.02±3.55	22.13±4.89	ns

### **Experiment 3**

#### Play and Social Contact

#### INTRODUCTION

It has been noted that social isolation accompanies play deprivation, and that these two are often behaviorally confounded. It is therefore difficult to ascertain what function each condition is responsible for. Holloway and Suter (2004) acknowledge that isolation eliminates social experiences other than play, increases stress, alters sensory stimulation, and may reduce motor activity. Temperature regulation may also be impacted as the animals have no opportunity to huddle. Wilson (2001) affirms that negative social situations increase stress, whereas positive social situations reduce stress (as measured by levels of cortisol/corticosterone). When adult female and male squirrel monkeys were in an established social group, they had lower levels of cortisol than animals that were individually housed (Lyons, Ha & Levine, 1995). Also, adult male Siberian dwarf hamsters housed with a female or a female and pups were found to have lower levels of prolactin (a hormone moderated by corticosterone) than have males housed alone (Castro & Matt, 1997). Wilson (2001) posits that the psychological stress associated with stressors is reduced by the presence of a social partner. It is possible, therefore, that the stress experienced by play deprivation is not specifically that of a lack of play, but at least partially due to social isolation.

Varlinskaya et al., (1999) suggest that play and social behaviors unrelated to play may be mediated by different neural systems, indicating that there is something distinct about play as opposed to merely being in contact with a social partner. Indeed, short term social isolation of juvenile rats leads to a specific increase in social play, rather than a general increase in social behavior following reintroduction (Panksepp, 1981; Panksepp & Beatty, 1980). Holloway and Suter (2004) found that play rebound occurred even in the absence of social deprivation. Play-deprived subjects were housed in identical cages that had been divided into two equal-sized areas by wire mesh which allowed for some physical contact between cagemates, but did not permit rough and tumble play. When reunited, this group of animals exhibited far more play behavior than the subjects that were not play deprived. Hole (1991) conducted a similar experiment, however he compared three groups: control group (members had free access to each other); restricted physical access group (members of a pair were separated by a wire partition, allowing for visual, tactual, and olfactory contact, grooming and to some extent, huddling); no physical access group (identical to the Restricted group except the partition was made of transparent plastic rather than wire). There were no significant differences between the Restricted and No Access groups, and both conditions showed a marked rise in the amount of play when the deprived animals were again allowed to interact socially. It has been demonstrated that animals which have been play deprived (hence socially deprived) have higher stress levels than those which have a social partner. If it is the play component which is causing the lower levels of stress, we should see lower levels of CORT in the animals which are allowed to play than those which are allowed social

contact and no play. That is, the playing reduces the stress of having been socially isolated.

## MATERIALS AND METHODS

### Subjects

The protocol is the same as in the first two experiments.

### Procedure

At 25-26 days of age (depending on day of birth), 24 Long-Evans male rats were removed to a separate room and placed in pairs of similar weight in 46 x 25 x 20 cm polyethylene tubs. A radio turned to low volume was used for background noise. The position of the cages in the rack was changed daily to equate experience of different levels of illumination.

Each pair in the “Play” (P) and “Social Contact” (SC) groups was given 30-min habituation periods in the test enclosure (50 x 50 x 32 cm for the P group, 35 x 29.5 x 15 cm for the SC group), commencing between 0900 and 1000 hr., for four days. On Day 5, one rat from each of the six pairs was removed leaving six isolated rats in the P group, along with the six isolated rats in the SC group. After twenty four hours elapsed, the pairs of rats were reunited with their original partner, the P group pairs in the “Play”

enclosure, and the SC pairs in the “No Play” enclosure. The “No Play” enclosure was equipped with a plastic partition whereby the animals could smell, hear, huddle, and see each other, but were prevented from playing. The testing sessions began between 0830 and 0900 hr and proceeded for three hours. Each session lasted for 30 minutes. It is known that the levels of ACTH affect behavior relatively quickly and return to normal rapidly, whereas corticosterone levels increase more slowly and remain high for a longer duration (Kant, Bauman, Anderson and Mougey, 1992). It is therefore important to allow enough time to elapse prior to testing to ensure accurate reflection of CORT levels.

However, depending on the type and duration of stress, CORT levels can remain elevated for a number of days (Kant et al., 1992). It is also known that maximal corticosterone response is easily reached (that is, maximal binding has occurred in the hippocampus), but graded responses are possible with relatively mild stressors (File & Peet, 1980).

Because the social isolation is considered to be a mild stressor, we would expect maximal CORT responses to be reached within a 30-min time frame. Weiss et al. (2004) found that CORT levels were returning to baseline by 60-min post-stressor, and were almost completely normal by 120-min post-stressor. Some animals had reached levels that were below basal levels after 1 h. It is thus necessary to choose a time frame whereby both treatments can affect CORT levels that accurately reflect the stress experienced, but also prior to CORT levels returning to baseline. Hence, a 30-min testing period was chosen.

Immediately after the 30-min test session, one rat from each pair was decapitated in an adjacent room. Blood was collected in 1 ml tubes, and plasma was obtained by centrifugation at 5000 rpm for 5 minutes. The sample was stored at -20C. Plasma CORT

concentrations were determined by radioimmunoassay using commercial kits (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA).

## RESULTS AND DISCUSSION

The rats which were allowed to play had significantly lower corticosterone levels, and so presumably were less stressed than those allowed to have social contact but no play.

(Figure 2.5)

It has been seen that social isolation in juvenile male rats increases stress which results in increased play. This experiment demonstrates that stress levels are reduced as a *result* of having played, and that the buffering due to social contact is not adequate to lower CORT. These results support the notion that it is *not* the social contact which causes a reduction in stress, but there is something distinct about play that is socially rewarding. This is consistent with results found by Hole (1991) and Holloway and Suter (2004) which both determined that play rebound occurred despite having social contact, but no opportunity to play. It was noted that the social deprivation also decreased the overall level of physical activity (Holloway & Suter, 2004). Furthermore, play is typically a vigorous activity involving repeated bouts of wrestling and chasing (Poole & Fish, 1975), and so can provide considerable exercise. It is therefore possible that the reduced levels of CORT in the rats that played versus those that didn't was due to the physical exercise experienced by the former. That is, the play has its stress-reducing effects via

### The Effect of Play vs. Social Contact on Corticosterone

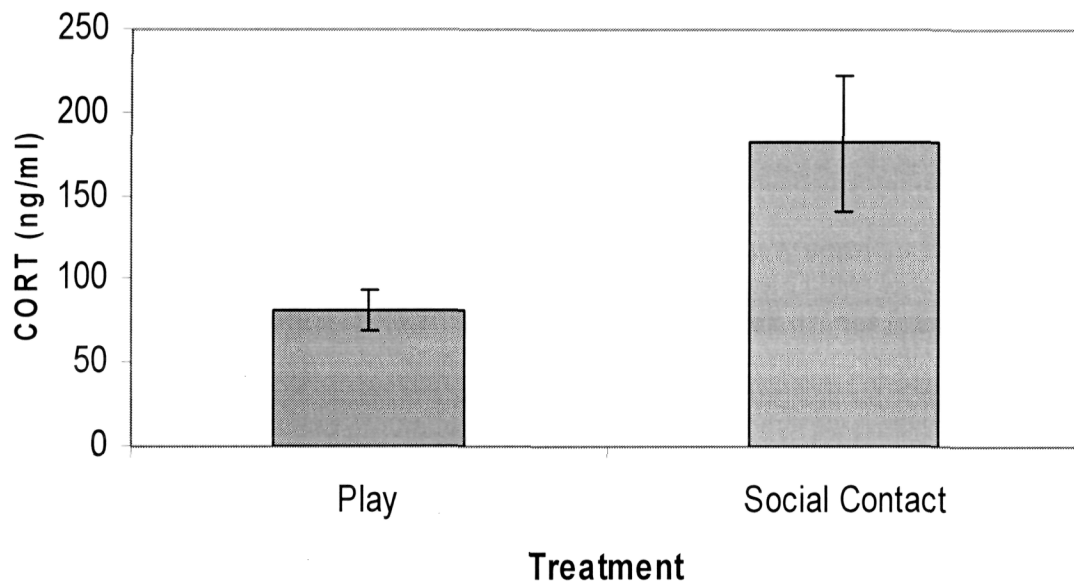


Figure 2.5. Male juvenile rats which were isolated for twenty-four hours, then reunited with their play partner had significantly lower levels of serum CORT than those allowed social contact, but no play (\* $U = 6(6,6)$ ,  $p < 0.005$ ).

exercise, and so not due to some other properties of play. If the stress-reducing benefits accrue from exercise, we would expect to see lower levels of CORT in animals that are allowed to exercise, but not play.

## **Experiment 4**

### Play and Exercise

#### INTRODUCTION

There is abundant literature about the benefits of exercise. Many consider exercise to be a great stress-relief and often speak of going to the gym to work off our frustrations. Certainly, the benefits of exercise are well-documented. Binder, Droste, Ohl, and Reul (2004) determined that anxiety-related behaviors in mice were reduced with the opportunity to exercise. Mice that were previously allowed to exercise presented a decreased corticosterone response to novelty (Droste, Gesing, Ulbricht, Muller, Linthorst, & Ruel, 2003). Cotman and Berchtold (2002) claim that exercise is believed to be a behavioral strategy used to relieve stress, and can reduce depression and anxiety. It has been shown that, in gerbil forebrain ischemia, there is reduced mortality and brain damage after locomotor activity (see Cotman & Berchtold, 2002). Voluntary exercise can increase resistance to brain insult of different etiologies, and improve learning and mental performance (Cotman & Berchtold, 2002). As we have seen, increased glucocorticoids (CORT) can result in a worse outcome for brain injury, as well as negatively impact learning and memory. Traustadóttir, Bosch, and Matt (2005) found that, in unfit women,



aging is associated with greater HPA axis reactivity to psychological stress, and that higher aerobic fitness among older women can attenuate these age-related changes as indicated by a blunted cortisol response to psychological stress. It seems logical, therefore, that the relationship between exercise and stress be further explored. If it is the exercise component of play that is reducing stress levels, we should see a lower level of corticosterone in the group that exercised but did not play. If, however, play is responsible for reducing stress rather than exercise per se, then we should see no differences between the groups.

## MATERIALS AND METHODS

### Subjects

The protocol is the same as in the first three experiments.

### Procedure

At 25-26 days of age (depending on day of birth), 24 Long-Evans male rats were removed to a separate room and placed in pairs of similar weight in 46 x 25 x 20 cm polyethylene tubs. A radio turned to low volume was used for background noise. The position of the cages in the rack was changed daily to equate experience of different levels of illumination.

Each pair in the “No Exercise” (NE) and “Exercise” (E) groups was given 30-min habituation periods in the test enclosure (45 x 45 x 45 cm for the NE group, 40 x 34 x 29 cm for the E group), commencing between 0900 and 1000 hr., for four days. In the “Exercise” condition, a running wheel was provided. The running wheel was 17 cm in diameter, metal, and free-standing. By the end of the habituation trials, the rats from the E groups ran in the running wheel for most of the 30 min test session, and similarly did so in the experimental test session. On Day 5, one rat from each of the six pairs was removed leaving six isolated rats in the NE group, along with the six isolated rats in the E group. After twenty four hours elapsed, the one rat from each condition (NE or E) was placed in the appropriate test boxes whereby the NE habituated rat was in the NE enclosure, and the E rat was placed in the E enclosure with the running wheel. The testing sessions began between 0830 and 0900 hr (when endogenous corticosteroid levels are low) and proceeded for three hours. Each session lasted for 30 minutes.

As in Experiment 3, immediately after the 30-min test session, each rat in the test condition was decapitated in an adjacent room. Blood was collected in 1 ml tubes, and plasma was obtained by centrifugation at 5000 rpm for 5 minutes. The sample was stored at -20C. Plasma CORT concentrations were determined by radioimmunoassay using commercial kits (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA).

## RESULTS AND DISCUSSION

There were no significant differences in CORT levels between the running wheel group and the no-exercise group ( $p > 0.25$ ). Adolescent male rats which are given the opportunity to exercise do not differ in stress levels from those who are not given the opportunity to exercise, thus supporting the hypothesis that it is the play component that reduces stress.

In both experimental conditions, the rats were isolated during testing. The only difference between the two groups was that one animal had access to a running wheel. It has been demonstrated that isolation is stressful for the animals, so if indeed exercise was able to reduce stress, it should be reflected in a significantly lower level of CORT. This was not the case.

From the last two experiments it has been seen that adolescent male rats that are given the opportunity to play experience less stress than those which have social contact but no play, and adolescent male rats which are able to engage in solitary exercise do not differ from those without that opportunity. Therefore, the data support the hypothesis that it is specifically the experience of play (not social contact or exercise) that reduces stress. This result is consistent with Holloway and Suter's (2004) finding that play rebound occurs only from play deprivation and not from other factors associated with isolation, despite differences in housing area.

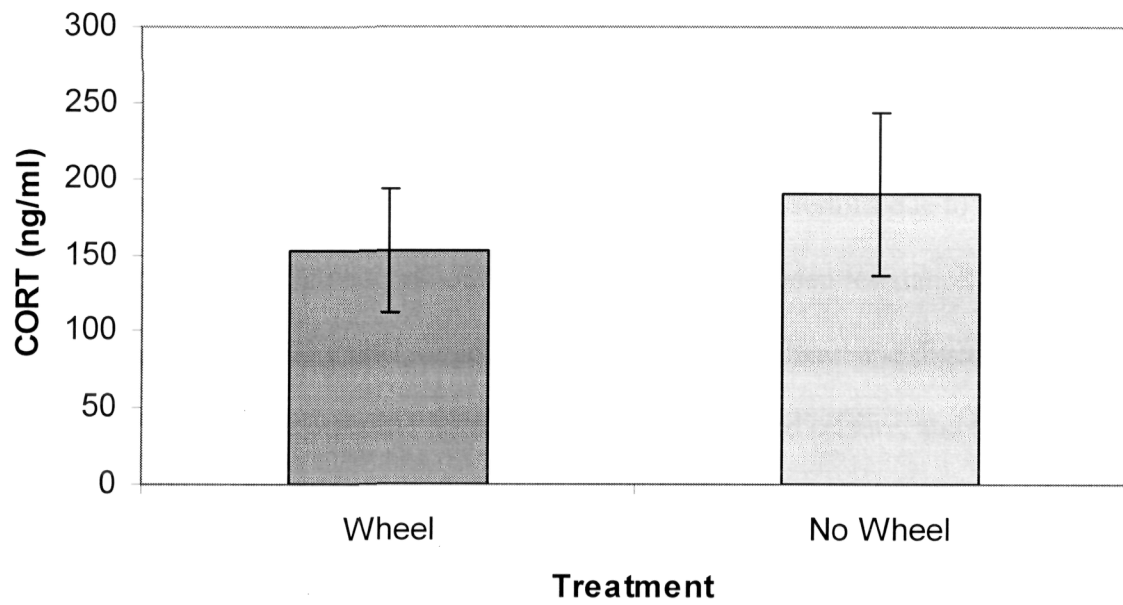


Figure 2.6. There were no significant differences in corticosterone levels between groups that had voluntary exercise using a running wheel and those that did not have a running wheel ( $p>0.251$ ).

## CHAPTER 3

### DISCUSSION

There are volumes of literature dealing with various aspects of stress, ranging from descriptions of the very basic stress response, continuing through to topics involving highly complex cellular and neuronal effects. In general, when an organism experiences a “stress response”, it is adaptive in that the main purpose is to provide a burst of energy to ensure the animal can deal with the immediate threat. The consequences of continued long-term stimulation to the stress response system can be quite serious due to the chronic elevated levels of glucocorticoids. These include decreased resistance to cancer and disease, memory impairments, impotence, increased risk of heart and digestive problems, a less favorable outcome from the effects of stroke and seizure, and the death of brain cells. Therefore, stress can be seen as either adaptive or harmful, depending on the relative level of exposure.

Play is a similarly complex topic, and many attempts have been made to describe and explain this behavior. It has proven to be a frustrating endeavor because play occurs in such a wide range of species, and often takes on many forms. In an effort to provide an inclusive definition, researchers have depicted several common features of play or conditions under which it is seen. One condition they have frequently included in these definitions is a “lack of stress” in the environment, whereby the basic needs such as food, safety and shelter, are met. One conjures up images of lions sunbathing in the Savannah, bellies bloated, flies buzzing, and youngsters frolicking near-by. Play has been observed,

however, in somewhat less idyllic environments. In fact, there are instances whereby low to moderate levels of stress enhance play. In this particular study, it was observed that social isolation for a brief period of time is stressful for male adolescent rats, yet when they are reunited, the frequency of play is enhanced. This obviously does not fit with many current play theories. It appears, then, that there is more to be explored in the relationship between stress and play.

Upon a closer review of the literature, it becomes apparent that there are many situations which could be construed as generating a mild level of stress, such as a novel environment or object, which actually induce play. Some suggest that brief stressors may have a stimulating effect on the occurrence of play. It has even been proposed that play actually serves as a means to reduce stress, or act as some form of coping mechanism.

As mentioned, male adolescent rats were indeed stressed after 24 hours of isolation. There are many instances describing an increase in play after pairs are re-united (play rebound). In this study, it was hypothesized that, if the stress of isolation had a causal relationship with play, we should be able to replicate the increase in play by “artificially” increasing the amount of stress the animal is experiencing via ACTH injection, which activates the HPA axis, resulting in increased serum CORT. Indeed, a concomitant escalation in play was seen. Therefore, a causal relationship between stress and play was demonstrated. To test the theory that play may reduce stress, play-deprived juvenile males were compared with pairs that were allowed to play. The males allowed to play had lower levels of CORT, indicating that there was something special about play

causing the reduction in stress that was separate from social contact. Given that play provides a form of exercise, and exercise has been shown to have stress-reducing properties, it was decided to compare individuals which had access to a running wheel to those which did not. It was shown previously that social isolation is stressful, so if exercise provided the stress-relief, we should have seen lower levels of CORT in that group. However, if play, rather than exercise were responsible for the decrease in stress, there should be no differences between the groups and that was, in fact, seen. The general conclusion from these experiments was that there is something unique about play that provides stress relief, and this is separate and distinct from benefits related to social contact and exercise. These findings have implications for our understanding of the functions of play.

### The Function of Play

Providing definitions of play has always been challenging. As previously indicated, even generalized descriptions are difficult due to the variety of species and behaviors expressed during playful encounters. Going one step further to determine the *function* of play is even more onerous. There have been debates on this topic for decades, with thirty or more hypotheses and little consensus emerging (Baldwin & Baldwin, 1981; Burghardt, 2005; Fagen, 1981). An exhaustive review is in excess of the confines of this thesis; however, a brief summary is instructive.

Play has been construed as having either minor functions and so likely irrelevant in terms of developing normal behavior, or critical for the development of various social, physical,

or emotional skills (Pellis & Pellis, 2006). Play behavior has been evolutionarily selected for in a wide range of species, and it has also been seen as very costly in consumption of energy and potential risk of injury. Hence, it has been widely accepted that there must be large benefits due to the purported high energy expenditure associated with this activity (Martin & Caro, 1985). In support of this position, Siviy and Panskepp (1984) determined that food deprivation was sufficient to reduce play, and a single meal increased the behavior over pre-feeding levels. Martin and Caro (1985), however, claim that the amount of energy expenditure relating to play is still uncertain. Martin (1984) argues that the energy expenditure for play is in fact quite small. He determined that the amount of energy expended on play in kittens only accounted for between 4% and 9% of total daily output, despite the subjects being well-fed, disease-free, and predator-free, and in an environment which was known to induce play in the typically playful species. Additionally, Martin and Caro (1985) note that there were no pathological or dysfunctional consequences when play was reduced to zero due to environmental pressures (e.g., food shortage) in normally playful monkeys.

Nonetheless, a wide range of studies on the effects of play deprivation in rats and monkeys indicate that the absence of play experience in the juvenile phase leads to adults that are hyperdefensive (Einon & Potegal, 1991), are cognitively impaired (Einon, Humphreys, Chivers, Field, & Naylor, 1981), socially incompetent and have an exaggerated stress response (Von Frijtag et al., 2002). The direct link between play in the juvenile phase and adult behavioural and cognitive performance is well illustrated in species that continue to use play in adulthood (Pellis and Iwaniuk, 1999, 2000). For



instance, several studies on rats have revealed that as adults use this playful form of social interaction to assess and manipulate each other, but doing so in a way so as to avoid escalation into serious fighting (Pellis & Pellis, 1991, 1992; Pellis, Pellis, & McKenna, 1993; Smith, Fantella, & Pellis, 1999). Furthermore, play in the juvenile is organized in a manner so that it may be used to facilitate the development of the emotional and cognitive skills needed for using play in such a manner in adulthood (Pellis, Pellis, & Foroud, 2005). Similarly, Spinka, Newberry, and Bekoff (2001) hypothesize that play offers the opportunity to “train for the unexpected”. They suggest that animals attempt to create unexpected situations in play by using self-handicapping, whereby control over their movements is deliberately relaxed, or they put themselves in a disadvantageous situation or position. According to this hypothesis, emotional flexibility is developed by rehearsing the emotion of surprise or disorientation, and that this ability will help deal with unexpected events as adults. In support of such hypotheses, Von Frijtag et al. (2002) have shown that animals with play experience are better equipped to handle aversive social stimuli.

#### *Immediate or Long Term Benefits?*

Traditionally, emphasis has been placed on the long-term benefits of early experience; that is, play has immediate costs but delayed benefits (Fagen, 1981; Martin & Caro, 1985). Gomendio (1988), however, suggests that there may indeed be a particular “immature niche”, as the developing animal progresses through different stages and therefore faces different challenges socially and environmentally at different times during its ontogeny. He proposes that play might allow juveniles to deal with the current

problems relative to both social and physical needs in the immediate world. Fagen and Fagen (2004) agree that there may be an immediate benefit of play. They found that survival in brown bears tended to increase as play increased, which implied that increased play also increased survival. Juvenile chimpanzees have heightened levels of play pre-feeding, a time when tension is normally high (Palagi, Cordoni, & Borgognini Tarli, 2004). It was hypothesized that this behavior had immediate as well as long term benefits. The immediate benefit was that of more relaxed feeding and potentially discouraged attacks by adult relatives. In the long run, social bonds could be assessed between adult players and adults related to immature playmates which could cause more “fair play” with the immature individual.

It was also noted that the play could serve as a stress reducer for juveniles who have yet to develop appropriate grooming behaviors (which is known to release  $\beta$ -endorphins). Palagi et al. (2004) observed that grooming among immature animals is rarely seen, and therefore is probably not used to reduce tension at this stage of life. They did find that, if an adult chimpanzee interacts with an immature subject, they may use a different behavioral strategy in “conflict management” as opposed to grooming. Indeed, an increase in frequency of play between adults and unrelated immature individuals during the pre-feeding period was seen. Further, because play is the main activity of the juvenile phase, they hypothesized that this behavior represents a good indirect “contact point” between adults and immature animals. Among unrelated immature animals, play behavior also occurred more frequently in the pre-feeding phase. They suggested that play may be used by unrelated youngsters to reduce tension and prevent the escalation of

conflict in high excitement contexts. This implies another immediate benefit that may not be involved with the promotion of co-feeding.

The results in this thesis are consistent with this suggestion, that play functions to reduce stress in juvenile rats and thus that play has immediate benefits in the juvenile phase of development. However, since there are long-term consequences to elevated levels of stress, the immediate function of play may well have long-term ramifications. A closer examination of the long-term effects of play deprivation in the juvenile phase will help link these immediate short-term effects with the long-term effects.

### Behavioral Consequences from Juvenile Experiences

#### *Play Deprivation Studies*

When juvenile animals are deprived of play during the critical period between weaning and prior to the onset of puberty (approximately 21-40 days old), aberrant social behaviors have been observed in the adult. Studies have reported that social isolates are abnormally reactive to handling and are hyperemotional (Weiss et al., 2003), and hyperactive as adults (Von Frijtag et al., 2002). van den Berg, Hol, Van Ree, Spruijt, Everts, and Koolhaas (1999) observed that depriving male rats of early social experiences permanently altered their ability to display appropriate socio-sexual interactions. They exhibited less social exploration (Hol, Van den Berg, Van Ree, and Spruijt, 1999) and social interest, which resulted in decreased anogenital sniffing, although there were no deficiencies in the ability to perform sexual acts. The play-deprived animals did not show the appropriate immobility behaviors when confronted with a dominant male, thus they

were unable to defuse potential aggressive encounters and incurred multiple attacks. Using a similar paradigm, Von Frijtag et al. found that individually reared rats were bitten more frequently by the resident than socially reared rats. Again, the individually housed rats lacked the correct behavioral responses when exposed to an aggressive resident. Socially reared rats appeared to seek safety on top of the resident's cage, whereas individually reared rats did not display this response to the same degree. Takahashi (1986) determined that, when 21-day old male rats were housed either independently or in pairs post-weaning, agonistic exchanges appeared earlier and more frequently when tested as adults in the singly reared animals, and these males were less able to defend themselves. Other deficits from play deprivation include slow reversal of a previously learned discrimination (Einson, Morgan, & Kibbler, 1978), delayed rates of habituation, increased latency to habituate to a new environment (Einson & Morgan, 1977), and slow learning in certain spatial tasks (Wongwitdecha & Marsden, 1996). It is fairly apparent that early social experiences are vital for aggression, sexual, and social interactions with conspecifics later in life (van den Berg et al., Von Frijtag et al.).

Perhaps more closely related to the findings of this thesis, is that there is evidence that play deprived juvenile rats have an impaired stress response when they are adults. They experience heightened CORT and adrenaline concentrations when confronted with territorial aggression as adults, and the levels of CORT remain high for a prolonged period of time, whereas CORT returned to a normal level more rapidly in socially reared animals (van den Berg et al.). This implies that isolated juveniles experience greater stress as adults than socially reared animals. Vanderschuren, et al. (1997) affirm that

during adulthood, previously isolated animals exhibited severely disturbed behavioral and neuroendocrine responses when confronted with a social stressor. All of the above studies indicate that play deprivation somehow involves alteration to the HPA axis which appears to behaviorally manifest itself in the adult animal. The role of experience in the development of the HPA axis has been more definitively examined in the early postnatal period.

### *Early Handling and Maternal Deprivation Studies*

Early adverse experiences can also have lifelong effects: increased levels of glucocorticoids during stress and an impaired recovery at the end of stress. There is enhanced anxiety and changes to the amygdala in postnatally stressed rats. In addition, there is impaired development in terms of learning and memory (Sapolsky, 2004).

Maternal separation during the first three weeks of life has shown to increase basal and stress induced ACTH concentrations and decrease CRH binding in the anterior pituitary (Kaufman, Plotsky, Nemeroff, & Charney, 2000). Other effects include enhanced CRH expression in the amygdala and increased stress-induced activation of the noradrenergic systems.

In previous sections, it was seen that various environmental conditions such as social isolation, play deprivation, maternal deprivation, and housing conditions during the postnatal and juvenile period resulted in behavioral deficiencies in adults. There is evidence that these defects can also be seen as a result of immature rats being exposed to elevated glucocorticoids. When young adult rats were exposed to corticosterone at doses

sufficient to mimic the elevated hormone levels observed following exposure to mild stress, learning impairments were seen when they were mid-aged adults (Bodnoff, Humphreys, Lehman, Diamond, Rose, & Meaney, 1995).

There is evidence that mild stressors during the postnatal period can have positive effects during adulthood. When rat pups are separated for differing periods from their mother as pups (a mild stressor), then reunited, the mothers lick the pups differentially. Liu et al. (1997) demonstrated that ACTH and corticosterone concentrations were lower before, during, and after a 20-minute restraint stress in adult rats that had received a high amount of maternal licking than in those that received a low amount. Similar to maternal licking, neonatal “handling” in rodents has also been shown to reduce the stress response as adults. It has been suggested that these types of early experiences can “imprint” the stress-response, and that there is a developmental critical period that can affect how adults respond to stress for the rest of their lives (Sapolsky, 2002). Rats that were handled postnatally for 15 minutes daily for 3 weeks had smaller stress-responses and a quicker return to baseline after the end of stress. An increased sensitivity of the brain and pituitary to circulating glucocorticoids has been suggested as a mechanism by which the stress response system becomes more efficient in later life. Vallée, Mayo, Dellu, Le Moal, Simon and Maccari (1997) have shown that, when postnatally-handled rats become adults, they revealed low anxiety-like behavior which was correlated with low secretion of corticosterone in response to stress. They report that postnatal handling did not affect cognition or learning, but it did improve memory performance in old rats.

Certain types of mild stress appear to enable a rat pup to cope better with stress later in life (Nelson, 2000; Sapolsky 2002). This has been referred to as “stress inoculation”. Young squirrel monkeys that were temporarily deprived of all forms of contact with their natal group on an intermittent basis showed diminished anxiety when exposed to a novel environment (Parker, Buckmaster; Schatzberg, and Lyons, 2004). Compared with controls, the intermittent stress monkeys had lower basal plasma ACTH and cortisol concentrations and lower corticotrophin and cortisol concentrations after stress. It was suggested that the experience of low-levels of stress produces competence in the management of and increased resistance to future stressful circumstances. Further, the research indicated that exposure to one type of stress confers a type of “cross-immunization”, and may strengthen resistance to different stressors encountered later in life.

Play deprivation, maternal deprivation, and early handling all appear to impact the HPA axis, some in a positive manner and some resulting in behavioral deficits. Each of these experiences affects adult behavior, implying that there is a degree of flexibility in the development of the stress response during the early stages of life.

### Plasticity and Adaptation

Deprivation and handling studies have been typically divided into two time periods: early infancy and adolescence. The “stress inoculation” effect ceases after three weeks of age in rats, prior to the onset of play behaviors in rats. If the deprivation and handling effects on the HPA axis have similarities in causal mechanisms, we must look further into

the developmental aspects occurring during the postnatal and juvenile phases to justify this apparent discrepancy. Pellis and Pellis (2006) contend that the infant does not have sufficient sensory, motor and cognitive maturity to enable it to link the mild stressors normally experienced due to the vicissitudes of life to specific features of its current environment. Further, they suggest that play in the juvenile phase enables fine-tuning of the emotional response system, allowing the animal to make associations between specific stimuli and environmental conditions with particular affective states. Play fighting experience provides such an opportunity because in the juvenile phase both the attack and the defense components of play are engaged in such a way as to prevent the performer from maintaining its advantage and so leading to moment to moment unpredictability in the actions of the partner, or for that matter in its own movements (Foroud & Pellis, 2002, 2003; Foroud, Whishaw, & Pellis, 2004). The coping mechanisms learned as a result of play fighting in the juvenile can thus be seen as independent of, and additive to, the experiences resulting from early handling. In this way, play in the juvenile period provides an important fine-tuning mechanism for the development of coping strategies.

Consistent with the hypothesis by Pellis and Pellis (2006), Laviola, Adriani, Morley-Fletcher, & Terranova (2002) note that, although the number of neurons and axons and final brain size is generally thought to be established early in infancy, there is still considerable plasticity in adolescents due to overproduction and elimination of axons and neuronal connections. This period is characterized by particular behavior patterns, consisting of hyperactivity, high attraction to novel stimuli, as well as heightened levels



of play. Additionally, Laviola et al. (2002) found that there are age-related discontinuities in the response of the hypothalamic-pituitary-adrenal axis. They suggest that the “set up” of the HPA axis is physiologically elevated during adolescence, and experiences during this time frame may potentiate the subsequent reaction to acute stress in adult rodents.

Heuther, Doering, Ruther, and Schussler (1999) posit that the stress reaction process is adaptive, and results in the modification and reorganization of neuronal networks. They affirm that the neural connectivity of the brain is subject to lifelong adaptive changes, which have been referred to as “experience-dependent plasticity”. Although the details responsible for this “plasticity” are beyond the scope of this thesis, a superficial description is presented here. As noted in a previous section, Laviola et al. (2002) state that this plasticity in adolescents is due to overproduction and elimination of axons and neuronal connections. Parker et al. (2004) claim that “manageable” stress exposure early in life may temporarily activate the HPA axis, and provide a neural basis for programming stress resistance. Heuther et al. (1999) suggest that adaptive modifications and reorganizations may be triggered by persisting environmental demands and stresses, and that repeated *controllable* stress allows the individual to adapt to environmental demands via the stabilization and facilitation of suitable patterns of appraisal and coping. This may be the mechanism involved in the previously discussed “stress inoculation”, whereby repeated low levels of stress at an early age strengthens stress resistance encountered later in life.

The terms, “manageable” and “controllable” imply that there is a positive outcome arising from the behavioral choices made as a result of the perceived stressor. This reflects the sentiment expressed by Spinka et al. (2001) during their discussion on the functions of play. To reiterate, they claim that animals seek and create unexpected events by putting themselves in disadvantageous situations by any number of behavioral or environmental manipulations, thus enabling the individual to develop emotional flexibility. As noted above, the work by Pellis and his colleagues shows that, during the peak play period, rats interject maneuvers that cause some uncertainty as to which behavior will arise next. They term this “structured flexibility” (Pellis & Pellis, 2006). The behaviors exhibited by both adults and juveniles can be seen as similar, but the order in which they are displayed differ. Generally, play fighting in rats involves attack and defense of the nape. Successful contact results in gently rubbing with the nose (Pellis & Pellis, 1987). Small degrees of flexibility are seen when a minor deviation in attack or defense results in a compensatory deviation by the other. These small deviations allow the individuals to experience moderate amounts of novelty and potentially, stress. If the novelty induces too much stress, the subject can then revert to a more stereotypical play pattern which is seen as calming (Pellis & Pellis, 2006). Indeed, it has been demonstrated in the research presented here that the experience of play reduces stress.

It is therefore proposed that play can serve to activate the HPA axis in a somewhat *controlled* or *manageable* manner (as in self-handicapping), which would stimulate the outgrowth of axons resulting in new neuronal connections. Repeatedly stimulating the same neural pathways would therefore establish these connections and provide for a

better outcome when these same individuals are exposed to stressful or unexpected situations as an adult. This is consistent with the data presented in this thesis. The HPA axis is stimulated by the injection of ACTH which enhances the frequency of play. This replicates behavior we see after 24 hours social isolation, which is a stressful experience. The increase in play as a result of a minor stress potentially facilitates axonal outgrowth and allows for the adaptive neural connectivity to develop. The repetitive exposure of minor stress associated with unpredictable play may be sufficient to reinforce adaptive neuronal changes required for successful behavioral strategies involving the stress response as adults.

### Conclusion

As stated in earlier sections, the immediate benefit of play for juveniles can be seen as a reduction in stress. The potential long-term benefits, however, lay in the reorganization of the stress response system, thus developing the behavioral flexibility required to successfully cope with various social and environmental encounters.

Heuther et al. (1999) state that “the experience of stress . . .of the right quality, of the right intensity, in the right context and at the right age is a prerequisite of normal development”. There have been a multitude of examples presented here which demonstrate the behavioral deficits which result from play deprivation, which we have seen is stressful. It is suggested that the experience of play provides just the right quality of stress, the right intensity, in the right context and at the right age, to prepare the adult

animal for appropriate responses to stress as well as the rigors of daily life. In short, play teaches one how to be, and that is, indeed, powerful.

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