ABSTRACT

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The purpose of the current study was to investigate genetic and experiential contributions to social buffering between juvenile non human primates. A second aim was to investigate the role of behavioral displays during social buffering, in order to explain social buffering deficits in primates with a history of early social deprivation (Winslow et al., 2003).

A total of 31 male rhesus macaques (mean age of 2 years) were videotaped during a Novel Cage Test with and without their homecage partner, and immediately following, blood samples were collected under anesthesia. Subjects were either reared with mothers and peers (mother reared, *n*=15) or without their mothers in the continuous presence of peers (peer reared, *n*=16). Cortisol concentrations and rh*5-HTT*LPR genotypes (long (*l)* and short (*s)* alleles) were generated from blood samples

(*l/l*=20, *l/s*=10, and *s/s*=1), and videos were coded for a variety of stress and affiliation behaviors.

Genotype and rearing differences in social buffering of stress behaviors and neuroendocrine function were assessed. Rates of social buffering were also compared between a group of high display subjects that exhibited frequent behavioral displays (*n*=21) compared to a low display group (*n*=10). Additionally, the behavioral data were subjected to a lag sequential analysis to examine levels of contingent responsiveness, or the likelihood of behavioral displays occurring before affiliative responses (Bakeman et al., 1997).

The results revealed social buffering deficits in the short allele, peer reared, and low display groups. Both the peer reared and low display groups were found to engage in less affiliative behaviors compared to the mother reared and high display groups respectively, while the short allele group appeared to receive less benefit from the presence of a familiar partner. Additionally, contingent responsiveness was identified as a feature of social buffering for the entire sample, but did not explain group differences in social buffering.

Taken as a whole, this study identifies genetic and experiential vulnerability factors for social buffering. Furthermore, it adds to our knowledge of how behavioral displays are used during social buffering.

FACTORS AFFECTING RATES OF SOCIAL BUFFERING IN JUVENILE RHESUS MACAQUES *(Macaca mulatta)*

By

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Dedication

To my grandmother Dorothy Hallas Herman who taught me the value of education

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CHAPTER 1: INTRODUCTION

Stress has been linked to the emergence of chronic diseases, psychopathology, and cognitive impairments (McEwen, 2000). One capacity that appears to buffer the experience of stress is social companionship (Gunnar et al., 2002). Yet, individuals vary in their ability to solicit and receive support from social partners in times of stress (Iarocci et al., 2007). Increasingly studies demonstrate both experiential and genetic contributions to the development of social abilities. Therefore, it is important to understand how social buffering capacities develop.

The concept of stress was first coined by Hans Selye (1950) to refer to physiological activation of the body to a series of nonspecific noxious stimuli. Since his discovery more than a half-century ago, researchers have noted that stimuli of this nature such as shock, noise, restraint, and novelty, commonly referred to as stressors, are all threatening and lead to increased emotional arousal (Lazarus, 1966; Levine et al., 1983; McEwen, 2000). Stressors are known to give rise to a host of emotional, behavioral, cognitive, and physiological changes that put an individual into a heightened state of readiness (Baum et al., 1999). Behaviorally, both humans and animals respond to stressors in certain characteristic ways. For instance, when faced with a novel environment or a stranger, individuals tend to display fear behaviors such as freezing and looking around for sources of danger (Kagan et al., 1988; Rheingold, 1969),whereas acute separation from a loved one tends to produce high levels of arousing behaviors including distress (e.g., crying), behavioral agitation, and motor activity (Ainsworth et al., 1978; Mineka et al., 1978; Rutter, 1979). Taken

together, it seems likely that common biological substrates give rise to psychological stress responses.

Stressors activate the body's two stress systems, the fast-acting sympathetic-adrenomedullary (SAM) system, and the Hypothalamic-Pituitary-Adrenocortical (HPA) axis (Gunnar et al., 2007; Ulrich-Lai et al., 2009). These two systems are connected at the level of the hypothalamus, and are innervated by multiple neural structures involved in emotionality. Also known as the 'fight or flight system', SAM activation stimulates the release of epinephrine from the adrenal glands which in turn elevate heart rate, blood pressure, and oxygenation of the lungs. In a matter of seconds, the SAM system prepares the body for survival responses such as running, fleeing, and fighting. By comparison, the HPA axis is a slower acting system that takes up to 10-12 minutes to reach peak levels of activation after exposure to a stressor (Ulrich-Lai et al., 2009). As a result, the HPA axis contributes to immediate stress responding via basal stress levels and supports sustained activities designed to promote survival. Also, given that the HPA axis has an established circadian rhythm, consisting of an early morning rise followed by a decline across the day leading to the lowest levels at night (Gunnar et al., 2007), the effectiveness of this system is impacted by the timing of stressors.

The HPA axis is activated when perceptual stimuli are interpreted as threatening by the brain (Gunnar et al., 2007; Ulrich-Lai et al., 2009). These messages are then relayed to cells in the paraventricular nuclei within the hypothalamus causing the cells to produce corticotrophin releasing factor (CRF) and arginine vasopressin (AVP). Small blood vessels carry the CRF to the anterior portion of the pituitary

gland where ACTH is released. Upon its release, ACTH travels via the bloodstream to the target organs, the adrenal glands. The outer surface of the adrenal cortex contains receptors that bind to ACTH and respond by releasing glucocorticoids. Nonhuman primates and humans produce the glucocorticoid cortisol, whereas rodents produce corticosterone (Levine, 2000, 2001). Glucocorticoids are then released into general circulation where they contribute to numerous functions of the brain and body via changes in gene expression (de Kloet et al., 1999). In turn, glucocorticoid levels are monitored by the pituitary, hypothalamus, and hippocampus, which allow the stress systems to return to baseline (pre-stressor) levels of functioning. Therefore, the HPA axis plays a crucial role in promoting homeostasis of physiological stress systems, a concept known as allostasis (McEwen, 2000). In this light, the HPA axis can be thought of as reflecting an individual's ability to cope with stress.

Coping consists of active strategies designed to master, manage, or mitigate the demands created by stressful events (Taylor et al., 2007). Coping resources can be conspicuous to others, such as displaying optimism, or support-seeking behaviors in times of stress, or it can take the form of internal thought processes that result in stress reduction. Factors in the environment that appear to promote coping include giving an individual greater control over his circumstances (Hanson et al., 1976; Rodin, 1980), providing feedback after a stressor to help him evaluate the effectiveness of a coping strategy (Weiss, 1968), and being able to predict when he will be free from the effects of a stressor, known as the safety signal hypothesis (Seligman, 1968). Thus, both endogenous and exogenous variables contribute to the ability to cope with stressors.

From the perspective of Selye's (1950) arousal model, behavior and physiology are expected to act in tandem, reflecting arousal in times of stress, and relaxation during non-stressful times. By contrast, a coping model posits that when behavior and physiology diverge, it reflects the existence of coping strategies (Levine et al., 1983; Spangler et al., 1998). For example, when infant primates display high levels of vocalizations, it is typically thought of as reflecting distress. However, Levine et al. (1984) found that despite displaying higher levels of vocalizations when infants were put in a cage adjacent to their mothers, their cortisol levels were not discernibly higher. Levine et al. interpreted these data as an active coping strategy designed to solicit maternal contact. Therefore, in order to evaluate the extent of coping that is taking place, it is important to measure both behavioral and physiological responses to stressors.

One definition of stress is the failure of coping strategies (Levine et al., 1983). More specifically, when an individual is subjected to a chronic stressor or repeated acute stressors, or is unable to return the body back to baseline levels, heightened glucocorticoid levels cause wear and tear on the body, a concept known as allostatic load (McEwen, 2000; Selye, 1950). In fact, elevated glucocorticoid levels have been linked to a variety of disorders including depression (Kendler et al., 1998; Levine et al., 1983), diabetes, obesity, heart disease, and strokes (McEwen, 2000). Chronic elevations of glucocorticoids also are associated with reductions in immune functioning, and rewiring of key neural structures including the hippocampus, amygdala, and the prefrontal cortex. Thus, it is crucial that we study coping

mechanisms that can downregulate stress responses and promote health over the long term.

For more than a half-century, researchers have observed that a variety of social species tend to affiliate with companions under stress (Kikusui et al., 2006). This has been observed for soldiers preparing for war and for individuals recovering from a natural disaster (Epley, 1974; Taylor, 2006). In addition, the presence of a conspecific is often associated with bolder responses and reduced emotion reactivity in rats and goats (Davitz et al., 1955; Latane, 1969; Liddell, 1950; Taylor, 1981). Based on this literature, Taylor (2006) hypothesized that similar to the fight or flight response, affiliating with companions and providing caregiving or "tend and befriend" promotes survival. Given this cross-species tendency to affiliate under stress, Bovard (1959) hypothesized that social companions serve a stress-reducing function, and labeled the concept social buffering. For the purposes of this project, social buffering will be defined as "the ability to use a (familiar) social companion to buffer the effects of a stressful environment (Winslow et al., 2003pp. 910)." While social buffering has been found for individuals within group settings such as daycare (Coe et al., 1982; da Costa et al., 2004; Gust et al., 1996; Rappolt-Schlichtmann, nd; Stanton et al., 1985; Stetler et al., 2008) (Dettling et al., 1999; Dettling et al., 2000), this project is focused on social buffering between dyads with established social bonds, and as a result, will review the effects of a single partner on stress levels.

While many valuable findings can be obtained by studying the impact of supportive companions on stress levels in naturalistic settings (Smyth et al., 1998; Wittig et al., 2008), researchers often study the phenomenon in laboratory settings in

order to exercise greater control over confounding variables. One common paradigm is separation and reunion of bonded partners, which allows researchers to investigate the extent to which a partner is able to reduce stress responses during reunion (Coe et al., 1978; Mendoza et al., 1978; Wiener et al., 1987). Other common paradigms involve exposing subjects to stressors (e.g., giving a public speech, or exposure to a live snake, or a novel room), and then measuring stress responses with and without a social companion present (Coe et al., 1982; Kirschbaum et al., 1993; Stanton et al., 1985; Winslow et al., 2003). Researchers studying social buffering tend to collect both behavioral and physiological measures of stress. Behavioral measures are centered on measuring levels of arousal and / or fear, while physiological systems involved in social buffering studies include the HPA axis, SAM, and immune function. However, measures of HPA function predominate in social buffering research. As a result, this project will focus on reviewing social buffering studies that employ measures of HPA functioning (Hennessy et al., 2009).

Affiliation is thought to play a central role in decreasing neuroendocrine and behavioral stress responses (Kikusui et al., 2006). The term affiliative refers to 'friendly' social interactions between companions that are designed to promote group cohesion (Maestripieri et al., 1997), including holding hands, hugging, grooming, and proximity without physical contact (Hertenstein et al., 2006; Kikusui et al., 2006). The physiological mechanisms by which affiliation impacts stress appears to closely implicate the neuropeptide oxytocin (Carter, 1998; DeVries et al., 2003; Kikusui et al., 2006; Taylor, 2006). In addition to affiliative contact stimulating oxytocin (Uvnas-Moberg, 1997), numerous studies have found that increases in oxytocin

suppress neuroendocrine function (Chiodera et al., 1991; Heinrichs et al., 2003; Heinrichs et al., 2009; Windle et al., 1997) as well as behavioral indicators of anxiety (Heinrichs et al., 2003). Additionally, opioids such as β-endorphin and the neurotransmitter dopamine are released in response to affiliative behaviors, which probably serve to reinforce social interaction and increase its future occurrence (Keverne et al., 1989; Nelson et al., 1998; Young et al., 2004). Thus, affiliation appears to provide individuals with a powerful mechanism for decreasing physiological responses to stress.

While many promising studies on social buffering are being carried out in humans (Ditzen et al., 2007; Heinrichs et al., 2003; Kirschbaum et al., 1995; Taylor et al., 2008), direct investigation is limited by ethical and practical concerns. By contrast, researchers working with animals are able to control and manipulate rearing conditions and then study individuals prospectively across development. Additionally, it is much easier to collect samples from biological systems influencing social buffering. Yet, the value of animal studies ultimately rests on the ability to identify general principles of behavior that are applicable to the human condition (Archer, 1992; Mears et al., 1975).

Many social buffering studies involve rodents and ungulates (Hennessy et al., 2009; Kiyokawa et al., 2004; Lyons et al., 1993; Wilson, 2001); however, they lack the cognitive and emotional complexity of humans. By contrast, nonhuman primates possess homologous neural structures, flexible cognitive abilities, and complex social behaviors that parallel humans (Nelson et al., 2009). As a result, research with non human primates is believed be more applicable to the human condition.

One species in particular, the rhesus macaque (*Macaca mulatta*; Figure 1), is an excellent model for studying social buffering, for members of this species form specific social bonds to both parents and peers (Suomi, 1999), and their neuroendocrine stress system functions similarly to humans (Coe et al., 1985). This species is particularly relevant for studying social buffering because members of this species are capable of forming a social bond to a single partner (Higley et al., 1992a; Mendoza et al., 1997). Therefore, the rhesus macaque provides an excellent model for studying the process of social buffering.

In the current study, individual differences in rates of social buffering were investigated between established pairs of juvenile rhesus macaques. The objectives of the current study were two-fold: 1) to investigate genetic and experiential variables, and 2) to examine the impact of behavioral displays on affiliative responses, in order to investigate the origins of social buffering deficits in primates with a history of impoverished social experience (Winslow et al., 2003).

CHAPTER 2: SOCIOEMOTIONAL COMPONENTS OF SOCIAL BUFFERING

The goal of this chapter is to review what is known about social buffering in humans and animals. A comparative perspective will be employed, and particular emphasis will be placed on studies carried out in humans and in rhesus monkeys. The chapter begins by describing the affectional systems and social skills that contribute to social buffering. Next, the ontogeny of social buffering is reviewed. In a subsequent section, the consequences of impoverished early social experience are discussed. Finally, a case is made for investigating the role of the serotonergic system on social buffering. This section reviews what is known about the consequences of impoverished social experience on serotonergic function, the impact of a polymorphism in the promoter region of the serotonin transporter (*5-HTT*LPR), and the potential role of G X E interactions on social buffering.

The Affectional Systems

Over the course of the lifespan, human social relationships involve several key affectional systems, including those formed between infants and parents, peers, and romantic partners (Cassidy et al., 1999; Harlow et al., 1965). By relationships, Hinde (1976) is referring to the types of behaviors exhibited by social partners, and the quality and patterning of these interactions over time. One central feature of close relationships is the social bond or attachment (Bowlby, 1969). Inspired by the work of early ethologists on imprinting (Lorenz, 1937) and the primacy of contact comfort (Harlow, 1958), Bowlby (1969) proposed attachment theory for thinking about the nature of social bonds that develop within close relationships. Relationships

characterized by enduring attachment bonds tend to promote ‘Secure Base’ behaviors such as exploration and play (Ainsworth et al., 1978; Bowlby, 1969; Bretherton, 1995; Feeney et al., 2010). Working around the same time as Bowlby, Harlow (1965) expanded on this concept by describing the features of attachment relationships that form between an infant and his parents, as well as between peers, and romantic partners.

Theory suggests that there are at least two major functions of established social bonds, only one of which is social buffering. The second major function appears to be coregulation or “psychological attunement” leading to synchrony in affect and physiology between bonded partners (Field, 1985; Hofer, 2006; Sbarra et al., 2008). For instance, coregulation is thought to mediate a phase of infancy in rats and humans known as the stress hyporesponsive period when stressors have little impact on the HPA axis (Meaney, 2001; Tarullo et al., 2006). It seems likely that coregulation of physiology would also be characteristic of established relationships at later points in the lifespan, including during childhood and adulthood. Much has been written about the consequences of being separated from a bonding partner, most significantly that it often can lead to dysregulation of affect and physiology, increased allostatic load, and various mental and physical health disorders (Hofer, 2006; McEwen, 2000). Therefore, it is important to be able to separate the effects of social buffering from coregulation.

Within attachment relationships with parents, infants regularly receive contact comfort, attention, and stimulation (Bowlby, 1969; Harlow, 1958; Hofer, 2006; Kraemer, 1992, 1997). It appears that infants learn the basic give-and-take of social

communication within primary attachment relationships. For instance, primate mothers and infants are known to engage in reciprocal interactions involving affiliative gestures known as ‘motherese’ (Fernald, 1989; Whitham et al., 2007). During these interactions, infants probably quickly learn that babbling and other affective expressions produce a response in their caregiver (Ainsworth et al., 1978). Thus, primary attachment relationships appear to provide infants with their first experiences with social contingencies (Van Egeren et al., 2001). Even as peers rise in importance, the infant continuously returns to the parent or ‘Secure Base’ for comfort and encouragement, and understands that the parent remains a ‘Safe Harbor’ in the event of danger (Bowlby, 1969). Not surprisingly, the quality of primary caregiving relationships is believed to impact an infant’s ability to explore and play with the physical environment and with peers (Ainsworth et al., 1978; Bowlby, 1969).

Harlow (1969; 1965) also proposed that humans and other social mammals possess a peer affectional system that facilitates social bonding with peers. The peer affectional system is thought to arise in late infancy as interests expand beyond parents, and continue to exert a powerful influence on relationships throughout childhood, adolescence, and adulthood. Unlike the parent affectional system which consists of ‘unconditional love’ for the infant, peer relationships are characterized as conditional and based on the mutual interests of the individuals involved. Due to this characteristic of peer relationships, bonds with peers are thought to be less specific and more ephemeral than those formed with parents.

Nevertheless, peer relationships are thought to provide a context for social buffering to take place (Bowlby, 1969; Harlow et al., 1965). Within the framework of

the peer affectional system, individuals might be expected to be less hesitant in a novel environment if accompanied by a preferred playmate. Another important point is that the meaning of friendships probably changes with increasing age, from simply being playmates to serving as a source of emotional support, suggesting that social buffering might be more likely with increasing age.

Given the common features of peer relationships, Harlow (1965) hypothesized that the peer affectional system provides the primary means for developing social competence. Social competence has been defined as the ability to achieve social goals while simultaneously maintaining one's position in a social group (Suomi, 1982). The 'non-serious' quality of social play is thought to provide individuals with an excellent opportunity to practice applying the social skills learned in relationships with parents and to develop and try out new social skills (Fagen, 1981; Pellis et al., 2006; Power, 2000). This is supported by Rubin's (1982) discovery that the play strategies of preschool children are associated with differences in social competence.

Within the context of play with peers, juveniles appear to learn which affiliative behaviors are most successful, as well as how to incorporate behavioral displays such as touch, relaxed open-mouth faces ("smiles"), and laughter (Blurton-Jones, 1967; Van Hooff, 1962). Also, the degree of matching or synchrony between partners also appears to be an important social skill that develops within relationships with friends (Field et al., 1992). The development of social skills probably result in part from reinforcement received from peer partners by learning which behavioral displays are met with success, and which lead to aggression and isolation from others (Bowlby, 1969; Crick et al., 1994; Lemerise et al., 2000; Skinner, 1938). Taken

together, social play and other affiliative interactions appear to contribute to the development of social skills important for social buffering.

Harlow (1965) also proposed that humans possess an affectional system that leads to the formation of romantic attachments and pair bonds. Activation of this system is thought to rise in importance around puberty and to be available to individuals throughout the adult lifespan. Individuals also might may to learn social skills important for social buffering within their romantic relationships. Similar to parents and peers, relationship security with a romantic partner or mate is thought to encourage individuals to explore and adapt within the outside world (Feeney et al., 2010). The mating system shares the nature of the peer affectional bond which is that it is voluntary and based on the development of mutual interests. Taken together, theory suggests that within social relationships, individuals learn the social skills necessary for engaging in social buffering (Iarocci et al., 2007).

Social buffering appears to be an active process that involves a number of important social skills. At a minimum, it is important for individuals to learn how to make use of affective expressions and affiliative behaviors to achieve relevant social goals. Also, while not considered a skill, individuals must be motivated to interact socially or at least not be averse to receiving affiliation from a partner.

In order to exercise greater control over independent variables, researchers have been investigating the direct impact of receiving a sensory message or affective expressions on stress reduction. However, in many cases, what we call behavioral display actually serve as social solicitation cues that convey information about an animal’s socioemotional state or future goals that is sent from one individual and

perceived by another (Kikusui et al., 2006). Therefore, what we attribute to the effects of a particular cue may also be arising from affiliative behavior. For instance, the Wilson et al. (2001) study attributes social buffering to the receipt of touch, but the authors rightly point out that the effects could be due to greater social play, and / or other affiliative interactions. Another example comes from wild baboons that are known to send grunts to subordinate troop members that often result in post-conflict affiliation behavior (Seyfarth et al., 2008). This suggests a need to study contingencies between behavioral displays and affiliative responses within the context of social buffering.

The Ontogeny of Social Buffering

Consistent with theory, social buffering has been noted for three types of social relationships across the lifespan: parents and infants, peers, and romantic partnerships (Hennessy et al., 2009; Kikusui et al., 2006). Besides partner familiarity, another factor that appears to influence the efficacy of social buffering is the degree of social bonding between partners (Gunnar et al., 2002; Hinde et al., 1977). Thus, when social buffering is not observed between partners in established relationships, it may reflect the existence of tenuous social bonds. An additional factor that is thought to influence social buffering is social skills.

The most robust evidence of social buffering has been noted for infants in early caregiving relationships. Many (but not all, see Gunnar et al., 1989) samples of human infants display neuroendocrine and behavioral evidence of social buffering by their mothers (Ahnert et al., 2004; Ainsworth et al., 1978; 2008; Gunnar et al., 1989;

Haley et al., 2003; Hertsgaard et al., 1995; 1993; Spangler et al., 1998). Similar benefits of the mother on physiology and behavior have been noted in rhesus macaque infants (Smotherman et al., 1979) and squirrel monkey infants (Mendoza et al., 1978; Wiener et al., 1987), as well as effects of the father in paternally-bonded titi monkey infants (Mendoza et al., 1997). In rodents, social buffering of corticosterone and distress behavior has been reported for mother infant domesticated guinea pig pairs (Hennessy et al., 1987) as well as rats (Levine, 2001). Thus, there is strong evidence for social buffering of infant stress responses by parents.

Social buffering has also been identified as a feature of peer relationships. This phenomenon has been noted for neuroendocrine and behavioral stress responses between preschool-aged friends (Goldstein et al., 1989; Rabinowitz et al., 1975), and for arousal between college-age friends (Epley, 1974). Additionally, several studies in animal models support the proposition that friends buffer the effects of stressful experiences. For instance, in rhesus macaques, both neuroendocrine and behavioral evidence of social buffering has been noted between peers (Higley et al., 1992a; Winslow et al., 2003), but only on a behavioral level between adult females (Gust et al., 1994). In rodents, social buffering at a behavioral level was documented for pairs of familiar and unfamiliar periadolescent rats (Armario et al., 1983a; Armario et al., 1983b). Therefore, social buffering appears to be a compelling feature of peer relationships in both humans and animals.

Additionally, there is evidence of social buffering between established pair bonds. In humans, social buffering of neuroendocrine activity has been noted between romantic partners (female romantic partners only: Ditzen et al., 2007), (male romantic

partners only: Kirschbaum et al., 1995). These findings are supported by research on pair bonding in animals. For instance, social buffering has been reported between pair bonded prairie voles but not montane voles (DeVries, 2002; DeVries et al., 1995), domesticated guinea pig harem members (Sachser et al., 1998), as well as in well-established zebra finch pair-bonds (Remage-Healey et al., 2003).These studies indicate that social buffering is a characteristic of established pair bonds. Collectively, research demonstrates that social buffering occurs throughout the lifespan, mostly within the context of established relationships.

While not as common, social buffering by a stranger also has been reported. In humans, Gunnar et al. (1992) noted social buffering of infant stress responses by sensitive unfamiliar experimenters but not by neutral experimenters. Furthermore, social buffering of neuroendocrine function in individuals receiving emotional support from an experimenter over video (Thorsteinsson et al., 1998). Studies in animal models also are consistent with the idea that unrelated adult females can buffer some aspects of the stress response. For instance, aunting by an unrelated female buffers behavioral distress in infants but does not prevent cortisol increases (Levine et al., 1988). In domesticated guinea pigs, social buffering of neuroendocrine activity can be achieved by unrelated female adults after weaning has taken place (Hennessy, et al., 2000). In most cases, bonds were capable of being formed between participating partners of the species under study. However, the crucial element appears to be the display of appropriate social skills. Thus, it is important to study the impact of early social experiences on the development of social skills involved in the social buffering process.

Consequences of Impoverished Early Social Experience

Impoverished social environments are believed to expose children to chronic levels of stress, with the increased allostatic load in turn having negative consequences on physical and mental health outcomes (Gunnar et al., 2007; Heinrichs et al., 2009). One form of impoverished early social experience is child maltreatment which also commonly involves neglect (Harden, 2004; USDHHS, 2003). Maltreated children are more likely to suffer from a number of poor outcomes including attachment disorders, social withdrawal around peers, aggressive behavior, depression and anxiety, cognitive and language deficits, impaired physical growth and development, and dysregulated (basal) HPA activity (Black et al., 1994; Bolger et al., 2001; Dozier et al., 2007; Gaudin, 1999; Smith et al., 2004). For these reasons, there is reason to believe that children who are subjected to maltreatment will be less able to benefit from social buffering.

Research shows that children with a history of maltreatment are more likely to be characterized as having a disordered/disorganized attachment with their mother (Main et al., 1986). The disordered/disorganized attachment classification reflects a child's mixed strategies of approach and avoidance when interacting with his caregiver, and is thought to reflect the fact that the caregiver is experienced both as a threat and a comfort (Ainsworth et al., 1978; Tarullo et al., 2006). In two separate investigations, Hertsgaard, et al. (1995) and Spangler, et al. (1993) found that the cortisol levels of children exhibiting a disordered/disorganized behavioral strategy did not decrease after reuniting with their caregivers, but also see Spangler et al. (1998) who failed to replicate the association. The Hertsgaard et al. finding is particularly

notable because the sample comprised a high risk group of mothers and infants living in poverty. Therefore, the current data provide mixed support for the theory that maltreatment compromises social buffering. Also it is difficult to attribute the absence of social buffering to maltreatment given that these children were subjected to multiple stressors at different points in development.

The link between maternal abuse and social buffering impairments is supported further by data from nonhuman primates. As discussed in Tarullo et al. (2006), McCormack found that infant macaques with a history of maternal abuse did not display typical affiliation behaviors during reunion with their mothers after a social separation, and their cortisol levels did not decline during the reunion period. Using a similar separation-reunion paradigm, Dettling et al. (1998) also did not find lower cortisol levels during reunion in Goeldi's New World monkey infants with a history of maternal abuse. Also, the abused subjects displayed *more* rather than less affiliation. Bonnet macaque infants with a history of apparent neglect (due to a variable foraging strategy where the foraging demand switched from high to low every few weeks) also displayed more contact-seeking behavior and did not explore and play as did infants from a typically housed environment (Andrews et al., 1991). These discrepant findings regarding affiliative behavior may result from a conflict between the normal role of a mother as a protector and an abuser. Thus, research in multiple species of primate supports the idea that maltreatment compromises social buffering of stress responses.

Children reared in orphanages provide an additional population of individuals that is subjected to impoverished early social experience, a practice that is still used in

many countries including Russia, India, and China as well as occasionally in the United States when foster care workers are in short supply (Harden, 2002; Zeanah et al., 2006). Post-institutionalized children tend to display attachment disorders, deficits in socioemotional functioning, cognitive impairments, motor and neurological problems, and dysregulated (basal) HPA activity (Carlson et al., 1997; Fries et al., 2008; Ghera et al., 2009; Gunnar et al., 2001; Marshall et al., 2004; Schuder, 2002). Many factors undoubtedly contribute to the variety of poor outcomes including high caregiver-to-child ratios, lack of cognitive stimulation, and nutritional deprivation (Zeanah et al., 2006). Furthermore, most of the children coming out of orphanage environments are adopted into supportive environments which produce immediate but often short-lived recovery in physical and behavioral milestones. Taken together, post-institutionalized children are considered at risk for poor social buffering.

Wismer Fries et al. (2008) recently investigated social buffering capacities in post-institutionalized children. In their study, four year old children were asked to play an interactive computer game with their adoptive mothers as well as experimenters on separate days. Children sat on the caregiver's lap and received similar levels of attention and tactile stimulation throughout. Immediately after the conclusion of each assessment, urine samples were obtained from the children which were subsequently assayed for cortisol. To begin with, urinary cortisol levels were found to increase from baseline for the entire sample which supports the interpretation that the assessment was stressful. They found that the post-institutionalized group displayed an increase in cortisol after the assessment with their adoptive mothers but not after the assessment with the experimenters. By contrast, the

comparison group of typically reared children displayed a decrease in cortisol levels after the assessment with their mothers, but a cortisol increase after interacting with the experimenter. Therefore, this study found that only the comparison children displayed evidence of social buffering.

Additionally, Wismer Fries et al. (2005) found that differences in neuropeptides levels were consistent with the group differences in social buffering. Using the same assessment and urine samples as above, the urine samples were also assayed for oxytocin and vasopressin levels. Interestingly, even though the children from both conditions received similar levels of attention and affiliative social contact during the assessments, the comparison children were found to have higher levels of both oxytocin and vasopressin compared to the post-institutionalized children. These findings demonstrate that the amount of benefit received from affiliative interaction is compromised by a history of an impoverished early social experience.

These findings are all the more remarkable given the heterogeneous nature of most samples of post-institutionalized children. For instance, while all of a similar age, the amount of deprivation experienced by these children varied considerably (7-42 mos). Multiple studies have found that duration of deprivation is positively correlated with developmental impairments in post-institutionalized children (Rutter, 2006; Zeanah et al., 2006). One advantage to studying post-institutionalized over maltreated children is that the experience of deprivation is in the past, whereas maltreated children most often remain with their parents. Thus, it seems possible that current environmental stressors including recent abuse and / or neglect may confound the effects of maltreatment history (Tarullo et al., 2006). Therefore, studying post-

institutionalized populations provides certain advantages over maltreated populations when studying the consequences of impoverished early social experience.

Researchers working with animal models are better able to control the type and duration of impoverished social experience and examine the impact on developmental outcomes such as social buffering. For instance, the pioneering work of Harlow (1958) revealed that infants deprived of social contact during the first six to 12 months after birth are typically deficient in species-specific mating and maternal behavior patterns (Arling et al., 1967; Suomi, 1991), display greater agonism when reunited with peers (Suomi, 1979), heightened levels of abnormal self-directed behaviors (Suomi, 1991), withdrawal behaviors resembling depression (McKinney et al., 1971) and heightened reactions to sensory stimuli (Sackett et al., 1967). These adverse outcomes only appear to be reversible by providing each impoverished subject with repeated social contact with a socially-reared younger conspecific (Cummins et al., 1976; Novak, 1979; Novak et al., 1975; Suomi et al., 1972).

Due to the devastating effects of social isolation, a milder manipulation of early social adversity known as peer rearing was developed by Harlow (1973; 1969) and has been adopted by the likes of Suomi (1991), Capitanio (2005), and Winslow (2003). Peer (only) rearing involves separating infants from their mothers at birth and providing them with continuous contact with two or three similar-aged conspecifics for most of infant period (Chamove et al., 1973). Infants reared without primary attachment figures are thought to lack the ability to regulate behavioral and physiological responses to social or environmental stressors (Hofer, 1994; Kraemer, 1997). Perhaps as a result, the HPA axis is thought to be chronically stimulated

leading to a variety of adverse consequences associated with increased allostatic load (McEwen, 2006). For instance, peer rearing produces structural and functional abnormalities in the brain, as well as dysregulated baseline cortisol levels (Capitanio et al., 2005; Kraemer, 1997; Sanchez, 2006; Shannon et al., 1998; Winslow et al., 2003). Some of the behavioral consequences of peer rearing include high levels of clinging, low levels of social play, less grooming and full mounting Kikusui et al., 2006), a greater incidence of self-directed behaviors and distress vocalizations when separated from conspecifics (Harlow, 1969; Suomi et al., 1970), and more frequent agonism in response to social bids (Kempes et al., 2008). Taken together, the nonhuman primate work demonstrates that impoverished early social experience has devastating effects on brain and behavioral development.

An additional and perhaps non-trivial factor contributing to the adverse outcomes of peer rearing is timing of exposure during development. All available evidence suggests that contact with parents and peers are expected features of the early social environment, which contribute to the formation of “experience expectant” neural circuits during sensitive periods for socioemotional development (Ames et al., 2001; Boccia et al., 2001; Greenough et al., 1987; Immelmann et al., 1981; Knudsen, 2004; Lorenz, 1937; Rutter, 2006; Thompson et al., 2001). Unlike “experience dependent” neural circuits that typically remain open to environmental input throughout the lifespan, “experience expectant” plasticity appears to characterize neural circuits during set time periods of development. Research in animal models has demonstrated that social input during these sensitive periods restructures the makeup of neural circuits in specific ways that become preferred over time (Knudsen,

2004). Furthermore, once a sensitive period has closed, it appears to become more and more difficult to restore plasticity to the neural circuit. Therefore, it appears that the timing of impoverished early social experience may contribute to social buffering deficits.

The effects of deprivation on social buffering in nonhuman primates can be traced back to the pioneering work of Harlow and Suomi. In Suomi et al. (1970), they removed infant rhesus from their mothers at birth and reared the four infants together as a social group. When the peer reared infants were approximately three months old, they subjected the infants to a series of separations from their peers. They found that similar to infants separated from their mothers, short-term peer separations led to increased behavioral distress, whereas reunion led subjects to display high levels of contact behaviors and clinging. Suomi et al.(1973) then extended this work by examining whether the presence of a peer mitigates the impact of separation from mother. Infant rhesus macaques were permanently separated from their mothers several months after birth and then housed either individually or with a single peer. They found that subjects housed with a peer displayed less locomotion and self-directed behavior compared to singly housed subjects, suggesting that the presence of a peer mitigates the effects of maternal separation on development.

Similar to the human data, the efficacy of social buffering also appears to be compromised by a history of impoverished early social experience. Winslow et al. (2003) studied the effects of a single familiar partner on social buffering of neuroendocrine function and stress behavior. Subjects selected for this study were from well established dyads and were either mother or peer reared. Male and female

juvenile rhesus macaques were subjected to a 20min novel cage test, with and without their homecage partner. All subjects received both a separation and a social condition on separate days, and blood samples were collected after both the separation and social conditions. A blood sample was also collected outside of the homecage environment to serve as a baseline measure of neuroendocrine function.

Winslow et al. (2003) found that in contrast to the mother reared group, the peer reared group did not appear to benefit from the presence of their partner. In particular, the peer reared group displayed similar levels of cortisol and abnormal behaviors during the two conditions. Similar to the human data, the peer reared group was found to have lower baseline levels of oxytocin in their central nervous system compared to the mother reared group. However, plasma oxytocin levels did not increase as a function of the novel cage test (Fries et al., 2005). Taken as a whole, this study provides compelling evidence of social buffering deficits in rhesus macaques with a history of peer rearing.

By contrast, Higley et al. (1992a) did not find that peer reared rhesus macaques were impaired at social buffering. In this study, infants were exposed to a novel environment with either the preferred, familiar, or novel playmate on separate days. Behavioral indicators of distress (self mouthing and vocalization frequency) were measured during all of the assessments. They found that similar to the mother reared group, the peer reared group also displayed low levels of distress in the presence of a preferred, but not a familiar or novel playmate. Furthermore, the differences in stress levels appeared to result from higher levels of affiliative behaviors including clinging, and proximity to partner. However, across conditions,

the peer reared group displayed more distress overall than the mother reared group, suggesting that they may have benefitted less from the presence of a preferred playmate.

What accounts for the discrepancies between these two studies? One similarity between Higley et al. (1992a) and Winslow et al. (2003) is that the peer reared group displayed higher levels of behavioral distress while in the presence of a companion compared to a mother reared group. This strongly suggests that peer reared monkeys derive less benefit from a companion under stress. One factor that differed between the two studies is the use of a social separation condition. In Higley et al. (1992a), behavioral responses from the preferred playmate condition were compared against a familiar and novel playmate condition, whereas in Winslow et al. (2003), responses from a social condition were compared against a separation condition. Needless to say, the differences in methodology make it difficult to ascertain why rearing differences in social buffering of distress behavior were found for Winslow et al. but not for Higley et al.

Interestingly, the effects of impoverished early social experience on social buffering do not appear to be limited to deficits in maternal attachment experiences. Research by Wiener et al. (1987) reported social buffering deficits in squirrel monkey infants subjected to early peer deprivation. During a brief separation from their mother, infants with a history of maternal and peer rearing displayed less locomotion, fewer vocalizations, and reduced cortisol levels in a familiar versus a novel cage. By contrast, no differences in behavior or neuroendocrine functioning were found for peer deprived infants. Importantly, the study did not assess the peer social buffering

capacities of peer-deprived subjects, presumably because introducing them to new social companions during the separation from mother would increase rather than decrease their stress reactivity levels. These findings demonstrate that the presence of peer companions led to a reduction in cortisol levels, but only in typically-reared squirrel monkeys.

In a study with the domesticated guinea pig, Sascher et al. (1998) investigated whether exposure to conspecifics around the timing of puberty influences social buffering by a peer. They found that guinea pigs with these specific experiences are more likely to provide social buffering for a partner than those subjects deprived of peer contact. In studies with rats, researchers have also reported heightened stress responding and freezing in rats that have been deprived of social interaction during periods of development characterized by high levels of peer play (Van den Berg et al., 1999). Thus, a good deal of evidence from primates and rodents indicates that rearing by mothers and peers improves the efficacy of social buffering.

What factors account for the social buffering deficits in individuals with a history of impoverished social experience? First, abnormally reared infants may have learned to prioritize alternative coping strategies due to difference experiences with reinforcement (Skinner, 1938). For instance, Suomi (1979) presents a compelling argument that rearing by other peers is no substitute for a mother. In a peer rearing environment, infants must learn to meet their own needs as well as those of other developing infants, and may have had more experience having their social bids rebuffed by peers under stress. As a result, when faced with a stressor, peer reared subjects tend to rely on less effective coping strategies such as self-directed behavior

(Nelson et al., 2009), which can further exacerbate the health consequences associated with allostatic load (McEwen, 2000).

Another possibility is that the absence of adult models and / or peers made it more difficult for developing individuals to learn the social skills necessary to engage in social buffering (Bandura, 1971). This is supported by observations that peer reared rhesus monkeys display fewer behaviors characteristic of social competence such as grooming and full mounting (Kikusui et al., 2006). It is important to point out that deficits in mounting behavior may also arise out of anxiety rather than impairments in social competence (Goy et al., 1979; Kempes et al., 2008).

One specific social skill that may be impaired by abnormal rearing experience is the ability to interpret behavioral displays accurately (Crick et al., 1994; Lemerise et al., 2000). This hypothesis is supported by the results of Kempes et al. (2008) who found that mother only monkeys are more likely to produce agonistic responses to social bids.

This proposition is supported by studies in humans with a history of impoverished experience. Pollak et al. (2002) found that maltreated children are more accurate at interpreting angry expressions of emotion than control children, and Wismer-Fries et al. (2004) found that post-institutionalized children were less skilled at distinguishing among fearful, sad, and happy expressions of emotion but were not more skilled at identifying angry faces. Taken together, both the animal and the human data indicate that early social adversity compromises the development of social skills important for social buffering.

Collectively, these studies demonstrate that social buffering can still develop in individuals with a history of impoverished social experience. With that said, the effectiveness of social buffering appears to be compromised relative to individuals reared in typical or optimal environments. One factor that has been noted across numerous samples is heterogeneity in developmental outcomes of children with a history of impoverished early social experience (Rutter, 2006; Zeanah et al., 2006). For instance, children reared in orphanages typically have deficits in cognitive and socioemotional functioning, but developmental outcomes range from severe impairments to better than average outcomes. One possible explanation is that the heterogeneity in outcomes are due in part to biologically-based differences in temperament (Goldsmith et al., 1987). Consistent with this possibility, a number of human and animal studies have reported that individuals with a reactive or fearful temperament tend to be more strongly impacted by unresponsive caregiving in a stressful environment (Gunnar et al., 1992; Nachmias et al., 1996; Spangler et al., 1998; Suomi, 1987). These findings strengthen the rationale for examining the role of genetic markers in social buffering.

CHAPTER 3: A ROLE FOR SEROTONIN IN SOCIAL BUFFERING

The goal of this chapter is to discuss the relevance of serotonin to social buffering. After providing an introduction to the serotonergic system, the first section describes how this system is involved in stressful behaviors as well as affiliation. The next section discusses what is known about the impact of impoverished social experience on serotonergic function. A subsequent section considers the impact of genetic variations in serotonergic function on social buffering, with a particular emphasis on a polymorphism in the promoter region of the serotonin transporter protein gene (*5-HTT*LPR). Finally, the last section examines the role of Gene X Environment (G X E) interactions involving the *5-HTT*LPR on social buffering. Understandably, much of what is known about the serotonergic system comes from work in animal models. Therefore in keeping with the previous chapter, a comparative approach will be employed, particularly with an eye for studies carried out in the rhesus monkey. Research on all of these fronts strongly indicates that serotonin is involved in social buffering.

Overview of the Serotonergic System

Serotonin, or 5-hydroxy-indoleacetic acid (5-HT), is one of the central neurotransmitters of the brain. Serotonin is produced in the dorsal and median raphe nuclei of the midbrain, with serotonergic neurons projecting to a wide range of neural circuits throughout the central nervous system, particularly those neural structures involved in emotionality and social behavior (Holmes, 2008; Insel et al., 1998; Kalueff et al., 2007). Activation of this system is modulated by a complex of

receptors known to release and reabsorb serotonin, which serve to regulate the amount of serotonin available within the synaptic space.

One important characteristic of the serotonergic system is that it is highly responsive to stressors. For instance, monkeys and rodents subjected to acute separations from bonded companions display heightened HPA activity and decreased central serotonergic function (Erickson et al., 2005; Heim et al., 2001; Insel et al., 1998; Meaney et al., 1994). A number of the limbic structures known to contain serotonergic receptors such as the amygdala and the septum also possess receptors that are sensitive to the action of glucocorticoids (Insel et al., 1998). This may help explain why mood disorders, alcoholism, and substance abuse are associated with dysregulation of both the HPA axis and the serotonergic system (Higley et al., 1991, 1996b; Maestripieri et al., 2006; Way et al., 2010).

Additionally, researchers have found that pharmacological manipulation of the serotonergic system leads to alterations in stress levels. At a behavioral level, researchers have found that blocking serotonin uptake leads to fewer separation calls over the short term, whereas stimulating the serotonergic system leads to more frequent separation calls (Insel et al., 1998). One interpretation of these findings is that the ability to cope with social stressors appears to depend on the availability of serotonin. At a physiological level, depleting the serotonergic system with precursors of serotonin, such as tryptophan, results in increased stress reactivity at multiple levels of the HPA axis, including pituitary and especially hypothalamus (Dinan, 1996). Thus, manipulations of the serotonergic system appear to impact both behavioral and physiological indicators of stress.

The HPA axis also appears to exert regulatory influences on the serotonergic system, as researchers have noted that serotonergic neurons within the dorsal raphe nucleus are responsive to glucocorticoids (Lanfumey et al., 2008). More specifically, stimulation of the HPA axis has been found to increase the synthesis and release of serotonin within the central nervous system. Therefore, the serotonergic system and the HPA axis have important bidirectional influences within stressful contexts.

Additionally, research suggests that the serotonergic system is responsive to affiliative behaviors. For instance, Field et al. (2005) recently found that massage increased serotonin and dopamine levels as well as decreased cortisol levels in humans. This pattern of results suggests that the increased serotonin produced by affiliative behaviors such as nonthreatening touch may decrease activity within the HPA axis via inhibiting the action of CRF neurons in the hypothalamus (Lanfumey et al., 2008).

The serotonergic system also appears to exert regulatory influences on social behavior. For instance, tryptophan depletion has been found to produce social impairments including increased aggression and irritability (Knutson et al., 1998; Young et al., 2002). By contrast, studies administering pharmacological agents designed to enhance serotonergic activity tend to produce higher levels of affiliative behaviors. For instance, Raleigh et al. (1985) found that administering the Selective Serotonin Reuptake Inhibitor (SSRI) fluoxetine or tryptophan produced higher levels of affiliative behaviors such as grooming in rhesus monkeys, which in turn appeared to improve their dominance status within their social groups. Naturalistic research with rhesus macaques has documented that higher levels of affiliative behavior tends

to be accompanied by lower levels of the serotonin metabolite, 5-hydroxyindolaceatic acid (5-HIAA) (Mehlman et al., 1995). In vervet monkeys, the SSRI fluoxetine led subjects to display less impulsivity toward an unrelated dominant male (Fairbanks et al., 2001). Finally, administering the SSRI paroxetine to humans who are not clinically anxious or depressed has been found to produce higher levels of affiliative behavior in humans (Knutson et al., 1998). Taken together, these findings demonstrate that the availability of serotonin influences the expression of affiliative behavior.

Interestingly, the effects of SSRIs appear to be developmentally dependent. Studies have found that administration shortly after birth leads to higher levels of emotional behaviors in mice, whereas no discernable effect was found during administration later in development (Ansorge et al., 2008; Ansorge et al., 2004; Homberg et al.). This suggests that experiences of an adverse or positive nature will have a greater effect on stress and affiliation during early development than at later stages of the lifespan.

Impoverished Early Social Experience and the Serotonergic System

Individuals with a history of impoverished early social experience typically produce low levels of serotonergic activity. For instance, lower levels of 5-HT and 5-HIAA have been noted in human and animal subjects with a history of abuse, maternal rejection, and / or peer rearing (Ichise et al., 2006). A recent positron emission tomography (PET) imaging study also reported a lower serotonin binding potential in peer reared compared to mother reared rhesus macaques (Heim et al.,

2002). This may help explain why HPA dysregulation has been noted in similar populations with a history of significant adversity or abuse (2008). However, differences in HPA function do not always accompany stress-induced changes in serotonergic activity.

Recent studies have reported that mother reared but not peer reared infant macaques are able to increase peripheral serotonin transporter (5-HTT) expression levels during a social separation (Kinnally et al., 2009; Miller et al., 2009). However, 5-HTT levels were unrelated to cortisol levels during the separation, and were positively correlated with cortisol levels during a post-stressor assessment. Similar impairments in peripheral 5-HTT expression have been noted in humans with a history of childhood abuse, as well as macaques receiving high levels of maternal aggression (Kinnally, 2007). These consistent findings suggest that peripheral measures of 5-HTT, which are considerably easier to obtain, may be able to serve as a proxy for central effects. With that said, it is important to mention that peripheral measures of 5-HTT expression do not necessary correspond to levels within the central nervous system (Murphy et al., 2008).

Effects of the Serotonin Promoter Gene-Linked Polymorphic Region

There is also considerable evidence that genetic variations in the serotonin system lead to differences in stress and affiliation. Research involving ‘SERT knockout mice’ where serotonergic activity has been genetically reduced (SERT +/-) or eliminated (SERT -/-), leads to blunting of the HPA axis (Jiang et al., 2009; Lanfumey et al., 2000; Li et al., 1999), but greater HPA responsivity to stressors, and

a sluggish return to baseline levels of activity after a stressor. In addition, SERT knockout mice display markedly lower levels of affiliation, locomotion and exploration, while at the same time increased anxiety-like behaviors (Kalueff et al., 2007; Moy et al., 2009). Similar to SSRI treatment, research with conditional knockout mice reveal that disabling SERT activity during the first four weeks of life produces a (somewhat) anxious outcome whereas the same conditional knockout has opposite effects during adulthood (Ansorge et al., 2004; Gross et al., 2004; Holmes et al., 2003; Homberg et al., 2009; Leonardo et al., 2006).

Taken together, these findings demonstrate that pronounced HPA activity and / or low affiliative behavior originate in part from deficiencies in serotonin function.

Similar findings have been reported for a naturally-occurring polymorphism within the promoter region of the *SLC6A4* gene, which is analogous to the SERT knockout subjects, and is responsible for producing the 5-HTT protein. In turn, 5-HTT is responsible for regulating the amount of serotonin available at the synaptic space, by reabsorbing any excess serotonin (Murphy et al., 2004). The *5-HTT*LPR polymorphism consists of short (*s*) and long (*l*) alleles that differ in the number of repeating elements (Heils et al., 1996). A homologous polymorphism has been identified in rhesus macaques called the serotonin transporter gene promoter region (rh*5-HTT*LPR) , consisting of a 21 base pair insertion / deletion event that gives rise to *s* and *l* alleles (Lesch et al., 1997).

In both species, the *s* allele confers a decrease in the level of transcriptional efficiency of 5-HTT relative to the more common *l* allele *in vitro* (Bennett et al., 2002; Heils et al., 1996). Some (Kalin et al., 2008), but not all studies have reported

differences in serotonergic activity in the living brain (Christian et al., 2009; Shioe et al., 2003), yet both human and macaque studies have documented structural and functional differences in prefrontal, limbic, and anterior cingulate circuitry in carriers of the *s* allele (Hariri et al., 2005; Hariri et al., 2002; Jedema et al., 2009; Pezawas et al., 2005). This suggests the intriguing possibility that the primary effects of 5-*HTT*LPR on behavior has been through lasting effects on the morphological development of the brain.

Recently, rh5-*HTT*LPR has been linked to differences in social buffering in rhesus macaques. McCormack et al. (2009) found that at two months of age, infant carriers of the *s* allele displayed increases in cortisol in the presence of the mother while no rise was found in subjects homozygous for the *l* allele. These differences in HPA activity appeared to be due to the fact that *s* allele infants were held less by their mothers and displayed more behavioral distress in the novel testing environment. However, these findings should be interpreted cautiously due to the small sample size and to the confounding effects of maternal abuse experienced by some of the infants.

In a similar vein, Bethea et al. (2004) tested nearly 100 rhesus macaque infants and found a higher level of fear-grimaces, lipsmacking, and passivity when subjects homozygous for the *s* allele were placed in a novel environment with their mothers compared to infants heterozygous or homozygous for the *l* allele. Yet, no significant genotype differences were found in the amount of time that infants spent away from their mothers. Thus, this effect appears to result from the mere presence of a mother figure rather than from affiliative behavior. Additionally, juvenile rhesus macaques with one or more copies of the *s* allele tend to display less social play

compared to subjects homozygous for the *l* allele (Barr et al., 2003), but similar genotype differences were not found for children in a laboratory playroom environment (Fox et al., 2005; Schmidt et al., 2002).

Interestingly, effects of rh*5-HTT*LPR on social behavior may be due to differences in the ability to interpret social information. In one recent study with rhesus macaques, carriers of the *s* allele spent less time looking at the eye region of another monkey's photograph and were less likely to view images of other high status males compared to subjects homozygous for the *l* allele (Watson et al., 2009). Also, the presentation of a high status male's photograph also led to greater dilation of the same subjects. This suggests that *s* allele subjects may display less assertive social strategies due to heightened levels of attention, fear or anxiety (Hariri et al., 2002; Jedema et al., 2009).

One issue that remains unresolved is the extent to which *5-HTT*LPR effects vary depending on the severity of environmental stressors or social variables. For instance, higher ACTH and cortisol levels have been noted in adult carriers of the s allele compared to adults homozygous for the *l* allele under both baseline (Chen et al., 2009; Wust et al., 2009), and stressful conditions (Gotlib et al., 2008). A similar association has been found for the *s* allele and higher cortisol levels, along with impaired coping skills in rhesus macaques subjected to a social separation (Barr et al., 2004b; Spinelli et al., 2007). However, these genotype effects may have emerged due to a reduction in HPA activity that has been noted across repeated social separations in rhesus macaques (Erickson et al., 2005; Gunnar et al., 2008).

Additionally researchers have noted *5-HTT*LPR genotype differences in temperament across species in the absence of familiar social companions. For example, higher levels of negative emotionality have been noted in human infants as reported by their mothers (Auerbach et al., 1999), a finding that is likely affected by the quality of the mother-infant relationship. However, findings across species argue against this possibility. For example, when tested away from their mothers, rhesus macaque infants with the *s* allele displayed poor state control over emotions compared to infants with both copies of the *l* allele (Champoux et al., 2002), and children with one or both copies of the *s* allele displayed higher levels of shyness again compared to children homozygous for the *l* allele (Battaglia et al., 2005). Several meta-analyses also have reported links between the *s* allele and personality traits such as Neuroticism and Harm Avoidance in adults (for a review, see Hariri et al., 2006).

However, it is important to point out that not all studies have found associations between *5-HTT*LPR and social behavior (Dettmer, 2009; Izquierdo et al., 2007; Rogers et al., 2008; Schmidt et al., 2002), personality traits involving fear or anxiety (Canli et al., 2007; Hariri et al., 2005), behavioral displays (Bethea et al., 2004), or the incidence of depression (Risch et al., 2009). Collectively, there is mixed evidence linking *5-HTT*LPR to stress and social variables.

G X E Interactions

Research on G X E interactions involving *5-HTT*LPR has largely focused on testing two competing theoretical models. The diathesis-stress model argues that

exposure to early adversity exacerbates the effects of a genetic vulnerability or 'diathesis' such as the *5-HTT*LPR *s* allele (Sameroff, 1975; Thomas et al., 1970; Wachs, 1983). More recently, Belsky (1997; 2005) developed an alternative model of differential susceptibility, where carriers of the *s* allele are hypothesized to be more sensitive to the effects of both adverse and supportive early experiences. In other words, the *s* allele may both be a risk factor in adverse environments and source of advantage in supportive environments. Given that social buffering is impacted by early adversity as well as *5-HTT*LPR, it seems promising that G X E interactions will be involved in social buffering.

Researchers have found evidence of G X E interactions involving the *5-HTT*LPR for a variety of outcomes relevant to social buffering. Turning first to the diathesis-stress model, social support has been found to buffer the effect of the *s* allele on shyness (Fox et al., 2005). In this study, researchers found that carriers of the *5-HTT*LPR *s* allele were more likely to be reported as shy by their mothers, and to display reticent social behaviors around their peers, but only if their mothers received low levels of social support. The impact of a hurricane on the likelihood of depression also appears to be buffered by the presence of social support (Kilpatrick et al., 2007). Similarly, exposure to foster care has been linked to higher rates of depression in individuals homozygous for the *s* allele, but not if they reported having a supportive mentor growing up (Kaufman et al., 2004).

Recently, higher levels of social support have been linked to lower levels of depression in adults homozygous for the *s* allele (Taylor et al., 2006; Way et al., 2010). Furthermore, many studies, but not all (e.g., Risch et al., 2009), have found

that fewer stressful life events can mask the vulnerability of the s allele to rates of depression in adults (Caspi et al., 2003). The Risch et al. met analysis bears further mention, as there were no significant G X E interactions or even main effects on levels of depression. This lack of replication suggests the possibility that genetic and experiential factors may operate via independent biological mechanisms. Thus, there is compelling but inconclusive evidence for an association between the *s* allele and poor outcomes.

In parallel work with nonhuman primates, researchers have noted less social play, and higher levels of irritability, aggression, and alcoholism for heterozygous subjects, particularly if they had been peer reared (Barr et al., 2003; Barr et al., 2004a; Kraemer et al., 2008). During a social separation, an event that is known to be a potent stressor, carriers of the *s* allele displayed impaired coping skills relative to subjects homozygous for the *l* allele (Spinelli et al., 2007). To my knowledge, support for the differential susceptibility model is currently lacking in rhesus macaques, but this may have more to do with the fact that researchers largely focus on studying the effects of adversity rather than enrichment on development (Belsky, 1997; 2005). In sum, studies at both the biological and behavioral levels demonstrate that a history of early adversity exacerbates effects of the *s* allele in rhesus macaques.

Furthermore, research with nonhuman primates is providing some fascinating insights into the biological mechanisms underlying G X E interactions. For one, reduced transcriptional efficiency of the *s* allele compared to the *l* allele has only been found in peer reared subjects (Bennett et al., 2002). In the same study, researchers noted a G X E interaction on concentrations of the serotonin end-product 5-HIAA:

Heterozygous subjects had lower 5-HIAA levels, but only if they were peer-reared. This suggests that early adversity may exacerbate the effects of reactive temperament via changes in the serotonin system. With regard to HPA activity, a study in rhesus monkeys has also documented higher ACTH and lower cortisol levels after a social separation in heterozygous subjects, but again only if they had been subjected to maternal deprivation (Barr et al., 2004b), or abuse (McCormack et al., 2009). Taken together, G X E interactions involving rh*5-HTT*LPR have been demonstrated for a variety of outcomes relevant to social buffering.

Summary

The aim of this review has been to discuss what is known about social buffering in humans and animals, with a particular emphasis on studies carried out in rhesus monkeys. The chapter began by describing the affectional systems and social skills that appear to be influencing social buffering. Then studies examining the ontogeny of social buffering were reviewed. Subsequently, early social adversity was identified as a factor that compromises social buffering, and its component social skills. In the last section, the role of the serotonergic system on social buffering was reviewed. In particular, the discussion focused on the effects of impoverished early social experience on serotonergic function, the role of *5-HTT*LPR, and the likelihood of G X E interactions arising from impoverished social experience and *5-HTT*LPR.

Statement of the Problem

Why do some individuals display deficits in social buffering? Part of this answer appears to be a history of impoverished early social experience. Winslow et al (2003) demonstrated that a history of peer rearing produces social buffering deficits in rhesus monkeys. Another likely contributor is genetic variation. This seems likely given the wide variation in outcomes noted for individuals reared in impoverished early social environments (Rutter, 2006; Zeanah et al., 2006). Studies also have found that behaviorally inhibited infants depend more on responsive caregiving from their mothers, and display deficits in social buffering when they receive unresponsive caregiving (Nachmias et al., 1996; Spangler et al., 1998; Suomi, 1987). Also, studies in multiple species have documented that those individuals with the *5-HTT*LPR *s* allele are less likely to seek companionship in stressful environments and are more likely to display elevated levels of fear behaviors and cortisol (Barr et al., 2003; for a review, see Iarocci et al., 2007; Nachmias et al., 1996; Spangler et al., 1998; Way et al., 2010; Weinstein et al., 2008). However, there is little direct evidence of genetic influences on social buffering in a controlled environment.

Additionally, social buffering deficits may reflect the action of G X E interactions. The heterogeneity noted in most populations of subjects with a history of impoverished early social experience raises the possibility that genetic vulnerabilities compromise social buffering by exacerbating the effects of impoverished early experience. Given that prior studies in macaques have reported both independent and joint effects of genetics and social adversity on social, affective, and neuroendocrine

outcomes, it seems promising to investigate whether G X E interactions influence rates of social buffering.

Finally, we also know very little about the role of communication skills in social buffering. For instance, social buffering deficits may be due to a reduced motivation to interact socially, elevated anxiety levels, and / or deficits in social skills. Studies to date have found associations between poor social buffering and deficits in social competence (Kikusui et al., 2006), as well as differences in the use of behavioral displays (Kamarck et al., 1990; Rukstalis et al., 2005; Wilson, 2000, 2001). However, more studies are needed to investigate whether affiliative behavior mediates the impact of behavioral displays on stress responses. Collectively, there is a pressing need for studies designed to investigate the origin of individual differences in social buffering.

Overview of the Current Study

Based on a review of the social buffering literature, the serotonin system was identified as a potential factor leading to variations in rates of social buffering (Figure 2). One source of variation within the serotonergic system is the *s* allele of the serotonin transporter gene polymorphic region, rh*5-HTT*LPR (Lesch et al., 1997). Approximately 31 percent of captive rhesus macaque populations possess one or more copies of the *s* allele at rh*5-HTT*LPR (Barr et al., 2003; Champoux et al., 2002; Spinelli et al., 2007; Way et al., 2010). The *s* allele has been linked to higher levels of distress, poor coping responses, social reticence and impulsive aggression (Bethea et al., 2004; Champoux et al., 2002; Kraemer et al., 2008; Lesch et al., 1997). However,

little is currently known about associations between the *s* allele and social buffering. Therefore, the first aim of the current study is to examine the impact of the rh*5-HTT*LPR *s* allele on rates of social buffering.

Another powerful factor impacting rates of social buffering appears to be impoverished early social experience (Hennessy, 1984; Higley et al., 1992a; Winslow et al., 2003). Winslow et al. (2003) reported social buffering impairments of neuroendocrine function and abnormal behavior in rhesus macaques reared with continuous exposure to peers compared to socially reared subjects. However, they did not analyze which component emotional systems make up the abnormal behaviors exacerbated by peer rearing during social buffering. Studies of peer rearing have documented higher levels of arousal behaviors, such as elevated distress, and a greater incidence of self-directed behaviors (Higley et al., 1992a; Higley et al., 1996a), as well as fearfulness, as demonstrated by increased reticence, and elevated startle responses (Sanchez et al., 2005; Suomi, 2004a). Therefore, it appears that both arousal and fear based behaviors are impacted by peer rearing, yet it is currently unknown whether one or both systems underlie social buffering deficits in peer reared subjects. For these reasons, aim two is to identify the type(s) of abnormal behaviors most strongly impacted by peer rearing within the context of social buffering.

Recent work strongly suggests that that the *s* allele also may present a risk-factor for poor social buffering in conjunction with a history of early adversity (Barr et al., 2003; Bennett et al., 2002; Caspi et al., 2003). Evidence of social buffering deficits in subjects with a history of impoverished early social experience has been noted (Winslow et al., 2003), along with poor social skills, higher distress, impaired

coping mechanisms, and elevated neuroendocrine function evidence in socially-deprived carriers of the *s* allele (Barr et al., 2003; Champoux et al., 2002; Spinelli et al., 2007; Suomi, 2004b). Therefore, the third aim of this study is to test for G X E interactions involving rh*5-HTT*LPR and rearing history on rates of social buffering.

An additional factor that may underlie social buffering deficits is poor communication skills. More specifically, an impoverished early social environment and / or genetic vulnerability factors, such as the rh*5-HTT*LPR *s* allele, may compromise the ability of an individual to initiate or respond to behavioral displays effectively, thereby making social buffering less likely to occur. Complicating this issue is the difficulty of determining when a behavioral display (e.g., a vocalization) functions as a social solicitation cue or as an affective expression. It can be argued that if the incidence of behavioral displays during social buffering differentiates social buffering abilities, then it provides support for the interpretation that the displays are being used as social solicitation cues during social buffering. Therefore, the fourth aim of this study is to compare rates of social buffering between high and low display groups of subjects.

Finally, little is known about the potential contributions of contingent responsiveness and social information processing skills to social buffering between peers. In typical rearing environments, infants experience contingent responsiveness with their mothers and peers almost continuously (Van Egeren et al., 2001). This suggests that under normal circumstances, behavioral displays become positively reinforced by affiliative responses from social partners (Skinner, 1938). By contrast, infant monkeys growing up without adult role models and / or peers may lack the

same history of contingent responding, and / or their behavioral displays may be negatively reinforced with agonistic behavior. As a result, subjects with a history of impoverished early social experience may learn to appraise behavioral displays as threats rather than as affiliation bids or safety signals which are defined as sensory cues with direct stress-alleviating properties (Figure 3).

This interpretation is supported by a study in which monkeys with a history of impoverished early social deprivation responded to behavioral displays with disproportionately aggressive or fearful acts rather than affiliative acts (Kempes et al., 2008). Genetic vulnerability factors such as the *s* rh*5-HTT*LPR allele also may be associated with reductions in contingent responsiveness, due to a heightened threat bias originating in increased excitability within fear anxiety circuits (Fox et al., 2007). Therefore, the fifth aim of the current study is to employ lag sequential analysis techniques to examine individual differences in rates of contingent responsiveness during social buffering (Bakeman et al., 1997). Rearing and genotype differences in contingent responsiveness will also be examined in order to investigate the origins of social buffering deficits.

Research Questions

1) Does the rh*5-HTT*LPR polymorphism influence social buffering?

   Hypotheses:

   a) The long allele but not the short allele group was expected to display lower levels of cortisol, arousal, and fearfulness in the presence of a companion compared to a separation condition.

b) The long allele but not the short allele group was expected to display higher levels of passivity in the presence of a companion compared to a separation condition.

c) The long allele group is hypothesized to display more affiliation and behavioral displays than the short allele group.

2) Does a history of peer rearing impact social buffering?

Hypotheses:

a) The mother reared but not the peer reared group was expected to display lower levels of cortisol, arousal, and fearfulness in the presence of a companion compared to a separation condition.

b) The mother reared but not the peer reared group was expected to display higher levels of passivity in the presence of a companion compared to a separation condition.

c) The mother reared group was hypothesized to display more affiliation and behavioral displays than the peer reared group.

3) Does a history of peer rearing exacerbate effects of the rh*5-HTT*LPR *s* allele on social buffering?

Hypotheses:

a) The peer reared long allele but not the peer reared short allele group was expected to display lower levels of cortisol, arousal, and fearfulness in the presence of a companion compared to a separation condition.

b) The peer reared long allele but not the peer reared short allele group was expected to display higher levels of passivity in the presence of a companion compared to a separation condition.

c) The peer reared long allele group was hypothesized to display more affiliation and behavioral displays than the peer reared short allele group.

4) Do low levels of behavioral displays impact rates of social buffering?

Hypotheses:

a) The high display but not the low display group was expected to display lower levels of cortisol, arousal, and fearfulness in the presence of a companion compared to a separation condition.

b) The high display but not the low display group was expected to display higher levels of passivity in the presence of a companion compared to a separation condition.

c) The high display group was hypothesized to display more affiliation than the low display group.

5) Are social buffering deficits due to impairments in contingent responsiveness?

Hypotheses:

a) After sending a social cue, subjects are hypothesized to receive affiliation more often than any other behavioral response from their partner (contingent responsiveness).

b) Lower levels of contingent responsiveness are expected in the peer reared versus the mother reared group, the short allele versus the long allele group, and the peer reared short allele versus the peer reared long allele group.

c) Contingent responsiveness is expected to be a stronger predictor of low cortisol levels during the social condition than affiliative events or behavioral displays separately.

CHAPTER 3: METHODS

Subjects

The current study consisted of 32 juvenile male rhesus macaques (*Macaca mulatta*) acquired from four birth cohorts (2004 to 2007) at the Laboratory for Comparative Ethology in Poolesville, MD. These data were collected as part of a larger study being carried out by the Nonhuman Primate Core facility at the National Institute of Mental Health, as part of an ongoing longitudinal study of brain and behavioral development. All research was approved by the Institutional Animal Care and Use Committee at the National Institutes of Health, and by the Institutional Animal Care and Use Committee at the University of Maryland College Park.

Rearing History

Half of the subjects assigned to the study were mother reared (n=15) and half had a history of peer rearing (n=16).Subjects were assigned to either rearing condition on a random basis, except for the rare circumstance when exceptions were made to balance out the gender makeup of nursery cohorts. However, this selection bias was of no consequence when analyses were performed across multiple cohorts of infants.

For the first six to seven months after birth, mother reared infants were reared by their biological mothers in social groups consisting of approximately six to eight mothers and their infants, as well as two adult males (Barr, et al., 2008). These mother reared groups were housed in large indoor/outdoor runs measuring 2.44 x 3.05 x 2.21 m (inside room) and 2.44 x 3.02 x 2.44 m (outside room), and all animals received daily enrichment as standard practice .

Peer reared subjects were removed from their mothers within the first two days after birth and were raised in a nursery according to standardized procedures (Shannon et al., 2005). For the first two weeks after birth, infants were housed individually in plastic incubators that were maintained at a temperature of around 27º C and 50-55º percent humidity. The floor of the incubators were covered in fleece and infants were each provided with a surrogate 'mother' made of a wire cylinder wrapped in an electric heating pad and covered with fleece. Infants were hand fed a diluted mixture of Similac (Ross Laboratories, Columbus, OH) and Primilac (Bio-Serv, Frenchtown, NJ) formulas by human caretakers until they were old enough to feed themselves, and a bottle of milk was strapped to the cage at all other times. At two weeks of age, the infants were transferred with their surrogates to larger individual cages within the nursery.

Approximately 35 days after birth, peer reared infants were placed in social groups with three other similarly-reared age mates. Between six and seven months after birth, infants were subjected to a series of social separations. For mother reared infants, the separation paradigm consisted of the mothers being removed from the social group for four days from Monday through Friday morning, followed by a three-day (weekend) reunion in between (Erickson et al., 2005). For the peer reared infants, partitions were added between the four quadrants of the homecage, preventing the infants from seeing or making physical contact with each other (Spinelli et al., 2007). These separations were performed for four consecutive weeks.

At approximately eight months of age, all mother and peer reared infants from that particular year's birth cohort (approximately 60 subjects) were removed from

their social groups and housed together in one of two large social groups, along with infants from other impoverished rearing histories (Dettmer, 2009). One enclosure was an indoor-outdoor circular corncrib, measuring 5.03 m wide by 5.49 m tall, and the other was an enclosure consisting of two indoor rooms measuring 7.3 x 3.4 x 3.7 m. Both enclosures contained perches, swings, and other forms of enrichment. The groups were rotated between the two enclosures as needed by the animal care staff.

Approximately 2 years after birth, subjects in the current study were removed from the mixed social groups and housed indoors with one familiar age mate of the same gender and rearing history.

Genotyping

Genotyping for the serotonin transporter gene promoter region (rh*5-HTT*LPR*)* was performed using standard procedures either by the California National Primate Research Center (Kinnally et al., 2008) or at the New England Primate Center (Vallender et al., 2008). Whole blood samples were collected from the subjects when they were anesthetized with ketamine (15 mg/kg), and then DNA was extracted from white blood cells. Standard primers were used to amplify the rh*5-HTT*LPR region. Gel electrophoresis was used to separate the amplicons, and ethidium bromide staining was used to identify the short (*s*=388 bp) and long (*l*=419 bp) alleles. Subjects were classified as possessing the *l/l*, *l/s* or *s/s* genotype. See Table 2 for the distribution of genotypes in the current study. The *s/s* genotype was so rare (*n*=1) that it was analyzed together with the *l/s* genotype, forming a short allele group, while subjects with the *l/l* genotype comprised the long allele group. Approximately 36% of

the subjects possessed at least one copy of the short allele, a distribution that is in accord with the Hardy-Weinberg equilibrium (Barr et al., 2004b). The degree of relatedness between subjects in the colony at the Laboratory for Comparative Ethology has previously been determined to be less than 1.45%, a value that is comparable to many human studies.

Novel Cage Test

When subjects were approximately 2-years old, they were subjected to Novel Cage Tests, both alone (separation condition), and with their homecage partner (social condition) on separate days (Winslow et al., 2003). The order of the conditions was counterbalanced across the sample. Subjects remained in the cage for 20 minutes, a time period that has found to be long enough to obtain peak levels of cortisol after exposure to a stressor (Ulrich-Lai et al., 2009). Testing was carried out between the hours of 10:15 a.m. and 2:30 p.m. The subjects were videotaped using a digital videocamera facilitating subsequent behavioral coding and data analysis, and then blood samples were collected immediately after testing ended.

Plasma Cortisol

After the conclusion of the Novel Cage Test, subjects were anesthetized with Ketamine (5mg/kg) and Telazol (4-5 mg/kg) (Winslow et al., 2003). Immediately after the anesthesia took effect, blood samples were drawn from each of the subjects; the interval between the end of the Novel Cage Test and biofluid collection was no longer than 5 min. During the month leading up to the Novel Cage Test, blood

samples were also collected straight outside the homecage at 0600 h, 1200 h, and 1800 h. Given the slow-acting nature of the HPA system, the homecage samples served as baseline values of cortisol activity (Ulrich-Lai et al., 2009). Blood samples were immediately placed on ice, then centrifuged at 4°C and stored in a -80°C freezer. Cortisol levels were assayed using an ELISA kit (CalbiotechTM; Catalog no. CO103S). Assays were run in triplicate, and acceptable intra assay coefficients of variation were obtained (<10 percent). Finally, cortisol levels were computed using a log- logit equation that incorporates standards as well as optical density values.

Ethogram

Behavioral data was scored from previously collected media files using Observer 5.31 (Noldus, 2006). A comprehensive ethogram developed by Winslow and colleagues (2003) was used to score a variety of social and non-social behaviors (see Appendix). Each subject's behavioral states comprised a mutually exclusive and exhaustive set of activities. A number of behaviors were scored as point events without duration and were scored whenever they occurred.

Preliminary Data Analysis

Data Management

Before beginning this project, a plan was developed to systematically name and organize data files acquired at different stages. A written record of the most important tasks and decisions carried out has been maintained throughout the project. During the data acquisition phase, observational data files from the inter-rater

reliability process were stored in separate folders for each scorer. Observational data files (including media files) were labeled before scoring began with random numbers ranging from S01 to S32 in order to ensure blind scoring. For the social data files, both the animal's collar color and the partner's number were added to the observational data file label (e.g., S0130_YELLOW.odf). Information identifying the subjects was sealed until after scoring was complete. Several media files terminated early, and the behavioral data from these files were normalized to 20min before running any analyses. All analyses were performed on copies of master data files, and corresponding syntax was retained. Pertaining to the sequential analysis, copies of the edited observational data files, the corresponding compilation files, and all syntax needed to recreate the contingency variables were retained.

Observer Training and Reliability

Twenty percent of the digital video files were scored by two coders. Inter-rater reliability was assessed by calculating Cohen's Kappa scores for the following behavior composites: behavioral displays, affiliation, arousal, fearfulness, and passivity (Cohen, 1960). Only after obtaining acceptable levels of inter-rater reliability ($\kappa \geq .70$) were remaining media files scored (Bakeman et al., 1997). For the social condition, Cohen's Kappa scores ranged from $\kappa = .71$-$.91$. For the separation condition, Kappa scores ranged from $\kappa = .77$-$.89$.

Creation of Composite Variables

Composite variables were generated by taking into account the correlation matrix for the raw variables, as well as considering how other studies have grouped similar variables (Erickson et al., 2005; 2003). The composites were formed by summing all the component variables of interest. The following behavior composites were employed in the study: behavioral displays, affiliation, arousal, fearfulness, and passivity (Table 1). Fearfulness and passivity were also analyzed together as a fear-withdrawal composite. However, passivity appeared to obscure the effects of fearfulness. Therefore, the effects of the two composites are reported separately.

Descriptive Statistics

Before running any analyses, descriptive statistics (central tendency, spread, and shape) for age, cortisol, and the original and composite variables were examined to determine whether the variables had adequate variance, and to identify outliers greater than +/-2 *SD* (Tables 1 & 2). As duration and frequency measures were positively and significantly correlated during both the social and separation conditions, only results of duration measures will be reported (Table 3). Across groups, all of the composite variables possessed adequate variance and did not fail normality tests (Table 4). Unless otherwise mentioned, all analyses were run using SPSS v17.0 (SPSS Inc., Chicago, IL).

One mother reared *l/l* subject (ZF49) was excluded from the analyses due to an abnormal rearing history: unlike the other mother reared subjects, this individual had less exposure to peers, no social separations, and no experience living in a mixed

rearing group (M. Schwandt, & M.F.X. Novak, personal communication, January 27, 2009). This subject also had extreme scores greater than +/-1.5 *SD* from the mean for fearfulness, passivity, and affiliation.

Data Analysis Part 1: Group Differences in Social Buffering

Multivariate Analysis of Variance

To examine social buffering influences on the construct of stress, a multivariate analysis of variance (MANOVA) was employed. This technique is useful means for controlling Type 1 (false positive) error rates. The dependent measure, stress, comprised the following four variables: cortisol, arousal, fearfulness, and passivity. Between-subjects variables included rh*5-HTT*LPR (long vs. short), and rearing (mother vs. peer). The within-subjects variable was condition, which allowed for comparisons of stress levels across separation and social conditions. Bonferroni-corrected post hoc comparisons were employed to decompose significant interaction effects. Only after obtaining a significant multivariate effect was analysis of variance (ANOVA) used to investigate group differences in the individual stress measures.

Analysis of Variance

To analyze social buffering influences on each of the stress behavior composites separately (cortisol, arousal, fearfulness, and passivity), a series of mixed-effects ANOVAs were employed. For the cortisol analysis only, a baseline measure of cortisol was also included as a third level of the condition variable, making it possible to examine changes in stress levels due to the Novel Cage Test stressor. To

test for group differences in affiliation and behavioral displays, a factorial ANOVA with genotype and rearing as the between-subjects factors was conducted. Similar Bonferroni-corrected post hoc comparisons were employed to decompose significant interaction effects.

Creation of Behavioral Display Groups

Behavioral display groups were formed by classifying each subject into a high or a low display group. A cutoff score of five behavioral displays was used to differentiate subjects into the high and low display groups. The high display group did not disproportionally reflect genotype, rearing, or G X E group membership (Table 10).

Behavioral Display Group Analyses

To analyze behavioral display group differences in social buffering, a similar sequence of MANOVA followed by mixed-effects ANOVAs was employed. The between-subjects variable was display group (high vs. low display groups), and the within-subjects variable was condition, allowing for comparisons of stress levels across separation and social conditions. For cortisol only, a baseline measure of stress reactivity was also included in the analysis. Analyses of group differences in affiliation employed a one-way ANOVA using behavioral display (high vs. low display groups) as the between-subjects variable. Similar Bonferroni-corrected post hoc comparisons were employed to decompose significant interaction effects.

Power Analysis

Before the study began, an *a priori* power analysis was run in G*Power 3.0 in order to evaluate the power associated with the available sample size (Bakeman et al., 1997). To assess group differences as well as effects of condition, G*Power recommended a minimum sample of 32 subjects with >.70 minimum power and a small effect size (Cohen's *d*=.25) as parameters. Thus, the design supplies adequate power for identifying group differences in social buffering.

Data Analysis Part 2: The Impact of Contingency on Social Buffering

Lag Sequential Analysis

Lag sequential analysis was used to investigate contingencies between behavioral displays and receiving an affiliative response from a partner. More specifically, an event-lag approach was used to generate odds ratios between behavioral displays and affiliative responses in the General Sequential Querier Program (GSEQ) (Bakeman et al., 1997). A lag of +1 was used for these analyses, which involves examining the number of times that a particular target behavior directly follows a given behavior.

Generating Odds Ratios

GSEQ was used to generate the following four variables for each subject: (a) the number of times behavioral displays led immediately to receive affiliation; (b) the number of times behavioral displays led directly to non affiliation; (c) the number of other non behavioral display events preceding affiliation from partner; and (d) the

frequency of all other behavioral transitions (Bakeman et al., 1997). These four variables (a through d) comprised a 2 x 2 matrix of values that was used to generate a separate odds ratio (OR) for each subject in SPSS (Figure 14).

As recommended by Bakeman and Gottman (1997), when subjects had fewer than five instances of behavioral displays or receive affiliation, the OR was regarded as missing for that subject. It was important to discard subjects with few behavioral displays because ORs tend to be highly skewed with fewer than five instances of either a given or target behavior. A majority of subjects (66 percent) had sufficient data to calculate an OR, and the percentage of subjects did not differ with regard to rearing, genotype, or G X E group membership.

Before running any analyses, descriptive statistics for the raw ORs were examined. Because ORs extend from zero to positive infinity, they are almost always positively skewed (Cote et al., 2008). For this reason, the ORs were subjected to the square root and log 10 transformations. The variable best approximating a normal distribution was selected for use in all subsequent analyses: the log odds ratio (LOR).

Log Odds Ratio Analyses

Following Cote et al. (2008) a one sample *t*-test was employed to investigate whether LORs differed significantly from zero. If a LOR is significantly greater than zero, it signifies that behavioral displays were more likely to be followed by an affiliative response from a partner than any other behavioral response, and provides support for contingent responsiveness (Bakeman et al., 1997). One sample *t*-tests were also run separately for each group (mother reared, peer reared, long allele, and

short allele). A factorial ANOVA with rearing (mother vs. peer reared) and genotype (long vs. short allele) as the between subjects variables, as well as independent groups *t*-tests were also employed to examine group differences in the LOR for contingent responsiveness. As a last step, Pearson product-moment correlations were examined between the LOR, receive affiliation, behavioral displays, and the cortisol variables.

Temporal Contingencies

An exploratory analysis was carried out to investigate whether behavioral displays and affiliative events were synchronized in time, in order to better understand the role of behavioral displays during social buffering. While this analysis does not address whether these sensory events are used as cues, or are simply a form of affective expression, this temporal contingency may provide a useful indicator of social buffering proficiency. Using the techniques described above, an LOR was generated between behavioral displays and all affiliative events, without send or receive information. Subsequently, Pearson product-moment correlations were analyzed, followed by hierarchical regression models predicting cortisol against the LOR, after controlling for baseline cortisol, rearing, and genotype.

CHAPTER 4: RESULTS

Results Part 1: Group Differences in Social Buffering

The results in this section were produced from a MANOVA for the stress construct, and a series of mixed-effects ANOVAs for each stress measure separately, as described in the methods section, Data Analysis Part 1. First, results of genotype and rearing on rates of social buffering are outlined. Then effects of behavioral displays (high vs. low display) on rates of social buffering are reported. Descriptive statistics for the composite variables across groups are reported in Table 4.

Effects of Rearing and Genotype on Stress Construct

Results of the MANOVA for the construct of stress are summarized in Table 5. The main finding of this analysis was a Rearing X Condition Interaction on the stress construct, $F(1,27)=9.48$, $p<.01$, partial $\eta^2=.26$. Post hoc comparisons revealed that both the mother reared and peer reared groups displayed lower levels of stress in the presence of a companion compared to a separation condition, *p's*<.001. During the social condition, the mother reared group displayed less stress than the peer reared group, $p<.05$. Additionally, a main effect of Rearing was found, $F(1,27)=8.81$, $p<.01$, partial $\eta^2=.25$, indicating a higher level of stress in the peer reared compared to the mother reared group.

A main effect of Condition was also identified, $F(1,27)=144.88$, $p<.001$, partial $\eta^2=.84$, which was due to lower levels of stress during the social compared to the separation condition. Additionally, a main effect of stress was noted, $F(1.97, 53.26)=61.17$, $p<.001$, partial $\eta^2=.69$, indicating differences between the individual

stress measures during the Novel Cage Test. No main effects or interactions involving genotype were found. For exploratory purposes, due to a particular interest in the role of rh5-*HTT*LPR on social buffering, genotype was included as a between-subjects variable in the mixed-effects ANOVAs for the individual stress measures.

Effects of Genotype

Descriptive statistics for the composite variables broken down by genotype group are reported in Table 6. Genotype effects from the ANOVA tests are reported in Tables 8-10 for each of the dependent measures separately. An Rh5-*HTT*LPR X Condition Interaction was found for fearfulness, $F(1,28)=3.95$, $p=.05$, partial $\eta^2=.12$ (Figure 7). Post hoc comparisons revealed that the interaction was due to the long allele but not the short allele group displaying lower levels of fearfulness in the presence of a companion compared to a separation condition, $p<.05$. No interactions or main effects were found for cortisol or arousal. There was no evidence of any interactions or main effects involving passivity. There were no main effects of rh5-*HTT*LPR for affiliation or behavioral displays.

Effects of Rearing History

Descriptive statistics for the composite variables broken down by rearing group are reported in Table 7. Rearing effects from the ANOVA tests are reported in Tables 8-10 for each of the dependent measures separately. A Rearing X Condition Interaction was identified for arousal, $F(1,28)=9.71$, $p<.01$, partial $\eta^2=.26$ (Figure 6). Post hoc comparisons revealed that both the mother reared and peer reared groups

displayed lower levels of arousal in the presence of a companion compared to a separation condition, *p's*<.001. During the social condition, the mother reared group displayed less arousal than the peer reared group, *p*<.05.

No interactions were found for cortisol or fearfulness, and no main effects of rearing were found for cortisol, arousal, or fearfulness. There was no evidence of any interactions or main effects involving passivity. A main effect of rearing was found for affiliation, such that higher levels of affiliation were observed in the mother reared than the peer reared group, $F(1,27)=8.43$, $p<.01$, partial $\eta^2=.24$ (Figure 8). No main effect of rearing was found for behavioral displays.

Effects of G X E Interactions

G X E effects from the ANOVA tests are reported in Tables 8-10 for each of the dependent measures separately. There was no evidence of any G X E interactions or main effects involving cortisol, arousal, or fearfulness. There was no evidence of any G X E interactions or main effects involving passivity. There was no evidence of G X E interactions or main effects for affiliation or behavioral displays. Visual histograms, depicting the distributions for all G X E groups separately, are illustrated for the stress measures in Figure 9, and the social buffering measures in Figure 10.

Effects of Display Groups on Stress Construct

Results of the MANOVA for the construct of stress are summarized in Table 11. The main finding of this analysis was a Display X Condition Interaction on stress, $F(1,29)=28.85$, $p<.001$, partial $\eta^2=.50$. Post hoc comparisons revealed that both the

high and low display groups displayed lower levels of stress in the presence of a companion compared to a separation condition, *p's*<.001. During the social condition, the high display group displayed less stress than the low display group, *p*<.001. Additionally, a main effect of Display was found, $F(1,29)=30.22$, $p<.001$, partial $\eta^2=.51$, indicating a higher level of stress in the low display compared to the high display group.

A main effect of Condition was also identified, $F(1,29)=186.95$, $p<.001$, partial $\eta^2=.87$, which was due to lower levels of stress during the social compared to the separation condition. Additionally, a main effect of stress was noted, $F(1.84,53.24)=63.84$, $p<.001$, partial $\eta^2=.69$, indicating differences between the individual stress measures during the Novel Cage Test.

Effects of Display Groups

Descriptive statistics for the composite variables broken down by display group are reported in Table 12. Rearing effects from the ANOVA tests are reported in Tables 13-14 for each of the dependent measures separately. A significant Display X Condition Interaction was identified for cortisol, $F(2,56)=4.08$, $p<.05$, partial $\eta^2=.13$. Post-hoc comparisons revealed that the interaction was due to the high and low display groups displaying lower cortisol values during baseline compared to a separation and a social condition, *p*'s <.001. No differences in cortisol levels were found between the separation and social conditions either across the group, or for the high or low display groups separately.

A significant Display X Condition Interaction was also found for arousal, $F(1,29)=6.16$, $p<.05$, partial $\eta 2=.18$. Post-hoc comparisons revealed lower levels of arousal for both the high and low display groups in the presence of a companion compared to a separation condition (p's $<.001$). During the social condition, lower levels of arousal were noted in the high display than the low display group, $p<.01$. A main effect of display was also evident for arousal, such that higher levels of arousal were found in the low display than the high display group, $F(1,29)=5.23$, $p<.05$, partial $\eta^2=.15$ (Figure 11).

Furthermore, a Display X Condition Interaction was present for fearfulness, $F(1,29)=10.90$, $p<.01$, partial $\eta^2=.27$ (Figure 12). Post-hoc tests revealed a decrease in fearfulness from the separation to the social condition for the high display group only, $p<.001$. During the social condition, lower levels of fearfulness were observed in the high display than the low display group, $p<.05$. There was no evidence of any interactions or main effects involving passivity. A main effect of display was found for affiliation, $F(1,29)=31.76$, $p<.001$, partial $\eta^2=.52$, such that higher levels of affiliation were found in the high display than the low display group (Figure 13).

Results Part 2: The Impact of Contingency Measures on Social Buffering

The results in this section are the product of lag sequential analysis techniques and a factorial ANOVA described in the methods section, Data Analysis Part 2. First, results involving a contingency between behavioral displays and affiliative responses are outlined. Second, as outlined in the methods section, exploratory analyses

involving a temporal contingency between behavioral displays and all affiliative events are reported.

Contingent Responsiveness Effects

Table 15 provides the untransformed odds ratios (OR representing contingent responsiveness scores for all groups of interest. Figure 15 illustrates the distributions of the individual contingent responsiveness ORs for the genotype, rearing, and G X E groups separately. Across groups, a one sample *t*-test revealed that the LOR for behavioral displays and receive affiliation was significantly different from zero, $t(16)=3.92$, $p<.01$. On average, subjects were found to receive affiliative responses to behavioral displays more often than any other behavioral response during social buffering. No effects of genotype, rearing, or G X E interaction were found for contingent responsiveness.

When one sample *t*-tests were run for each group separately, the LORs for the peer reared but not the mother reared group, $t(7)=3.48$, $p<.01$, and the long allele but not the short allele group were significantly different from zero, $t(11)=3.13$, $p<.01$ (Table 15). Thus, contingent responsiveness was only evident in the peer reared and long allele groups. When Pearson product-moment correlations were evaluated (Table 16), social cortisol values were uncorrelated with the frequency of behavioral displays, receive affiliation events, and the LOR for contingent responsiveness.

Temporal Contingency Effects

By contrast, the temporal contingency LOR between behavioral displays and all affiliative events was significantly and negatively correlated with social, r=.50, p<.05, and separation cortisol values, r=-.54, p<.05 (Table 16 & Figure 16). Results of a hierarchical regression analysis revealed that the LOR for behavioral displays leading to all affiliative events was a significant predictor of social cortisol values above and beyond the effects of baseline cortisol, genotype, and rearing, R^2=-,34, p=.05 (Table 17). No interactive effects involving the LOR, genotype, and rearing were identified for social cortisol. There was also a trend for the LOR to be predict lower cortisol levels after the separation condition, p<.10.

CHAPTER 5: DISCUSSION

Overview

The purpose of the current study was to investigate the impact of genetic and experiential variables on social buffering between juvenile nonhuman primates. A second aim was to investigate the use of behavioral displays during social buffering, in order to explain social buffering deficits in subjects with a history of early social deprivation (Winslow et al., 2003). Juvenile rhesus macaques, differing with respect to rh*5-HTT*LPR genotypes and early rearing history, were subjected to a 20min Novel Cage Test with and without their homecage partner on separate days, and behavioral and neuroendocrine responses were generated. The behavioral data were subsequently subjected to a lag sequential analysis in order to examine the likelihood of receiving an affiliative response after sending a behavioral display (Bakeman et al., 1997).

This study produced a number of important results. First, higher levels of stress behaviors were noted in the peer reared, short allele, and low display groups. Additionally, the peer reared and the low display groups engaged in less affiliation than the mother reared and high display groups, while no difference in the affiliation composite was noted between the short and long allele groups. Second, across the entire sample, contingent responsiveness was identified as a feature of social buffering, suggesting that behavioral displays are more likely to produce an affiliative response than any other response. Yet no group differences in levels of contingent responsiveness were found. Finally, a measure of temporal contingency involving behavioral displays and all affiliative events predicted lower cortisol concentrations after the social condition, and marginally lower cortisol concentrations after the

separation condition. Therefore, the incidence of behavioral displays during social buffering appears to provide a useful indicator of subjects with a stress resilient temperament.

Social Buffering of Neuroendocrine Activity

The current study found no evidence of social buffering of neuroendocrine activity. These results contradict the findings of Winslow et al. (2003) who found social buffering of cortisol and abnormal behavior in mother reared subjects only.

There are several possible reasons why social buffering of cortisol levels may not have been evident in the current study. To begin with, the lack of replication is unlikely to be due to measurement error. Samples were run in triplicate and low coefficients of variation were obtained which is similar to earlier studies (Winslow et al., 2003). In addition, the cortisol values obtained for both the separation and social conditions were more than double the values obtained at baseline. This indicates that the assays were sufficiently sensitive to detect the effect of environmental stressors.

Probably the most compelling explanation for the lack of replication are features of the mother rearing paradigm currently in place at the NIH Animal Center (Dettmer, 2009; Strand, 2006). In order to compare rearing conditions between the two studies, it is important to begin by describing the mother rearing paradigm employed in Winslow et al. (2003). For the entire first year after birth, mother reared infants were raised by their mothers in large mixed social groups, consisting of other infants, siblings, and adult females and males, at the Yerkes Field Station in Atlanta, GA.

These early experiences in the mother-infant relationship may have been crucial for learning when to rely on a partner for social buffering. In support of this assertion, naturalistic studies of rhesus macaques have found that infants below the age of a year (and even afterwards) tend to remain in close proximity to their mothers, and are also quickly aided by their mothers after the slightest provocation (Suomi, 2005). These months of experience learning to rely on their mothers as a 'secure base' in times of stress, were probably not trivial to their development (Bowlby, 1969). Therefore, it seems likely that the mother reared infants in the earlier study developed more proficient social buffering abilities through the relationships with their mothers, which were then applied to peer relationships during the juvenile phase of development (Harlow, 1969).

By contrast, the mother reared monkeys in the current study were subjected to a number of atypical rearing experiences that may have made them appear more like peer reared monkeys, both in terms of neuroendocrine function and behavior (Figure 4). To begin with, mother reared infants were subjected to a series of repeated short-term social separations around six to seven months of age. Along these lines, investigators have reported that repeated social separations can produce more stress reactive monkeys (Barr et al., 2004b; Higley et al., 1992b), but contrasting findings have also been noted (Erickson et al., 2005; Gunnar et al., 2008). Second, mother reared infants were removed from their mothers after the conclusion of the social separations, and from this point forward, were reared in large social rearing pens along with peer reared and surrogate peer-reared subjects from the same birth cohort (Dettmer, 2009).

Therefore, in contrast to the earlier Winslow et al. (2003) study, the current mother reared infants did not experience having their mothers available to them during the second half of their first year (Harlow, 1969). Also, studies have found that socially-deprived infants become more similar to mother reared subjects when housed together, both in terms of behavior and neuroendocrine activity(Strand, 2006). Therefore, it seems possible that the converse is also true: Through social learning mechanisms, mother reared infants may have adopted some of the patterns of responding typically seen in adversely reared monkeys (Bandura, 1971). Collectively, the non-trivial differences in mother rearing between the two studies provide a compelling explanation for the absence of social buffering at a neuroendocrine level.

Another potential explanation is variation in the time of day when testing occurred. In Winslow et al. (2003), testing was carried out in the early to mid-morning from approximately 9:30 to 10:30 a.m., whereas testing times in the current study ranged from 10:15am to 2:30 p.m., with baseline samples obtained at noon. The circadian rhythm of the neuroendocrine response is well-established in both humans and rhesus macaques. This rhythm typically consists of an early morning rise followed by a decline throughout the day, with effects of social deprivation most pronounced near peak activity levels (Barrett et al., 2009; Gunnar et al., 2007).

At the time of testing in the current study, peer reared subjects may have already been demonstrating ceiling levels of stress reactivity, and the Novel Cage Test may have simply had the effect of elevating mother reared cortisol levels to the point where they masked rearing differences. In support of this proposition, challenge

cortisol levels were more than double those found in a previous study, despite few differences in testing conditions (Winslow et al., 2003).

Subjects in the current study also were approximately one year younger than the subjects in Winslow et al. (2003). Perhaps two-year-old macaques are still in process of transitioning from infancy to the juvenile phase of development, and have not yet developed the ability to buffer neuroendocrine function of a partner? In support of this possibility, Maken et al. (2009) tested for social buffering of neuroendocrine function in domestic guinea pigs at four time points ranging from weaning to adulthood, and only found social buffering of neuroendocrine activity once guinea pigs reached full adulthood. Thus, stage of development may be an important factor in detecting social buffering of neuroendocrine activity.

Finally, the length of the Novel Cage Test also was shorter than Winslow's earlier study by 10min. This difference in timing may seem trivial, but given the sluggish nature of the HPA system (Ulrich-Lai et al., 2009), it is possible that testing did not last long enough to capture a decrease in cortisol levels resulting from affiliative behavior. Taken together, future work is needed on several fronts to investigate why neuroendocrine evidence of social buffering was not found in the current study.

Effects of Genotype

A novel finding of this dissertation is the discovery of an rh*5-HTT*LPR genotype difference on fearfulness that was specific to the social condition. Yet, it is important to mention that this interaction effect was not noted in the multivariate

analysis, but only in the individual analysis when fearfulness was examined separately. Given the number of separate analyses that were performed (cortisol, arousal, fearfulness, passivity, affiliation, and behavioral displays), there appeared to be nearly a one-in-five chance of identifying a false positive outcome (Sankoh et al., 1997). This brings into the question the validity of this interaction effect.

With that said, the validity of the interaction effect between rh*5-HTT*LPR and condition was strengthened by the pattern of results noted in the posthoc comparison tests: The genotype effect was due to the long (but not short) allele group display lower levels of fearfulness in the presence of a companion compared to a separation condition. Furthermore, the genotype effect was specific to fear behaviors, a link that has been well established in both nonhuman and human studies. Furthermore, researchers have noted positive correlations between the *s* allele and increased activity within fear circuitry such as the amygdala (Hariri et al., 2005). Collectively, these factors strengthen the case for rh*5-HTT*LPR genotype influences on social buffering.

What can account for the group differences in rh*5-HTT*LPR on fearfulness during the social condition? Unlike the rearing and display group differences, the genotype effect could not be attributed to variations in affiliation. Rather, the long allele group appeared to benefit more from the physical presence rather than proximity of a partner. This finding is supported by studies in sheep and humans that have found pictures or video of supportive partners to produce social buffering effects (Thorsteinsson et al., 1998). In particular, the presence of a social partner may have led to differences in the patterning of fearfulness events across the social buffering

paradigm. In support of this possibility, the long allele group appeared to take much longer to display the first fear event during the social condition compared to the short allele group (134.25 vs. 57.66 *s*). Taken together, it appears that partner presence may be sufficient for producing genotype differences in fear behavior during the social condition.

It is also important to address why effects of rh*5*-*HTT*LPR genotypes were not found during the separation condition. While earlier studies may have identified main effects and interactions involving rh*5*-*HTT*LPR (Barr et al., 2004b; 2007), these effects may have been due to a habituation effect of repeated social separations. In support of this assertion, rhesus monkeys subjected to repeated social separations have been found to have *lower* rather than higher stress at a behavioral and neuroendocrine level (Erickson et al., 2005; Gunnar et al., 2008). Taken together, this study builds on earlier work on social buffering in rhesus macaques by identifying rh*5*-*HTT*LPR genotype influences on social buffering.

Effects of Rearing History

Similar to Winslow et al. (2003), a rearing difference in the arousal composite was apparent for the social condition that was attributable to differences in the affiliation composite. Thus, multiple studies now report that peer rearing primarily compromises social buffering through its impact on locomotor activity and self-directed actions. Plus, numerous studies have reported higher levels of abnormal behavior in peer reared subjects in stressful non-social settings (for a review, see Nelson et al., 2009).

What can account for these rearing differences in social buffering? For one, an absence of maternal figures in the early social environment may have lead peer reared subjects to rely on alternative coping strategies: Instead of relying on a partner in stressful circumstances, they may engage in self directed acts designed to reduce anxiety (Nelson et al., 2009). Thus, even if the motivation for interacting socially is high, similarly high levels of arousal may produce an approach-avoidance conflict where peer reared subjects find it difficult to overcome an arousing state to the point where they are able to engage in social interaction (Asendorpf, 1991).

Another factor that appears to be impact rearing differences in social buffering is quality of affiliation. Similar to Kikusui et al. (2006), the current study found a higher incidence of grooming and full mounting events in the mother reared compared to peer reared group (13 events vs. 1 event). These findings are consistent with the theory that adult models are necessary to learn the social skills necessary for social buffering, but they may also support the proposition that deficits in social competence are the product of increased anxiety (Goy et al., 1979; Kempes et al., 2008; Lemerise et al., 2000), or perhaps a reduced motivation for social interaction (Nelson et al., 2002). Or perhaps affiliating with a partner is less effective for peer reared monkeys, due to reduced production of neuropeptides such as oxytocin (Nelson et al., 2002)? The low levels of affiliative behavior also support the idea that the degree of social bonding between peer reared subjects may be compromised compared to typically reared subjects.

Effects of G X E Interactions

No G X E interactions involving rearing and rh5-*HTT*LPR genotype were found in the current study. One possible interpretation is that interaction effects were not detected due to inadequate power associated with small sample sizes. In support of this interpretation, the distributions for the G X E groups (Figures 7 & 8) revealed that within the peer reared group, emotionality scores for the fearfulness and arousal composites tended to be lower and more tightly clustered in the long allele compared to the short allele group. By contrast, higher and more tightly clustered scores were noted for affiliation, behavioral displays, and passivity in the long allele compared to the short allele group. These general trends in the data have been supported by numerous G X E interaction studies in both rhesus monkeys and humans for similar measures of emotionality, social interaction, and depression (Suomi, 2004b). However, a recent meta analysis in humans was unable to replicate a G X E interaction for depression, highlighting the need to search for other possible explanations for the current findings (Risch et al., 2009).

For instance, the absence of statistical G X E interactions between rh5-*HTT*LPR genotype and rearing may in fact reflect the true state of affairs where genotype and rearing have independent rather than interactive effects on social buffering. Such an interpretation is supported by the fact that rearing and genotype influenced distinct measures of emotionality, with rearing influencing arousal, and genotype impacting the fearfulness composite.

While both are measures of emotionality, arousal and fear behaviors appear to involve distinct strategies for handling stress, and may operate under different neural

mechanisms (LeDoux, 2000). Regarding arousal, strategies appear to be focused on actions that are designed to mitigate or eliminate a stressor. For example, distress vocalizations emitted by an infant are arousing in the short term, but are a powerful coping strategy for successfully soliciting maternal contact (Levine et al., 1984).

On the other hand, the fearfulness composite involves freezing and withdrawal actions designed to facilitate hiding from predators and conserving energy. In humans, arousal and fear behaviors to stress may be comparable to approach and avoidance coping strategies (Taylor et al., 2007). Thus, arousal and fearfulness appear to reflect distinct strategies for coping with stressful experiences, an interpretation that weakens the case for identifying G X E interactions during social buffering.

Effects of Behavioral Display Groups

The low display group, characterized by fewer than five behavioral displays, displayed deficits in social buffering compared to a high display group. This suggests that the incidence of behavioral displays during social buffering can be used to identify individuals at risk of displaying poor social buffering. Importantly, the composition of the high display group was not predominated by any one genotype or rearing group, suggesting that behavioral displays identify a unique component of temperament.

These results are supported by similar findings in children. Several decades ago, Field et al. (1988) found that children previously characterized as displaying an active coping style were more talkative and active during play with their mothers and

subsequently required a shorter hospitalization after minor surgery. While there were no data on whether these children received more affiliative contact from their mothers, it seems possible that affiliation with mother may have facilitated a faster recovery in these children. Therefore, the incidence of display frequency was linked to differences in social buffering.

Contingent Responsiveness Effects

Across the entire sample, behavioral displays and receipt of affiliation from a partner were used contingently, meaning that behavioral displays were more likely to elicit an affiliative response from the partner than any other behavioral response (Bakeman et al., 1997). This finding demonstrates that behavioral displays and affiliation are synchronized in real time for individual subjects (Cote et al., 2008). Behavioral displays and affiliative events were also significantly correlated in the positive direction (r=.38, p<.05); however, measures of correlation only tell us that variables are related at the group level. Also, it is important to point out that affiliative behavior occurred in the absence of behavioral displays. On average, subjects displayed 21 instances of affiliative events that were not preceded by behavioral displays. On the basis of these data, it appears that behavioral displays are neither necessary nor sufficient to give rise to affiliative events. Taken together, the evidence suggests but does not establish that behavioral displays serve a communicative function during social buffering.

Contrary to expectation, no group differences in contingent responsiveness were found. Therefore, variations in contingent responsiveness do not appear to

account for social buffering deficits. Additionally, the long but not the short allele group displayed evidence of contingent responsiveness. However, this appeared to be due to greater variability rather than a particularly large odds ratio (Table 15). Given the small sample sizes, it is unwise to conclude anything about genotype differences in contingent responsiveness. Taken together, contingent responsiveness does not appear to explain rearing or genotype deficits in social buffering.

When rearing groups were assessed separately, the mother reared group did not display evidence of contingent responsiveness. One possible explanation is that mother reared subjects may be more flexible in their ability to use behavioral displays during social buffering. In other words, they may view responding with affiliation as simply one of a number of possible responses. For instance, in addition to using behavioral displays as bids for affiliation, mother reared subjects may use displays to express contentment after an affiliative state is already taking place. Thus, the flexible behavioral repertoires of mother reared subjects may make it more difficult to detect contingent responsiveness in this group.

Furthermore, no impairments in contingent responsiveness were found for the peer reared group (Cote et al., 2008). Thus, this suggests that social buffering deficits in monkeys with a history of early social deprivation do not result from an inability to integrate behavioral displays and affiliative responses in stressful contexts (Winslow et al., 2003). Perhaps this also indicates a reflexive contingency on the part of peer reared subjects rather than a fully developed ability to employ behavioral displays in appropriate contexts? In support of such an interpretation, when rhesus macaque

infants are reared by humans in lieu of their mothers, they retain the ability to engage in of neonatal imitation during the first week or so after birth (Ferrari et al., 2006).

Alternatively, peer reared subjects tend to be more highly stressed by novel environments (Nelson et al., 2009). As a result, they may be more likely to respond to invitations for social contact under stress, even if the events do not last as long (Harlow, 1969). As Wismer-Fries et al. (2005) found for post-institutionalized children in a social buffering context, there is also the possibility that affiliative behavior is less effective against stress in peer reared subjects. For example, at a physiological level, the same affiliative event may lead to less oxytocin being released compared to mother reared monkeys (Winslow et al., 2003). Therefore, these findings indicate that factors besides behavioral displays account for peer rearing deficits in social buffering.

Temporal Contingency Effects

On another note, the results also revealed that a temporal contingency representing the synchronization of behavioral displays and affiliative events in time predicted significantly lower cortisol levels after the social condition. However, there was also a strong trend for this measure of temporal contingency to predict separation cortisol values, suggesting that this association is probably not causal in nature. Rather than reducing cortisol levels, higher temporal contingency scores may instead provide an indicator to other social partners that a subject is already in a relaxed state. Thus, the measure of temporal contingency appears to provide a sensitive indicator of stress resilient subjects.

Limitations and Future Directions

Several limitations to this study need to be addressed. To begin with, there were several limitations associated with the rh*5*-*HTT*LPR breakdown in the current sample. To begin with, there were very few subjects with the *s/s* genotype (*n*=1), making it difficult to investigate dosage effects of the *s* allele on social buffering. For example, it is possible that the absence of a genotype effect on affiliation may be due to the rarity of *s/s* subjects. Along these lines, some studies only have documented effects of the polymorphism in homozygous recessive monkeys (Bethea et al., 2004). Second, this study combined *l/s* and *s/s* subjects into a short allele group; however, this is not an ideal practice for it makes it difficult to determine whether the *l* or *s* allele predominates in determining outcomes. Finally, the shortage of peer reared *s* allele subjects (*n*=4) resulted in inadequate power for detecting most G X E interactions. Therefore, dosage studies with larger sample sizes are needed to fully test the role of rh*5*-*HTT*LPR to the social buffering process.

Additionally, there were a number of limitations inherent in interpreting the contingent responsiveness measures. To begin with, approximately one-third of the subjects were not included in the contingency analyses because they displayed fewer than five behavioral displays. Had they been included, the odds ratios generated for these individual subjects would likely have been skewed in magnitude (Bakeman et al., 1997). Given this limitation, the most advantageous solution was to exclude these subjects. Insufficient data also made it impossible to investigate whether there were group differences in contingent responsiveness for each behavioral display separately.

Second, this study employed an event-based contingency approach, meaning that the analysis was carried out without respect to the duration of time between given and target events. Yet, preliminary data from this study indicate that on average, behavioral displays were followed by an affiliative response very rapidly (within one second), suggesting that the absence of time information was not a major source of variation. Finally, certain software limitations made it impossible to generate time-lag contingency results. In order to adopt a time-lag approach when estimating contingencies, it would have been necessary to code behavioral displays independently of affiliation in a separate channel configured to capture both frequency and duration information (Cote et al., 2008). The manner in which the data was coded also precluded examining the likelihood of behavioral displays leading to agonistic responses from a partner. Thus, there are a number of ways that future studies can improve upon the contingency methodology employed in the current study.

Future research is also needed to address a number of questions that were raised by the current study. Probably most pressing is the need to identify variables that can account for group differences in social buffering deficits. Along these lines, it may prove valuable to investigate more subtle aspects of social communication such as differences in gaze or imitation. Another promising factor to investigate in nonhuman primates is emotional status or temperament profile of the partner. For example, prior studies in rats have noted bolder behavioral responses when subjects were accompanied by fearless compared to fearful rats (Davitz et al., 1955), as well as social buffering deficits in rats subjected to a startle paradigm and then tested in a

novel cage with and without a partner (Kiyokawa et al., 2004). Altogether, more research is needed to identify the cues underlying group differences in social buffering.

Additionally, there is a need to investigate physiological mechanisms underlying affiliative behavior, particularly as they relate to neuroendocrine function. Keverne et al. (1989) found higher levels of the opioid β-endorphin in non human primates who had engaged in longer periods of grooming. Along these lines, it would be extremely valuable to investigate whether opioids, or neuropeptides such as oxytocin, downregulate neuroendocrine function in a stressful context. Furthermore, given that polymorphisms such as *5-HTT*LPR do not act in isolation (Gottlieb, 2007), and effect sizes with outcomes are thought to be small (Rutter, 2007), it seems important to investigate what role other relevant genes such as brain derived neurotrophic factor (BDNF) have on social buffering. Given the impairments in social buffering noted in peer reared subjects (Winslow et al., 2003), it is important for future studies to examine whether any of these deficits can be reversed by pharmacological agents such as the SSRI fluoxetine. Related to this, perhaps the effectiveness of these agents would vary across different stages of development (Anderson, 2003).

Finally, there is a need to investigate whether ordinary variations in caregiving make lasting contributions to social buffering later in development. Cross fostering paradigms (e.g., Maestripieri et al., 2006; Suomi, 1987) provide researchers with an opportunity to rear infants with foster mothers characterized by high or low quality caregiving and then test them at older ages in stressful contexts with and without a

peer. Studies in both the rhesus monkey and in rodents demonstrate that rearing by highly nurturing “Supermoms” can buffer an infant’s behavioral and neuroendocrine responses to stress (Meaney, 2001; Suomi, 1987). Thus, it seems promising to investigate whether these early caregiving experiences would translate to differences in social buffering with peers later in development.

Conclusions and Contributions

This study was designed to investigate genetic and experiential influences on social buffering, as well as to examine the contribution of behavioral displays to social buffering. The results revealed that the short allele, peer reared, and low display groups displayed impairments in social buffering on a behavioral level. Across groups, contingent responsiveness was identified as a feature of social buffering, but this ability did not explain group differences in social buffering. Finally, a temporal contingency measure predicted lower levels of neuroendocrine function.

This study provides a number of implications for human development. To begin with, the discovery of social buffering of stress behaviors between juveniles, which translates to middle childhood in humans (Suomi, 2004b), supports the idea that childhood friends can be valuable partners for reducing fear or arousal responses in novel or frightening circumstances, such as on the first day of school (Fox et al., 1989), or after a natural disaster (Prinstein et al., 1996). Additionally, the discovery of a genetic risk factor specific to social contexts and supported by similar discoveries in humans (Way et al., 2010), suggests that some stress-related diseases may be partly

social in origin. In particular, individuals carrying one or more copies of the *5-HTT*LPR *s* allele may be predisposed to the effects of stress due to an inability to seek out companionship under stressful circumstances, similar to individuals with a history of abuse or neglect (Repetti et al., 2002). Finally, findings from this study suggest that individuals with superior social skills will display the most resilient outcomes under stress.

In conclusion, this study contributes to our knowledge of the genetic and experiential factors influencing social buffering. Furthermore, it adds to our knowledge of how behavioral displays are used during social buffering, and suggests that interventions designed to improve social skills may provide a means for interrupting developmental trajectories to stress-related diseases.

TABLES

Table 1. *Social Buffering Composites*

Category	Definition
Arousal	Walk/patrol, climb, stereotypy, thumbsuck, autogroom, self-genital exploration, manipulate object
Fearfulness	Hang, crouch, fear grimace
Passivity	Sit
Affiliation	Cling, huddle, side by side, allogroom, full mount
Behavioral displays	Coo, lipsmack, girn, touch (frequency)

Table 2. *Sample Characteristics*

Group	*n* (males/females)	Age in Days (*SD*)
	Genotype	
Long allele	20 (31/31)	694.60(25.31)
Short allele	11 (31/31)	681.73(51.44)
	Rearing	
Mother reared	15 (31/31)	688.87(49.75)
Peer reared	16 (31/31)	691.13(18.48)
	G X E	
Mother reared long allele	8 (31/31)	698.00(35.08)
Mother reared short allele	7 (31/31)	678.43(64.04)
Peer reared long allele	12 (31/31)	692.33(17.58)
Peer reared short allele	4 (31/31)	687.50(23.45)
	Behavioral Displays	
High display	21 (31/31)	690.81(41.60)
Low display	10 (31/31)	688.40(23.93)
Total sample	31 (31/31)	690.03(36.43)

Note. Behavioral display groups were formed by classifying subjects into high or low display groups using a cutoff score of five behavioral displays.

Table 3. *Pearson Correlations for Duration and Frequency Variables*

Variable	1	2	3	4	5	6	7	8
1 Arousal duration (*s*)	-							
2 Arousal frequency	.78**	-						
3 Fearfulness duration (*s*)	.10	-.01	-					
4 Fearfulness frequency	.34	.46**	.71**	-				
5 Passivity duration (*s*)	-.03	.20	.07	.26	-			
6 Passivity frequency	.11	.49**	-.07	.35	.79**	-		
7 Affiliation duration (*s*)	-.73**	-.62**	-.62**	-.71**	-.41*	-.35	-	
8 Affiliation frequency	-.59**	-.31	-.50**	-.32**	-.07	.08	.71**	-

Note. Similar results were obtained for both social and separation condition variables. Due to redundancies, only social condition variables are reported in the above table.
*Significant at the p<.05 level ** p<.01 ***p<.001

Table 4. *Total Sample Means and Standard Deviations*

	Condition		
Variable	Baseline	Separation	Social
Cortisol (µg/dl)	22.22a,b (10.14)	50.39a (26.37)	46.67b (19.04)
Arousal composite	-	703.24c (208.37)	293.72c (199.51)
Fearfulness composite	-	299.79d (177.50)	155.63d (147.55)
Passivity composite	-	192.76 (118.54)	153.41 (110.75)
Affiliation composite	-	-	597.93 (280.84)
Behavioral display composite	-	-	16.45 (17.93)

Note. Significant differences are designated by matching superscripts ($p<.05$).

Table 5. *Summary of MANOVA for Stress Measures*

Source	*df*	*SS*	*MS*	*F*	*p*	η^2
Condition	1	1106549.20	1106549.20	144.88	.00	.84
Condition X Genotype	1	18350.89	18350.89	2.40	.13	.08
Condition X Rearing	1	72372.80	73272.80	9.48	.01	.26
Condition X G X E	1	675.51	675.51	.09	.77	.00
Error (Condition)	27	206218.26	7637.71			
Stress	1.97	6462969.17	3276144.94	61.17	.00	.69
Stress X Genotype	1.97	189322.55	95969.53	1.79	.18	.06
Stress X Rearing	1.97	64763.59	32829.32	.61	.54	.02
Stress X G X E	1.97	47091.59	23871.21	.45	.64	.02
Error (Stress)	53.26	2852522.32	53554.54			

Note. Huynh-Feldt correction applied due to violations in sphericity. Partial eta squared (η^2).

Table 6. *Genotype Group Means and Standard Deviations*

	Long allele			Short allele		
Variable	Baseline	Separation	Social	Baseline	Separation	Social
Cortisol (µg/dl)	21.53 (9.64)	52.13 (29.60)	47.16 (20.86)	23.41 (11.33)	47.23 (20.11)	45.76 (16.08)
Arousal composite	-	662.76 (187.59)	263.14 (173.63)	-	776.84 (232.74)	349.32 (238.46)
Fearfulness composite	-	327.26a (160.34)	143.70a (121.22)	-	249.84 (177.30)	177.30 (191.28)
Passivity composite	-	203.61 (93.53)	167.38 (121.41)	-	173.04 (157.71)	127.99 (87.69)
Affiliation composite	-	-	624.46 (285.78)	-	-	549.71 (278.35)
Behavioral display composite	-	-	17.50 (20.32)	-	-	14.55 (13.17)

Note. Significant differences are designated by matching superscripts ($p<.05$).

Table 7. *Rearing Group Means and Standard Deviations*

	Mother reared			Peer reared		
Variable	Baseline	Separation	Social	Baseline	Separation	Social
Cortisol	18.31a	41.71	41.96	25.64a	58.53	51.08
(µg/dl)	(8.96)	(20.45)	(16.04)	(10.13)	(29.23)	(21.02)
Arousal	-	738.84b	222.45b,d	-	669.86c	477.34c,d
composite		(199.54)	(158.43)		(217.30)	(269.02)
Fearfulness	-	283.03	91.01	-	315.49	216.20
composite		(188.46)	(131.05)		(171.22)	(139.36)
Passivity	-	177.92	157.86	-	206.67	149.23
composite		(102.25)	(111.61)		(133.87)	(113.43)
Affiliation	-	-	726.56e	-	-	477.34e
composite			(239.00)			(269.02)
Behavioral display	-	-	19.73	-	-	13.38
composite			(17.26)			(18.54)

Note. Significant differences are designated by matching superscripts ($p<.05$).

Table 8. *Summary of ANOVAs for Stress Measures*

Source	*df*	*SS*	*MS*	*F*	*p*	η^2
Cortisol Values						
Genotype	1	12.56	12.56	.05	.83	.01
Rearing	1	870.33	870.33	3.44	.08	.12
G X E	1	1.68	1.68	.01	.94	.00
Subject (group)	26	6585.61	253.29			
Condition	2	10656.70	5328.35	24.63	.00	.49
Genotype X Condition	2	113.70	56.85	.26	.77	.01
Rearing X Condition	2	138.34	69.17	.32	.73	.01
G X E X Condition	2	337.41	168.71	.78	.46	.03
Subject X Condition (group)	52	11249.64	216.34			
Arousal Composite						
Genotype	1	95851.27	95851.27	3.19	.09	.11
Rearing	1	38285.99	38285.99	1.28	..27	.05
G X E	1	19392.32	19392.32	.65	.43	.02
Subject (group)	27	810494.95	30018.33			
Condition	1	2284142.54	2284142.54	131	.00	.83
Genotype X Condition	1	1094.37	1095.37	.06	.80	.01
Rearing X Condition	1	133732.78	133732.78	7.67	.01	.22
G X E X Condition	1	4577.07	4577.07	.26	.61	.01
Subject X Condition (group)	27	470791.14	17436.71			

Note. Huynh-Feldt correction applied due to violations in sphericity. Partial eta squared (η^2)

Table 9. *Summary of ANOVAs for Stress Measures-Continued*

Source	*df*	*SS*	*MS*	*F*	*p*	η^2
Fearfulness Composite						
Genotype	1	212.42	212.42	*.01*	*.91*	*.00*
Rearing	1	34382.13	34382.13	*2.0*	*.17*	*.07*
G X E	1	2709.87	2709.87	*.16*	*.70*	*.01*
Subject (group)	27	464365.80	17198.73			
Condition	1	189799.30	189799.30	*11.15*	*.01*	*.29*
Genotype X Condition	1	70644.88	70644.88	*4.15*	*.05*	*.13*
Rearing X Condition	1	64194.10	64194.10	*3.78*	*.06*	*.12*
G X E X Condition	1	8838.15	8838.15	*.52*	*.48*	*.02*
Subject X Condition (group)	27	459500.63	17018.54			

Note. Huynh-Feldt correction applied due to violations in sphericity. Partial eta squared (η^2).

Table 10. *Summary of ANOVAs for Social Buffering Measures*

Source	*df*	*SS*	*MS*	*F*	*p*	η^2
Passivity Composite						
Genotype	1	8697.50	8697.50	*.88*	*.36*	*.03*
Rearing	1	39.77	39.77	*.01*	*.95*	*.00*
G X E	1	1810.05	1810.05	*.18*	*.67*	*.01*
Subject (group)	27	266436.60	9868.02			
Condition	1	21568.72	21568.72	*2.55*	*.12*	*.09*
Genotype X Condition	1	1032.92	1032.92	*.12*	*.73*	*.01*
Rearing X Condition	1	4960.73	4960.73	*.59*	*.45*	*.02*
G X E X Condition	1	275.89	275.89	*.03*	*.86*	*.01*
Subject X Condition (group)	27	228714.36	8470.90			
Affiliation Composite						
Genotype	1	136507.18	136507.18			
Rearing	1	545761.83	545761.83	*8.43*	*.01*	*.24*
G X E	1	3130.07	3130.07	*.05*	*.83*	*.01*
Subject (group)	27	1748575.79	64762.07			
Behavioral Display Composite						
Genotype	1	156.01	156.01	*.46*	*.50*	*.02*
Rearing	1	403.01	403.01	*1.19*	*.29*	*.04*
G X E	1	11.67	11.67	*.03*	*.85*	*.01*
Subject (group)	27	9166.40	339.50			

Note. Huynh-Feldt correction applied due to violations in sphericity. Partial eta squared (η^2).

Table 11. *Summary of MANOVA for Stress Measures*

Source	*df*	*SS*	*MS*	*F*	*p*	η^2
Condition	1	929150.06	929150.06	186.95	.00	.87
Display X Condition	1	143359.70	143359.70	28.85	.00	.50
Error (Condition)	29	144132.45	4970.09			
Stress	1.84	6488117.70	3533929.43	63.84	.00	.69
Display X Stress	1.84	171235.17	93267.88	1.69	.20	.06
Error (Stress)	53.24	2947161.11	55353.49			

Note. Behavioral display groups were formed by classifying subjects into high or low display groups using a cutoff score of five behavioral displays. Huynh-Feldt correction applied due to violations in sphericity. Partial eta squared (η^2).

Table 12. *Behavioral Display Group Means and Standard Deviations*

	High display			Low display		
Variable	Baseline	Separation	Social	Baseline	Separation	Social
Cortisol	23.01a,b	45.15a	46.14b	20.39c,d	63.56c	48.28d
(µg/dl)	(10.81)	(5.63)	(4.29)	(8.69)	(8.60)	(6.56)
Arousal	-	686.00e	217.67e,g	-	739.44f	453.43f,g
composite		(215.95)	(148.95)		(197.35)	(203.83)
Fearfulness	-	332.15h	118.97h,i	-	231.83	232.61i
composite		(190.39)	(161.34)		(129.94)	(70.70)
Passivity	-	175.63	126.66	-	228.73	209.58
composite		(108.94)	(77.00)		(135.45)	(150.11)
Affiliation	-	-	735.77j	-	-	308.47j
composite			(161.31)			(260.12)
Behavioral	-	-	-	-	-	-
display						
composite						

Note. Behavioral display groups were formed by classifying subjects into high or low display groups using a cutoff score of five behavioral displays. Significant differences are designated by matching superscripts ($p<.05$).

Table 13. *Summary of ANOVAs for Stress Measures*

Source	*df*	*SS*	*MS*	*F*	*p*	η^2
Cortisol Values						
Display	1	127622.72	127622.72	.86	.36	.03
Subject (group)	28	21897.02	782.04			
Condition	2	14854.40	7427.20	39.54	.00	.59
Display X Condition	2	1532.83	766.41	4.08	.02	.13
Subject X Condition (group)	56	10519.75	187.85			
Arousal Composite						
Display	1	283287.92	283287.92	5.23	.03	.15
Subject (group)	29	1570436.86	54153.00			
Condition	1	1927368.97	1927368.97	105.38	.00	.78
Display X Condition	1	112589.93	112589.93	6.16	.02	.18
Subject X Condition (group)	29	530425.76	18290.54			
Fearfulness Composite						
Display	1	2839206.39	2839206.39	79.95	.00	.73
Subject (group)	29	1029925.04	35514.66			
Condition	1	152809.23	152809.23	10.74	.01	.27
Display X Condition	1	155047.39	155047.39	10.90	.01	.27
Subject X Condition (group)	29	412671.10	14230.04			

Note. Behavioral display groups were formed by classifying subjects into high or low display groups using a cutoff score of five behavioral displays. Huynh-Feldt correction applied due to violations in sphericity. Partial eta squared (η^2).

Table 14. *Summary of ANOVAs for Social Buffering Measures*

Source	*df*	*SS*	*MS*	*F*	*p*	η^2
Passivity Composite						
Display	1	62664.72	62664.72	3.70	.06	.11
Subject (group)	29	491293.99	16941.17			
Condition	1	15718.20	15718.20	1.96	.17	.06
Display X Condition	1	3013.55	3013.55	.38	.55	.01
Subject X Condition (group)	29	232551.21	8019.01			
Affiliation Composite						
Display	1	1236858.41	1236858.41	31.76	.00	.52
Subject (group)	29	1129342.38	38942.84			

Note. Behavioral display groups were formed by classifying subjects into high or low display groups using a cutoff score of five behavioral displays. Huynh-Feldt correction applied due to violations in sphericity. Partial eta squared (η^2).

Table 15. *Contingent Responsiveness Group Means and Standard Deviations*

Group	*n*	*M*(*SD*)
	Genotype	
Mother reared	12	1.92(2.04)
Peer reared	9	3.96(3.40)*
	Rearing	
Long allele	14	2.75(2.17)*
Short allele	7	2.89(4.04)
	G X E	
Mother reared long allele	7	2.58
Mother reared short allele	5	1.01
Peer reared long allele	7	2.92
Peer reared short allele	2	7.58
Total sample	21	2.79(2.82)*

Note. Untransformed odds ratios appear in the table while log transformed values were used in the analyses. * Significant at the $p<.05$ level

Table 16. *Pearson Correlations between Cortisol and Social Variables*

Variable	1	2	3	4	5	6	7
1 Basal cortisol (µg/dl)	-						
2 Separation cortisol (µg/dl)	.44*	-					
3 Social cortisol (µg/dl)	.54**	.67**	-				
4 Behavioral displays	.25	-.18	-.09	-			
5 Affiliative events	-.40*	-.44*	-.33	.38*	-		
6 LOR contingent responsiveness	.21	.40	.24	-.16	-.40	-	
7 LOR temporal contingency	-.29	-.53*	- .50*	-.23	-.02	- .32	-

Note. Log transformed odds ratio (LOR) *Significant at the p<.05 level ** p<.01 ***p<.001

Table 17. *Hierarchical Regression for Temporal Contingency and Cortisol*

	Social condition			Separation condition		
Model	β	ΔR^2	R^2	β	ΔR^2	R^2
Step 1 (df=2,18)						
Basal cortisol	.61*			.73*		
Rearing	-.11		.33	-.26		.44
Step 2 (df=1, 17)						
Basal cortisol	.62*			.70*		
Rearing	-.12			-.22		
Genotype	-.01	.00	.21	.13	.02	.46
Step 3 (df=1,16)						
Basal cortisol	.49*			.59*		
Rearing	-.02			-.13		
Genotype	-.07			.08		
LOR	-.41*	.14*	.47	-.36†	.11†	.57

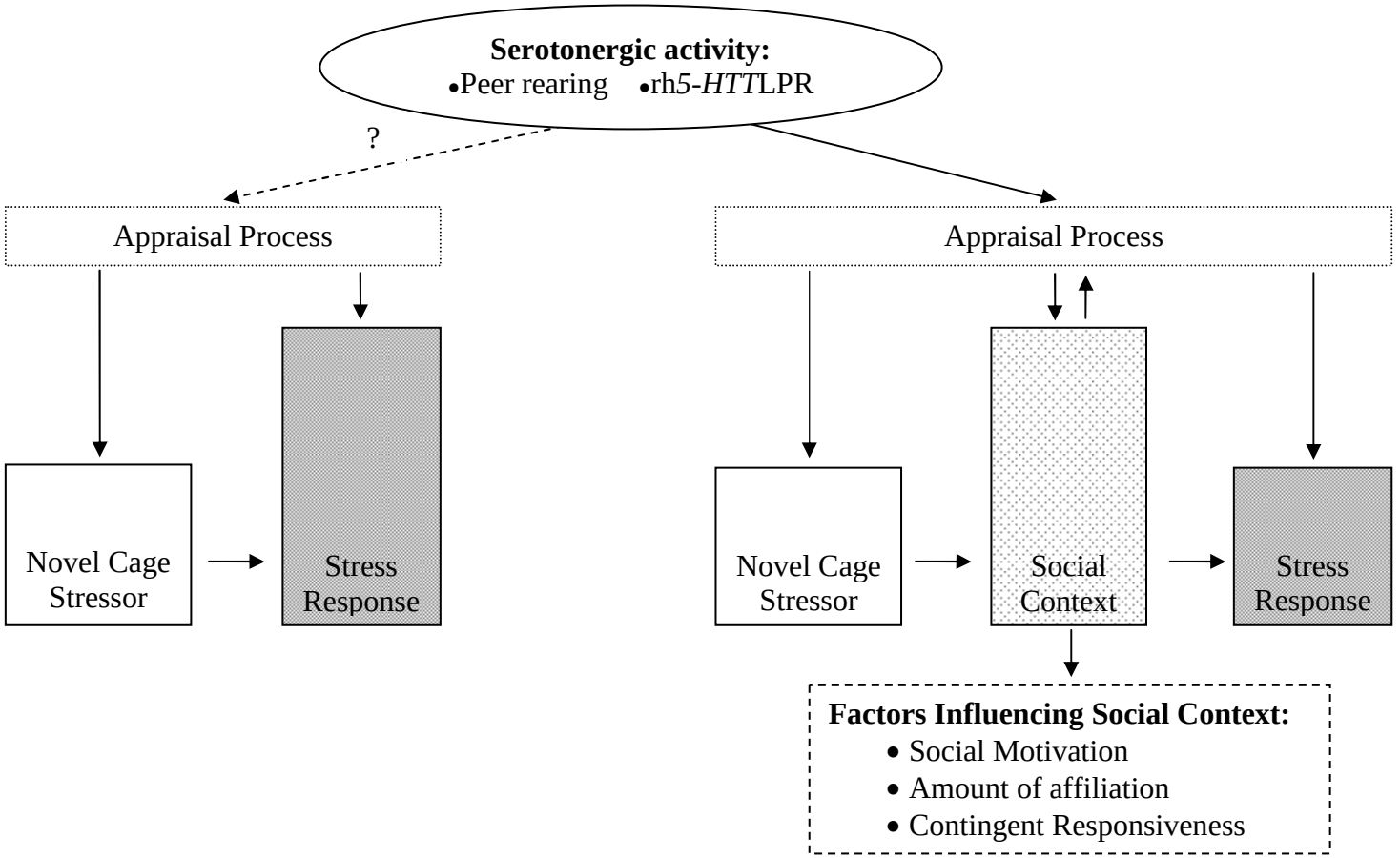
Note. R2 change (ΔR^2). Log transformed odds ratios (LOR) were used in the analyses. *Significant at the p=.05 level †p<.10

FIGURES

Figure 1. *Macaca mulatta model*

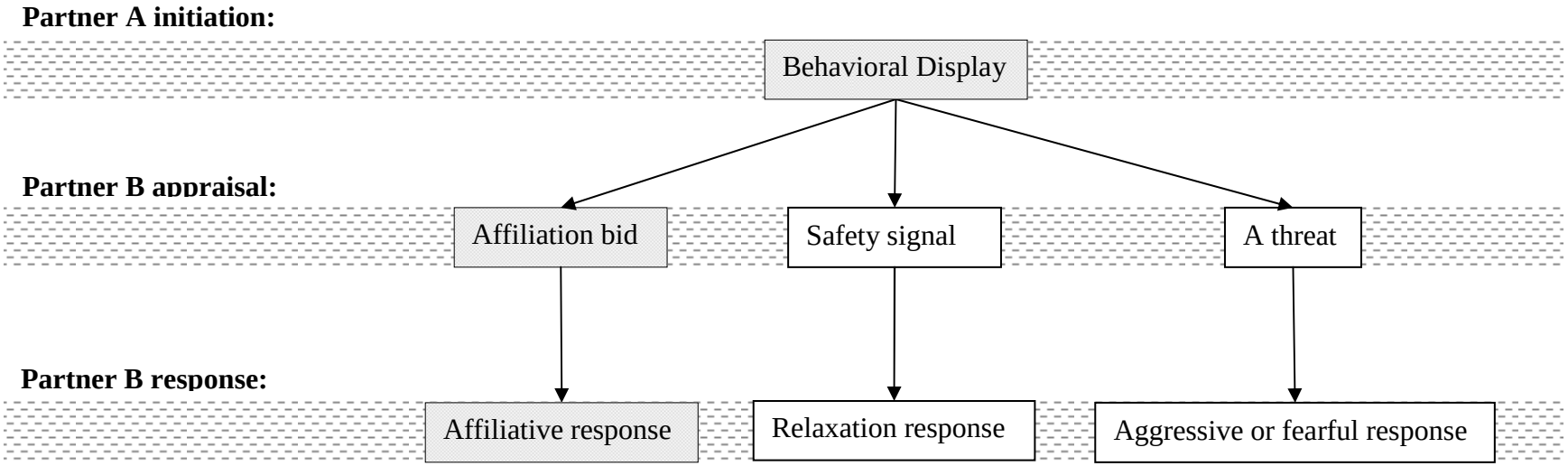
Note. *Macaca mulatta* is a highly social non human primate species whose members form attachment relationships to a variety of social partners including infants, peers, and adults (Suomi, 1979). Individuals of this species belong to family units as well as troops ranging in size from several dozen to several hundreds of individuals (Berman, et al., 1997). Troops are organized around matrilines, where most females remain within their natal group throughout the lifespan and tend to acquire their mothers' status, whereas the majority of adolescent males form male-only groups and emigrate to other troops (Suomi, 1982). *M. mulatta* are a highly adaptive Old World Monkey species living in a wide range of diverse geographical habitats ranging from the Himalayas to the forests of Europe to an island in the Caribbean (Suomi, 1982; 2004). One factor behind this behavioral flexibility appears to be orthologous polymorphisms in a number of candidate genes that facilitate adaptation to varied environmental conditions (Wendland, et al., 2006). These polymorphisms are also understood to influence behavioral and biological components of fearful and impulsive temperament (Suomi, 2004). Underlying neuroendocrine and immune systems of *M. mulatta* also respond quickly to stressors but are able to return to baseline levels of activity quite rapidly (Coe, et al., 1985). Taken together, these similarities make *M. mulatta* an excellent model for studying social buffering.

Figure 2. *A Model Linking the Serotonergic System to Social Buffering*



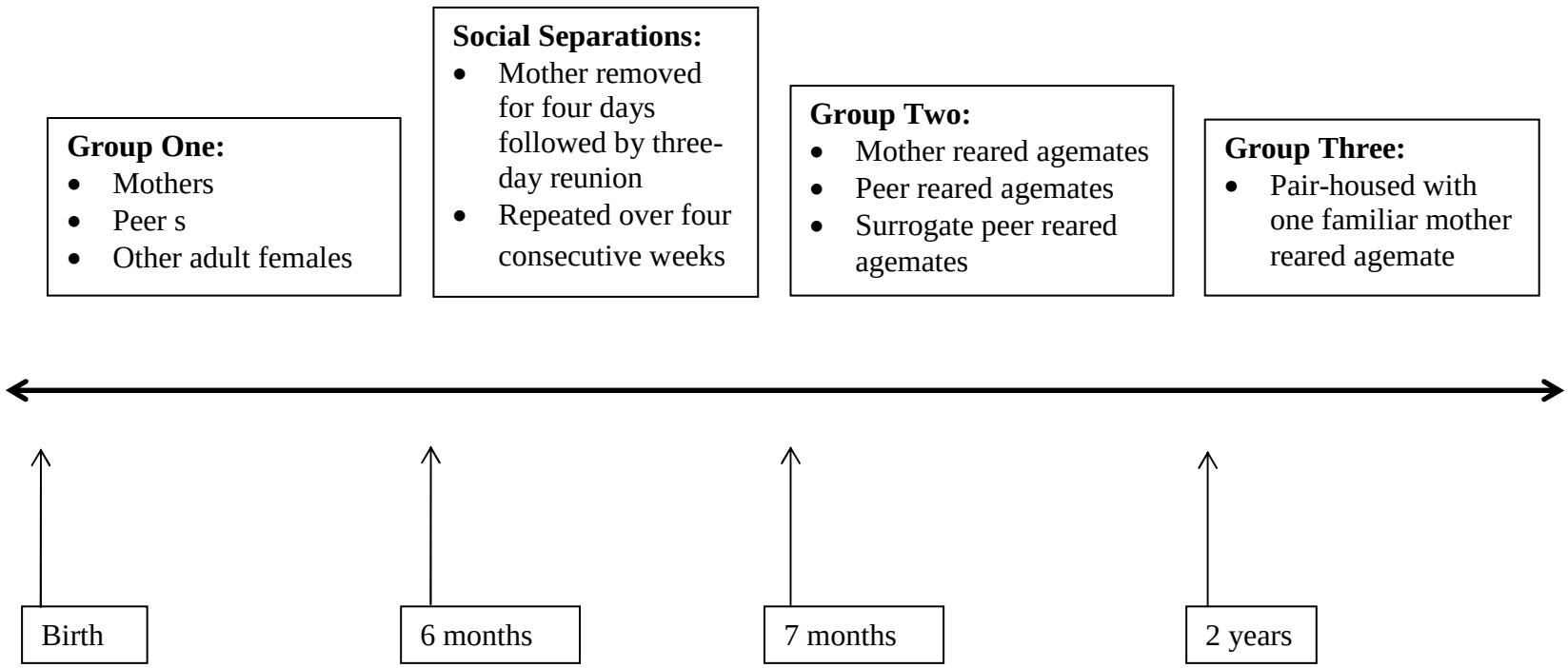
Note. In this diagram, reduced serotonergic activity is thought to impact the appraisal process by elevating threat detection in a novel environment, particularly within social contexts. In turn, affiliative behaviors in a social context are expected to mitigate the degree to which a stimulus is appraised as stressful, and thereby lead to a reduction in stress levels in the presence of a partner.

Figure 3. *A Model of Social Information Processing during Social Buffering*



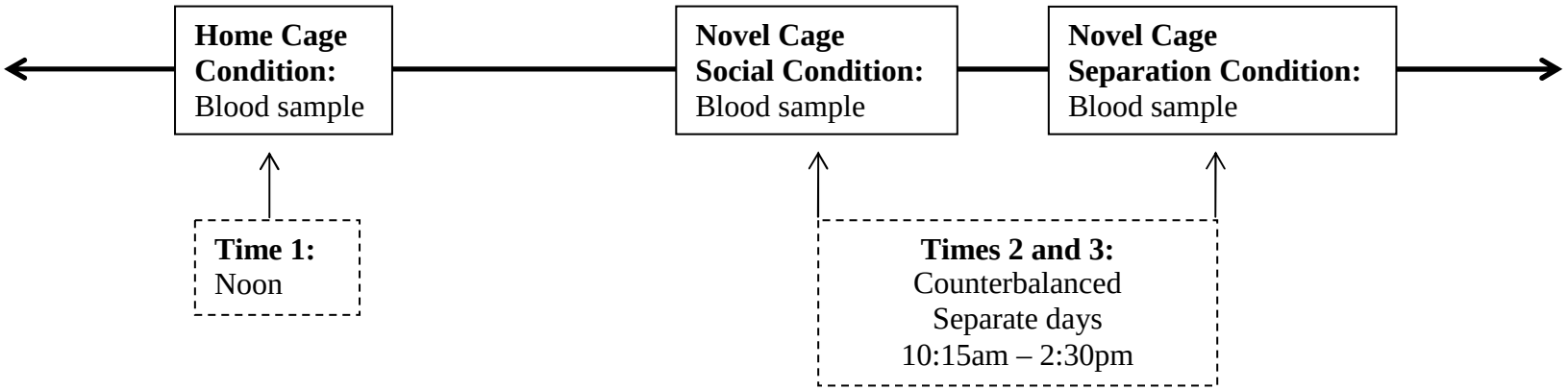
Note. A schematic illustrating several modes of social information processing between two partners, A and B. After Partner A has emitted a behavioral display, Partner B is expected to respond in one of three ways. First, if Partner B appraises the display as an affiliation bid, then affiliation is expected more than any other response. Second, if Partner B appraises the display as a safety signal, then behaviors indicating stress alleviation such as passivity are expected to ensue. Finally, if Partner B appraises the display as a threat, then agonistic (aggressive or fearful) responses are expected more than any other response.

Figure 4. *Mother Rearing Paradigm at the NIH Animal Center*



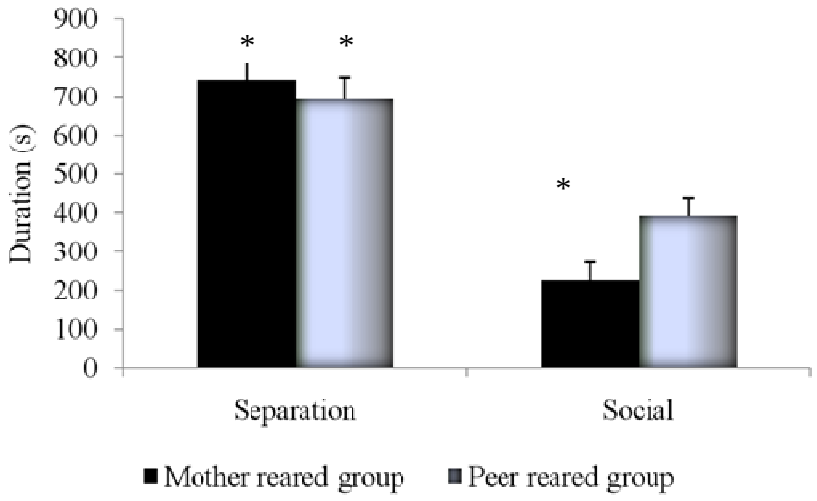
Note. A timeline representing the typical experiences of mother reared cohorts in the current study (2004-2007). Briefly, subjects were reared from birth with their mothers and other mother and infant pairs. At approximately six months of age, infants were subjected to a series of four weekly social separations. The separation paradigm consisted of the mothers being removed from the social group for four days, followed by a three-day reunion in between. After the separations were completed, mother reared subjects were moved to a mixed rearing group consisting of other mother reared agemates, as well as peer reared, and surrogate peer reared agemates. At two years of age, subjects were moved indoors and housed with one familiar mother reared agemate.

Figure 5. *Timeline of Novel Cage Test Experiments*



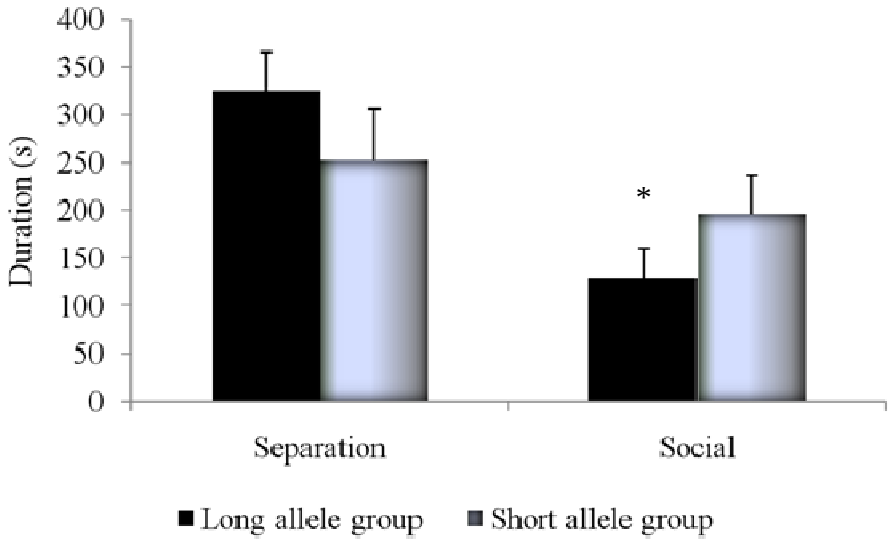
Note. A schematic of Novel Cage Test experiments. All subjects received both the social and separation conditions on different days and the order of the sessions were counterbalanced. A blood sample was collected from all subjects immediately following both conditions and the homecage which were later assayed for cortisol. All experiments were conducted as close to noon as possible.

Figure 6. *Rearing X Condition Interaction on Arousal Composite*



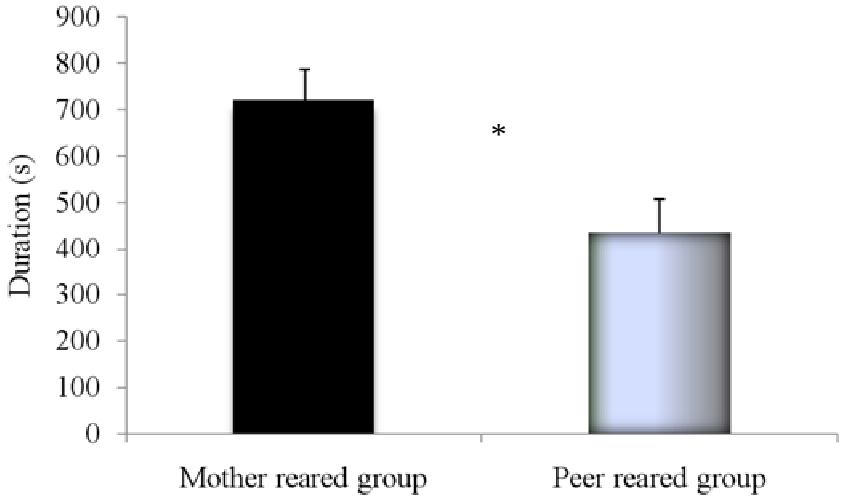
Note. The mean (+/- 1 standard error) duration (s) of arousal behaviors displayed by mother and peer reared groups during the social and the separation condition.
* Significant at the p<.05 level

Figure 7. *Rh5-HTTLPR X Condition Interaction on Fearfulness Composite*



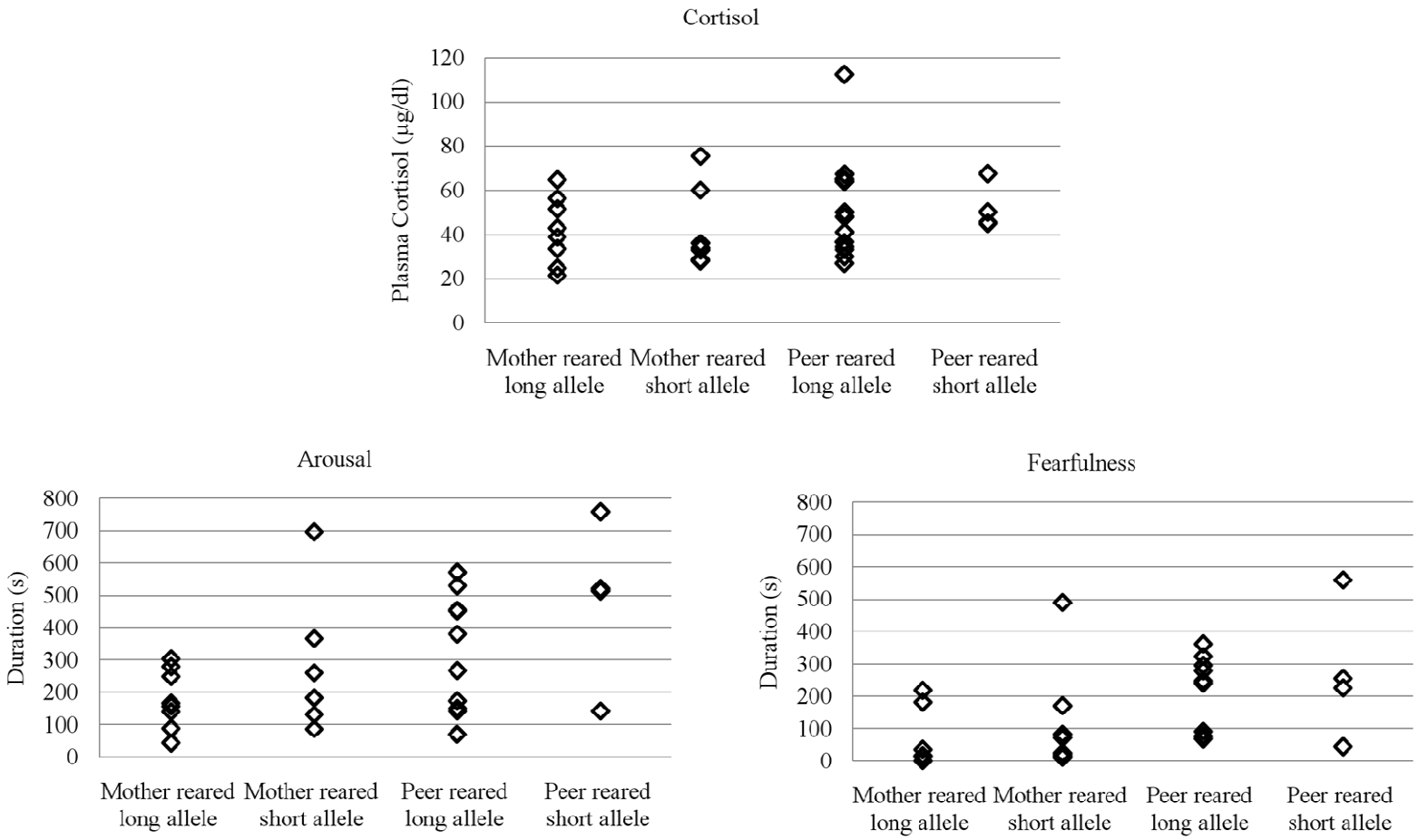
Note. The mean (+/- 1 standard error) duration (s) of fear behaviors displayed by rh*5-HTT*LPR long and short allele groups during the social and the separation condition. The short allele group included both *l/s* and *s/s* subjects, and the long allele group consisted of all *l/l* subjects. * Significant at the p<.05 level

Figure 8. *Rearing Differences in Affiliation Composite*



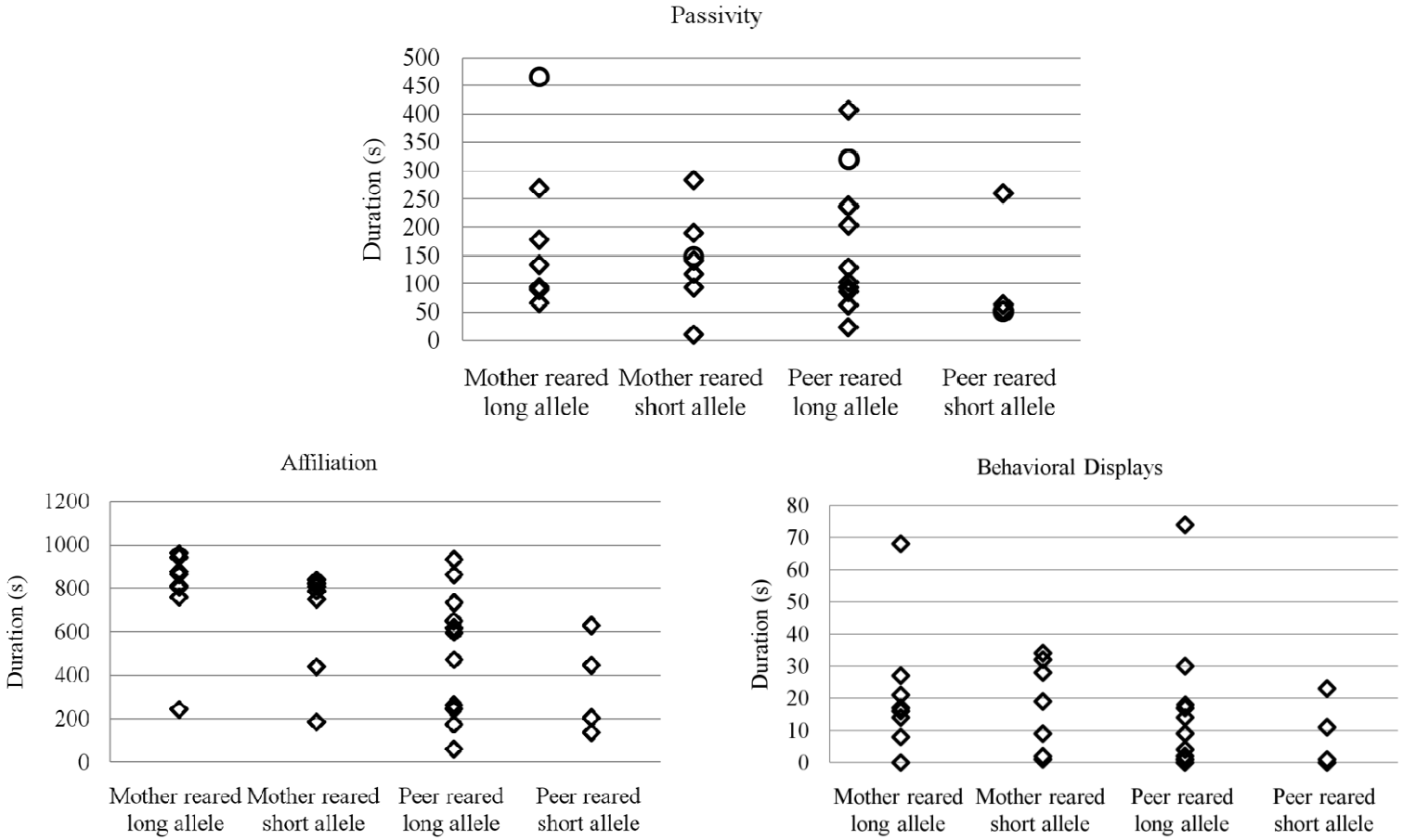
Note. The mean (+/- 1 standard error) duration (s) of affiliation behaviors displayed by mother reared and peer reared groups during the social condition. * Significant at the $p<.05$ level

Figure 9. *G X E Distributions of Stress Measures from the Social Condition*



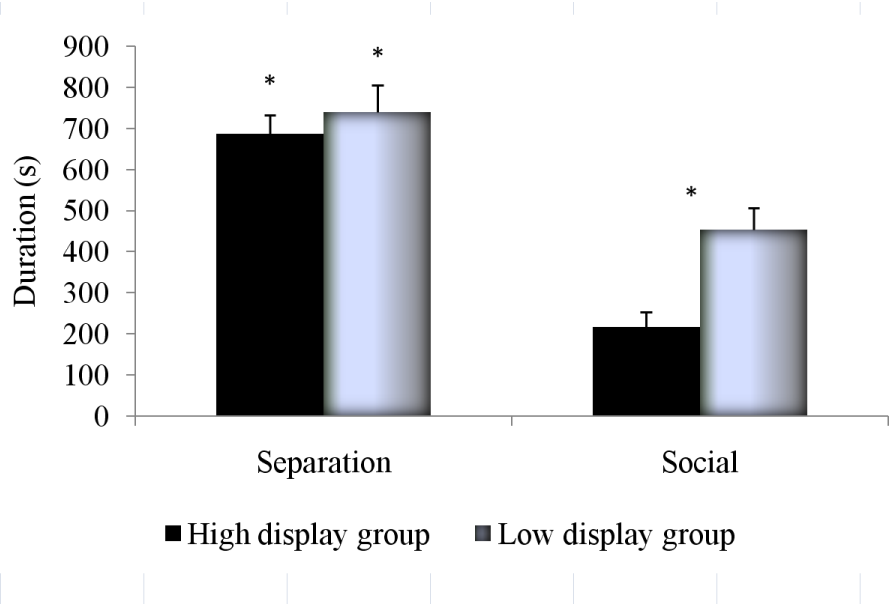
Note. Scores for individual subjects on the individual stress measures are reported for each of the G X E groups involving rearing and rh5-*HTT*LPR separately. Behavior is represented by duration (s) while cortisol is represented in micrograms per deciliter (µg/dl).

Figure 10. *G X E Distributions of Social Buffering Measures*



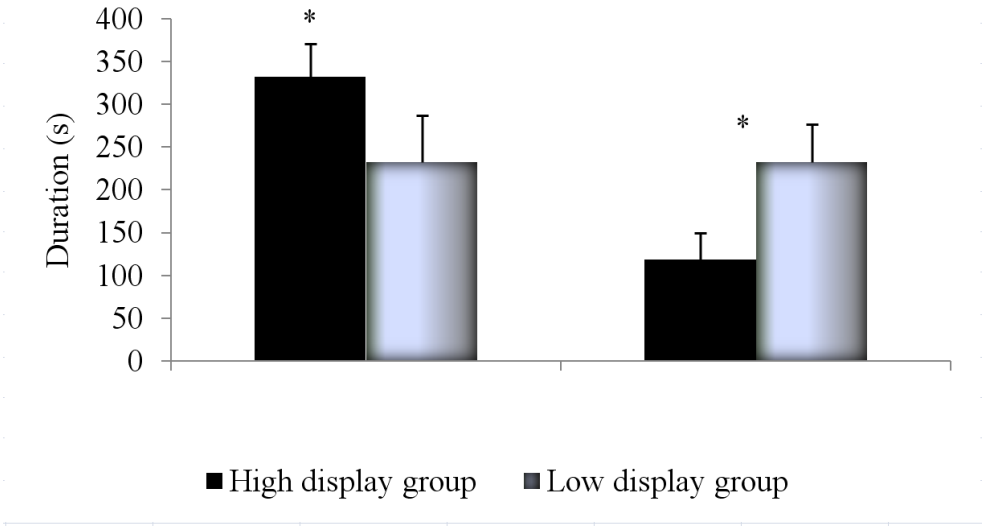
Note. Scores for individual subjects on the individual social buffering measures are reported for each of the G X E groups involving rearing and rh5-*HTT*LPR separately. Behavior is represented by duration (s).

Figure 11. *Display X Condition Interaction on Arousal Composite*



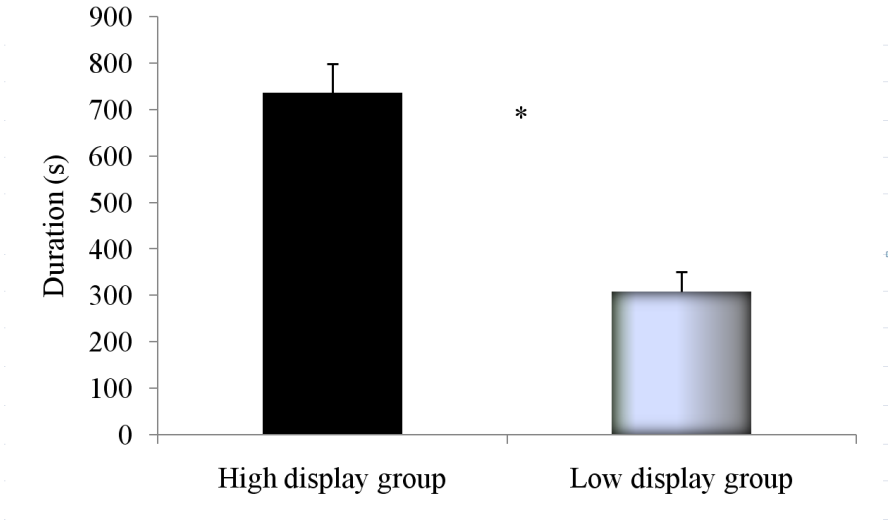
Note. The mean (+/- 1 standard error) duration (s) of arousal behaviors displayed by high and low display groups during the social and separation conditions. Behavioral display groups were formed by classifying subjects into high or low display groups using a cutoff score of five behavioral displays.* Significant at the $p<.05$ level

Figure 12. *Display X Condition Interaction on Fearfulness Composite*



Note. The mean (+/- 1 standard error) duration (s) of fear behaviors displayed by high and low display groups during the social and separation conditions. Behavioral display groups were formed by classifying subjects into high or low display groups using a cutoff score of five behavioral displays.* Significant at the $p<.05$ level

Figure 13. *Behavioral Display Group Differences in Affiliation Composite*



Note. The mean (+/- 1 standard error) duration (s) of affiliation behaviors exhibited by high and low display groups. Behavioral display groups were formed by classifying subjects into high or low display groups using a cutoff score of five behavioral displays. * Significant at the $p<.05$ level

Figure 14. *Event-lag Approach for Estimating Odds Ratios*

	B	~B
A	*a*	*b*
~A	*c*	*d*

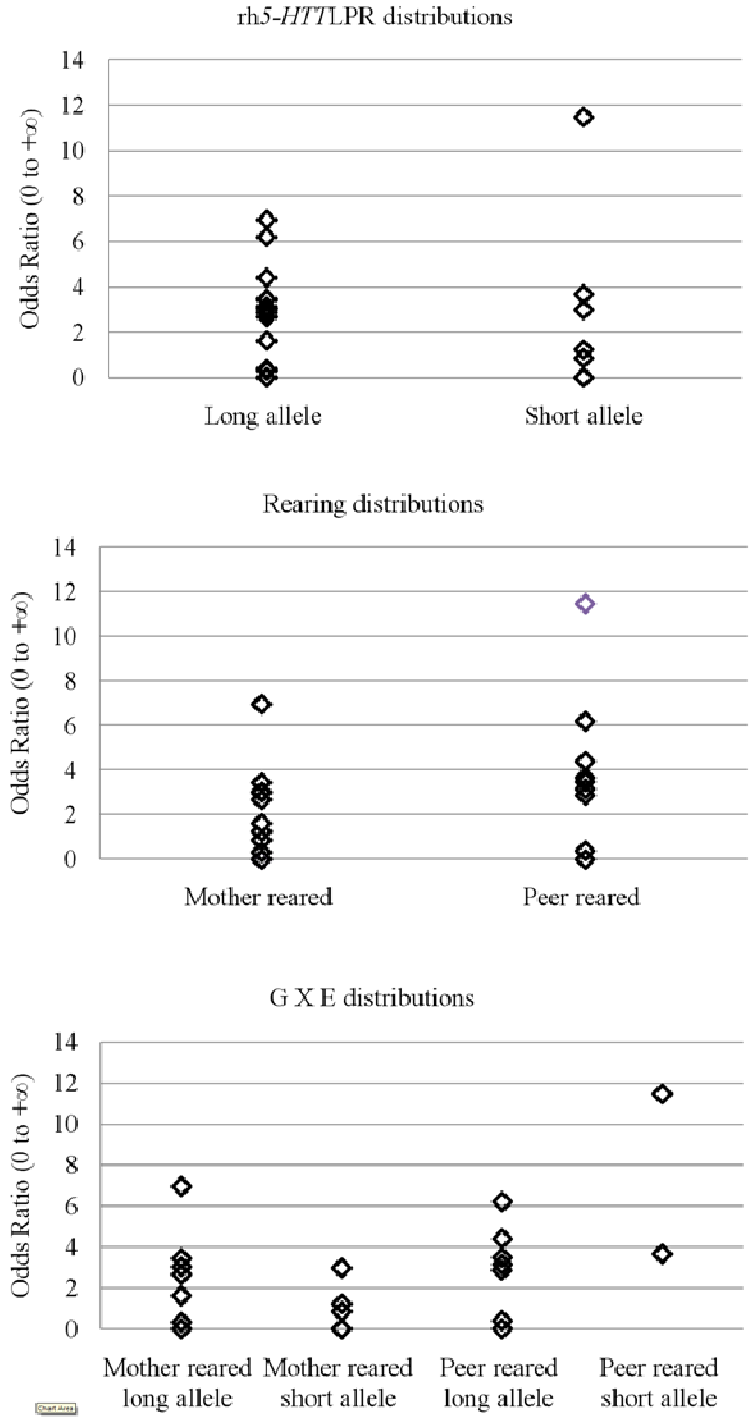
B=target
A=given

a=frequency of given leading directly to target
b=frequency of given leading directly to other
c=frequency of target preceded by other
d=frequency of neither behavior

Estimated odds ratio=(a/b)/(c/d)

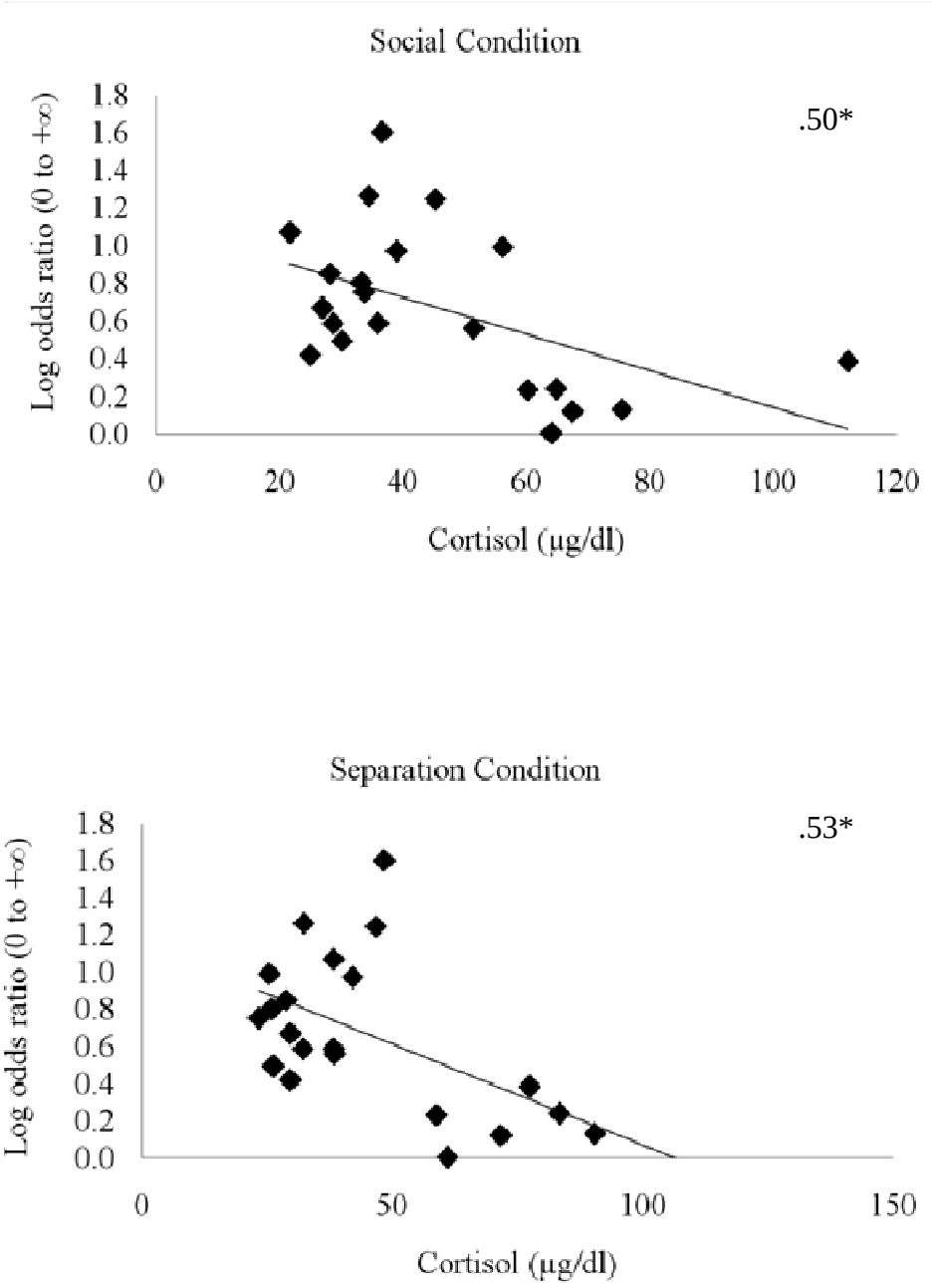
Note. Variables used to generate an odds ratio representing a contingency between any two behaviors of interest. Adapted from Bakeman and Gottman (1997).

Figure 15. *Group Distributions of Contingent Responsiveness*



Note. Contingent responsiveness scores for individual subjects classified into rearing, rh5-*HTT*LPR, and G X E groups separately.

Figure 16. *Scatterplots of Temporal Contingency by Cortisol Measures*



Note. Scatterplots representing correlations between a temporal contingency (behavioral displays and all affiliative events) and cortisol values after the social and separation conditions respectively. * Significant at the $p<.05$ level

APPENDIX

Social Buffering Ethogram

Behavior	Definition and Scoring Criteria
Coo	A clear tone of medium pitch and intensity. Can be a distress call, or a solicitation for contact (Frequency).
Lipsmack	Rapid movements of the lips, teeth are covered (Frequency).
Girn	Affiliative purring sound associated with close contact and comfort (Frequency).
Touch	Affiliative action, reaching out towards the other animal with a hand, and making light contact (Frequency).
iHo!	Short, semi-gutteral threat vocalization (threat-bark), often accompanied by small, stiff jumps (Frequency).
Screech	Shrill, high-pitched, high-intensity sounds that are short and repetitive. Submissive/fearful (Frequency).
Idiosyncracy	Repetitive or odd behaviors that fall under species-typical when not repeated, such as repetitive bouncing, or other repeated behaviors an individual may incorporate into his repertoire. Only code as idiosyncrasy if there are 3 repetitions of the behavior within a minute, then code every time it occurs thereafter (Frequency).
Swat At	Animal is in a crouched position, and attempts to slap partner – may or may not result in contact (Frequency).
Cage Shake	Focused grasping and shaking of shelving or walls resulting in noisy display (Frequency).
Head Bob	Head is jerked very quickly up and down often while staring at partner. The arms are sometimes flexed and extended, so that the gesture resembled a very rapid push-up. (Frequency).
Open Mouth Threat	Includes visual fixation, animal's head is thrust forward and the body appears rigid. Ears flattened, brow retracted, teeth partially exposed, round mouth (Frequency).
Yawn	Fully open mouth, lips retracted, and teeth showing (Frequency).
Scratch	Rapid scratching with hands or feet – not incorporated in a self-grooming session. Can be a sign of stress (Frequency).
Solicit Play	Includes crouch with open mouth, approaching partner and attempting to initiate contact with the apparent intent to engage the other in play. May also include reclining with belly exposed, with a playface expression (Frequency).
Solicit Groom	Consists of presenting back or rump for grooming (Frequency).
Mount Present	Orientation of hindquarters toward partner, usually accompanied by lowering of the forelimbs, lifting of the tail, or looking back over the shoulder (Frequency).
Self Bite	Intense or vigorous self-biting – may result in self-injury - does not include mild self-grooming activities (Frequency).
Walk/Patrol	Continuous locomotion involving 2 or more steps along the floor of the enclosure. Includes pacing.
Climb	Locomoting up off the floor – moving from place to place either horizontally or vertically along the sides of the enclosure.
Stereotypy	Extremely odd behaviors such as hair-plucking, self-injurious behaviors, or repetitive head rolls. Score after the third repetition of an act within 60 secs, and thereafter each time it occurs.
Manipulate Object	Focused exploration of object (>1 sec) with hands, feet, or mouth. Includes exploring parts of the cage such as pull squeeze mechanisms, pulling off water bottle, and licking and biting cage.
Autogroom	Self-grooming: scratching or picking at own fur or skin, may include light oral contact.

Appendix – Continued

Social Buffering Ethogram

Behavior	Definition and Scoring Criteria
Self Genital Explore	Manipulation of own genitals with hands and/or mouth.
Thumbsuck	Self-sucking of thumbs or toes.
Display	Vigorous shaking of cage or other objects, bouncing on all fours or off walls (<1 second).
Sleep	Huddled posture, eyes closed, no lateral or vertical gross motor movement, do not include pauses (<1 second) in locomotion.
Sit	Upright, no lateral or vertical gross motor movement, do not include pauses (<1 second) in locomotion.
Hang	Hanging from the top or sides of cage by the feet and/or hands, >1second. Includes being perched above ground.
Crouch	Stationary on all fours with the body held low to the floor.
Cringe	A submissive posture involving a crouched position where limbs are held beneath the body and the head is lowered. Associated with a fear grimace, usually performed while watching partner.
Fear Grimace	Teeth are bared by tightly pulling back muscles of the face, a grin-like facial expression involving the retraction of the lips exposing clenched teeth.
Scream	Shrill, high-pitched, high-intensity long sounds. Submissive/fearful.
Cling	Clinging hug with both arms involved in at least one of the partners. Includes dorsal-ventral or ventral-ventral contact.
Huddle	Involves one or both animals actively seeking contact with the other, but without the hugging with both arms that Cling involves.
Side by Side	Siting or standing in close physical proximity (<2-3 inches), may include physical contact.
Allogroom	Manipulation, brushing, or licking of the fur, eyes, or wounds of partner with hands and/or mouth.
Genital Explore	Smell, inspect, or touch genitals of partner.
Full Mount	Climbs rump of partner, should include grasping of partners rear legs.
Partial Mount	Climbs rump of partner, does not include grasping onto partners rear legs.
Playfight	Rough and tumble wrestling, may include mild biting and grasping, not usually associated with screaming vocalization or fearful/defensive facial expressions.
Attack	Vigorous biting and grasping, usually associated with screaming vocalizations, submissive posturing and face expressions. Also includes warning bites.

Modifier Codes	Definition and Scoring Criteria
Focal Sends	Focal animal initiates social interaction.
Focal Receives	Non-focal animal initiates social interaction.
Unknown / Mutual	Unclear which partner initiates social interaction.

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