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INTERACTIONS BETWEEN THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS, OXYTOCIN SYSTEM, AND BEHAVIOR IN DIFFERENTLY REARED RHESUS MONKEYS (*MACACA MULATTA*)

A Dissertation Presented

by

AMANDA F. HAMEL

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

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Neuroscience and Behavior

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DEDICATION

to Aaron,
for your limitless love and support

&

to CJ,
and all my other macaque friends

ACKNOWLEDGMENTS

ABSTRACT

INTERACTIONS BETWEEN THE HYPOTHALAMIC-PITUITARY-ADRENAL

(HPA) AXIS, OXYTOCIN SYSTEM, AND BEHAVIOR IN DIFFERENTIALLY

REARED RHESUS MONKEYS (*MACACA MULATTA*)

MAY 2015

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Adverse experiences that occur during early critical periods of development modify activity of neuroendocrine systems, such as the hypothalamic-pituitary-adrenal (HPA) axis and oxytocin system. This dissertation examines the effects of nursery rearing, an established model of adverse early experiences, on activity of the HPA axis and oxytocin system in infant and adult rhesus macaques (*Macaca mulatta*). In addition, influence of oxytocin system activity on the HPA axis and behavioral reactivity was examined. In infant monkeys, nursery-rearing was associated with lower HPA axis, yet higher oxytocin system activity, following the acute stress of developmental assessment. Nursery rearing may result in dysregulation of the HPA axis and a lower overall set point of activity. Mother-reared infants demonstrated higher reactivity during the assessment, which likely reflects a protest response to maternal separation and the strong attachment bond formed in mother-reared infants. Higher oxytocin system activity, lower HPA axis activity, and lower behavioral reactivity during separation and testing in nursery reared monkeys support interactions between HPA axis and oxytocin system activity and corresponds with the pattern of rodent neuroendocrine activity in response to an acute

stressor. At 8 months of age, infants were weaned and relocated to mixed-rearing social housing. Infants responded with increased HPA axis and oxytocin system activity that did not vary by rearing condition. Although there was no effect of rearing on overall oxytocin system activity, nursery-reared infants that had been reared with a surrogate-mother, versus rearing with peers, had lower hair cortisol concentrations than mother-reared counterparts at the time of weaning, further supporting the view that nursery-rearing confers a lower set point of HPA axis activity. In contrast, neither HPA axis activity, oxytocin system concentration, nor behavioral reactivity as measured by the Human Intruder Test varied as a function of rearing history in adult rhesus monkeys. This dissertation demonstrates that, although nursery rearing is associated with changes in HPA axis and oxytocin system activity in infant rhesus monkeys, early rearing experiences may not exert prolonged effects. Finally, these data provide some support for an interaction between the HPA axis and oxytocin system, although only during early development.

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THE EFFECTS OF EARLY REARING EXPERIENCE ON NEUROENDOCRINE SYSTEMS

1.1 Introduction

Infancy and childhood are periods of marked growth and development. Stressful experiences that occur during early developmental periods, such as abuse or neglect, have been demonstrated to result in abnormal activity within certain neuroendocrine systems, specifically the hypothalamic-pituitary-adrenal (HPA) axis and the oxytocin (OT) system. The following dissertation is an examination of the effects of adverse early experiences on these neuroendocrine systems, as well as on behavior, and the interactions between neuroendocrine activity and behavior. Nursery rearing of laboratory housed rhesus macaques has previously been demonstrated to be a causative agent of abnormal development and therefore serves as a model for adverse early experiences.

1.2 The Hypothalamic-Pituitary Adrenal (HPA) Axis

When a primate encounters a stressful experience, the central nervous system responds by activating a cascade of events within the HPA axis and the sympathomedullary pathway to help the individual manage the incident. Activation of the HPA axis causes the paraventricular nucleus (PVN) of the hypothalamus to secrete corticotrophin releasing hormone (CRH). CRH stimulates the pituitary gland to secrete adrenocorticotropic hormone (ACTH) into the blood stream. In response to activation by ACTH, the adrenal glands produce and secrete cortisol, a glucocorticoid steroid hormone. Cortisol provides negative feedback at the level of the PVN and the pituitary to reduce

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secretion of CRH and ACTH and also activates other downstream pathways that prepare the body to overcome the stressful experience.

Although this HPA axis response benefits the individual in the short-term, prolonged elevation of cortisol can have harmful consequences. Chronic exposure to glucocorticoids has been shown to result in a number of negative effects, including impaired cognitive function, suppressed thyroid function, reduced immune function, and activation of pro-inflammatory factors (Sorrells & Sapolsky, 2007). Specifically, exposure to glucocorticoids requires activation of allostatic processes, which function to stabilize the system and maintain homeostasis (McEwen, 2003). During a period of chronic stress, the system is exposed to a period of high allostatic load, which can have detrimental effects, including the desensitization of target receptors, tissue damage, and dysregulation of the HPA axis (McEwen, 1998). Changes to HPA axis activity can present as either hyper- or hypoactivation, both of which have been identified in human psychiatric disorders (Staufenbiel, Pennix, et al., 2013).   Individuals diagnosed with Generalized Anxiety Disorder (Mantella, Butters, et al., 2008) and Major Depressive Disorder (Parker, Schatzberg, & Lyons, 2003) have higher baseline levels of cortisol whereas individuals with Post-Traumatic Stress Disorder have lower baseline levels of cortisol than controls (Kellner, Baker, & Yehuda, 1997) compared to controls.

1.2.1 Regulation of the HPA Axis by the Oxytocin System

Activity of the HPA axis is regulated by a neurohypophysial hormone, oxytocin, both at baseline and during the response to a stressor. Oxytocin is synthesized in (and

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secreted by) parvocellular and magnocellular neurons within the PVN and the supraoptic

nucleus (SON) of the hypothalamus. Parvocellular neurons project to various extra-

hypothalamic regions within the brain, including limbic and autonomic areas whereas

magnocellular neurons project to the posterior pituitary. Neurons that project to the

pituitary terminate on capillaries, thereby secreting oxytocin into the bloodstream and

mediating peripheral effects (Sofroniew, 1983). Therefore, oxytocin is synthesized in

two discrete neural populations which have either central or peripheral effects.

1.2.1.1 The HPA Axis and the Oxytocin System Relationship in Rodent Models

Rodent research has demonstrated that, under normal conditions, oxytocin

attenuates HPA axis activity. At baseline, oxytocin released within in the PVN inhibits

release of CRH from nearby neurons. The result is inhibition of downstream ACTH and

corticosterone (the rodent glucocorticoid homologue of cortisol) release (Engelmann,

Landgraf, et al., 2004; Neumann, 2002) and overall dampening of HPA axis activity. For

example, rats administered oxytocin intracerebroventricularly (ICV) exhibited decreases

in basal plasma ACTH (but not corticosterone) (Windle, Kershaw, et al., 2004).

Conversely, blocking the activity of oxytocin results in *increased* HPA axis activity; local

infusion of an oxytocin receptor antagonist into the PVN of male rats resulted in

increased concentrations of plasma ACTH (Neumann, Krömer, et al., 2000) and

corticosterone (Neumann, Wigger, et al., 2000). In rodent models, oxytocin appears to

attenuate HPA axis activity during non-stressful periods.

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Under conditions of stress, oxytocin is released within the PVN. Central oxytocin concentrations were elevated when male rats were exposed to the shaker stress test, during which rats are placed in a small enclosure and exposed to short but unpredictable bouts of shaking. Oxytocin elevations were observed specifically within the hypothalamic PVN (Nishioka, Anselmo-Franci, et al., 1998), which is of note because stress-induced release of oxytocin within the PVN may affect HPA axis activity at the level of the hypothalamus. When the rats were subjected to restraint stress, ICV oxytocin attenuated the HPA axis response as demonstrated by blunted plasma ACTH (Windle, Kershaw, et al., 2004) and corticosterone concentrations (Windle, Shanks, et al., 1997). Similarly, mice exposed to the elevated plus maze demonstrated blunted ACTH and corticosterone following ICV oxytocin (Windle, Shanks, et al., 1997).

Mice that are oxytocin-deficient, oxytocin knockouts, display higher levels of plasma corticosterone than wild types following the shaker stress test (Amico, Mantella, et al., 2004) and higher levels of anxious behaviors on the elevated plus maze than controls (Mantella, Vollmer, et al., 2003). Furthermore, when oxytocin knockouts were supplemented with oxytocin ICV and exposed to the elevated plus maze, anxious behavior decreased to levels similar to that of wild type mice (Mantella, Vollmer, et al., 2003). These data support that oxytocin regulates activity of the HPA axis, not only during normal conditions, but also during conditions of stress. Increased oxytocin secretion during stress likely functions to regulate HPA axis activity.

1.2.1.2 The Relationship between HPA Axis and Oxytocin System Activity in Human and Non-Human Primates

The relationship between oxytocin and the HPA axis in human and non-human primates follows a similar pattern of that which has been observed in rodents. Naturally or experimentally elevating levels of oxytocin in human subjects has been associated with a decrease in HPA axis activity, both at baseline and in response to stressful situations. For example, natural increases in oxytocin occur during milk letdown, resulting in elevated circulating levels of oxytocin in lactating women (Grewen, Davenport, & Light, 2010). During breastfeeding, women experience an increase in plasma oxytocin that is correlated with a decrease in baseline levels of plasma ACTH and cortisol (Chiodera, Salvarani, et al., 1991; Amico, Johnston, et al., 1994). Furthermore, lactating women exposed to the physical stress of running on a treadmill (Altemus, Deuster, et al., 1995) or the Trier Social Stress Test (TSST) (Heinrichs, Meinlschmidt, et al., 2001) experienced a blunted plasma ACTH and cortisol response, as compared to non-lactating women. Overall, as levels of oxytocin increase, activity of the HPA axis declines, demonstrating that oxytocin is involved in the regulation of HPA axis activity in humans, at baseline and under conditions of stress.

Although some studies have reported increases in oxytocin concentrations within plasma of lactating women (Grewen, Davenport, & Light, 2010), it is unclear whether these changes in oxytocin concentration are also present within the brain. This illustrates a significant challenge in analyzing physiological oxytocin levels: central and peripheral domains represent different oxytocin systems. The blood-brain barrier, or collection of tight junctions and capillaries that surround the brain, prevents oxytocin from readily

passing between circulating blood and cerebrospinal fluid (CSF). Therefore, levels measured in plasma or saliva, which represent peripheral oxytocin levels, do not always predict central levels, as measured in CSF. Baseline levels of plasma oxytocin are not correlated with levels measured in CSF (Kagerbauer, Martin, et al., 2013) and increased concentrations of oxytocin in one matrix are often not observed in the other (Amico, Challinor, & Cameron; 1990).

Given that central and peripheral domains are essentially separate, it would be very difficult to experimentally manipulate central oxytocin concentrations in human and non-human primates without the use of invasive procedures. However, researchers have recently developed a non-invasive technique to experimentally elevate central oxytocin. Administering peptides intranasally results in central elevations because of the absence of a "nose-brain barrier". Gaps exist between epithelial cells within the nasal cavity (Balin, Broadwell, et al., 1996), allowing peptides to pass through. Peptides administered intranasally have been measured in CSF as early as 10 minutes post-administration (Born, Lange, et al., 2002) and in saliva 15 minutes post-administration (Weisman, Zagoory-Sharon, & Feldman, 2012). These data also demonstrate that intranasal administration results in elevations of both central and peripheral concentrations. Furthermore, intranasal administration of oxytocin has resulted in sustained elevations of peripheral levels; for example, van IJzendoorn et al. demonstrated that salivary oxytocin concentrations remained elevated at least 7 hours post-intranasal administration (2012).

Experimental elevations of oxytocin concentrations, like natural increases during lactation, also modulate HPA axis activity, whether administered peripherally or

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intranasally. The HPA axis consists of both central (hypothalamus and pituitary) and peripheral (adrenal glands) sites, allowing for regulation by oxytocin in either domain. Oxytocin administered to men intravenously decreased plasma ACTH and cortisol levels in a dose-dependent manner (Legros, Chiodera, et al., 1984) and, during a stress condition, blunted the response of the HPA axis, as reflected by lower plasma ACTH and cortisol levels (Legros, Chiodera, & Demey-Ponsart, 1982). As oxytocin does not cross the blood brain barrier, the results of these manipulations likely reflect regulation of the HPA axis at the pituitary or adrenal gland level, as demonstrated by alterations to levels of ACTH and cortisol. Intranasal oxytocin blunted the HPA axis response, as measured by salivary cortisol, to laboratory-induced couple conflict (Ditzen, Schaer, et al., 2009), to the physical stress of running on a treadmill (Cardoso, Ellenbogen, et al., 2013), and to the Trier Stress Test (but only when paired with social support) (Heinrichs, Baumgartner, et al., 2003). Intranasal oxytocin also blunts baseline HPA axis activity and response to a stressor in non-human primates. Infant rhesus monkeys demonstrated lower salivary cortisol concentrations after receiving intranasal oxytocin through a nebulizer, as compared to saline controls (Simpson, Sclafani, et al., 2014). Squirrel monkeys that received oxytocin intranasally had a blunted ACTH response to the well-established stress of relocation and isolation (Parker, Buckmaster, et al., 2005). The mechanism by which intranasal oxytocin modulates the HPA axis is a little less clear, as it results in both central and peripheral increases in oxytocin concentrations and could conceivably involve either central or peripheral domains.

1.2.2 Early Adverse Experiences and the HPA Axis

The timing of periods of high allostatic load plays an important role in determining the severity of the stressor's effect. Potent stressors that are experienced during certain sensitive periods of development, such as infancy or puberty, can have particularly adverse effects on an individual's health that are mediated by permanent changes to HPA axis functioning (Chrousos, 1997). These alterations manifest in altered levels of CRH, ACTH, and glucocorticoids, the stability of which are necessary for an appropriate stress response and general well-being.

Adverse experiences that occur during early development can alter neurobiological structures and processes of the HPA response. Women that report childhood sexual or physical abuse have higher plasma ACTH and cortisol concentrations following the Trier Social Stress Test, but not at baseline (Heim, Newport, et al., 2000). In addition, children in the foster care system demonstrated lower morning cortisol (Dozier, et al., 2006) while children in an orphanage showed a flattened circadian rhythm of HPA axis activity (Carlson & Earls, 1997). Changes resulting from early life stress have been hypothesized to be adaptive and signal the development of an alternative mode of operation that might better cope with an environment characterized by high levels of stress (Teicher, Andersen, et al., 2003). Yet, some evidence suggests that human subjects that report early life stress are more vulnerable to developing psychological disorders, such as depression, anxiety, or PTSD, each of which are characterized by changes to HPA axis activity (Heim & Nemeroff, 1999). This has led

authors to hypothesize that early life adverse experiences confer later vulnerability, not vitality, as a result of changes to the HPA axis.

Researchers have studied the effects of adverse early experiences in non-human primates by experimentally controlling the maternal foraging demand of bonnet macaques and, therefore, systematically manipulating infant early experience. Mothers are assigned to one of three feeding paradigms: low foraging demand (LFD) where food is obtained easily, high foraging demand (HFD) where mothers must complete a task in order to receive food, and variable foraging demand (VFD), which rotates randomly between LFD and HFD. The VFD environment is unpredictable, prevents mothers from developing stable foraging strategies, and results in mothers that are dismissive to their infants and that display erratic behavior (Rosenblum & Andrews, 1994). Infants reared in the adverse VFD environment form an unsecure attachment to their mothers, have decreased play and exploratory behaviors, and display a higher frequency of disturbance and depressive behaviors than LFD or HFD infants (Rosenblum & Andrews, 1994). As adults, VFD monkeys exhibit low baseline CSF cortisol levels (Coplan, Andrews, et al., 1996). These blunted levels of cortisol likely do not represent reduced stress but rather an overall dysregulation of the HPA axis resulting from the VFD environment and, more specifically, the maladaptive mothering received. The nature of this dysregulation could be an overall lower baseline level, or set point, of the HPA axis.

Similarly, stressful early experiences result in changes to the HPA axis in rodent models. Rats that had experienced maternal separations before postnatal day 14 demonstrated elevated hypothalamic CRF levels as adults (Plotsky & Meaney, 1993) as

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well as higher plasma levels of corticosterone, a glucocorticoid, in response to a restraint stress test (Plotskey & Meaney, 1993) and light handling (Kalinichev, Easterling, et al., 2002). In addition to demonstrating a heightened physiological stress response, maternally-separated rats demonstrated higher levels of anxious behavior during the elevated plus maze and an increased startle response (Kalinichev, Easterling, et al., 2002). Increased HPA axis activity at baseline and during a stress condition may reflect altered sensitivity of negative-feedback of the HPA axis in rats that experienced maternal separation.

1.2.3 Early Adverse Experiences and the Oxytocin System

Given that there is a connection between the HPA axis and the oxytocin system, and that HPA axis activity is modified by early life stress, it is not surprising that the oxytocin system is also altered by early life stress. However, previous reports of the effects of early experiences on the oxytocin system are not as numerous as those on the HPA axis. Adult men that reported having experience stressful events during childhood displayed lower levels of plasma oxytocin. In addition, oxytocin levels were negatively correlated with adult depressive symptoms and trait anxiety (Opacka-Juffry & Mohiyeddini; 2012). Similar patterns have been demonstrated by measuring central levels of oxytocin. Women that reported childhood trauma had lower CSF oxytocin concentrations, which were negatively correlated with both the number of traumatic exposures and ratings of anxiety (Heim, Young et al., 2009). Finally, men that reported early parental separation responded to intranasal oxytocin with an attenuated decline in

salivary cortisol (Meinlschmidt & Heim, 2007), suggesting that adverse early experience may modulate sensitivity of the HPA axis to oxytocin.

The mechanism by which early life stress produces relatively permanent physiological changes in HPA axis and oxytocin system activity is unclear at this time. Furthermore, it is uncertain whether early life stress alters HPA axis activity and the oxytocin system independently or whether these changes are associated. One model that could be valuable in assessing the effects of adverse early experiences on the HPA axis and oxytocin system is the non-human primate nursery rearing paradigm. Rearing infant monkeys without their mothers has reliably resulted in both physiological and behavioral modifications and provides a highly controlled environment in which to alter early experience.

1.3 Nursery-Rearing of Non-Human Primates

Infants born in a primate facility are typically reared either with their mother only or socially, with other mother-infant dyads. At the National Institute of Child Health and Human Development's Laboratory of Comparative Ethology in Bethesda, Maryland, infants are studied under various other early rearing experiences. In order to determine the importance of a maternal stimulus on infant development, infants are reared away from their mother in a nursery. The overall goal of the research is to determine the effects of differential early rearing on behavioral and physiological development. Nursery rearing also serves as an excellent model for impoverished early life. Although

one of a handful of primate facilities to rear infants in a nursery, the NICHD is at the forefront of the field of altered early rearing experiences.

In the late 1950s, Harry Harlow pioneered the technique of rearing rhesus macaque infants in a nursery. He reared infants in a variety of paradigms, ranging from total social isolation to rearing with peers only or with an inanimate surrogate mother. Early isolation produced infants that were withdrawn and lacked normal social behavior (Harlow & Harlow, 1962). Infants given access to surrogates (Harlow & Harlow, 1962) and peers (Harlow, 1962) developed strong bonds of attachment, yet displayed fearful and stereotypic behaviors. However, surrogate-reared infants also developed normal social behaviors, whereas social behaviors of peer-only reared infants were impaired (Chamove, Rosenblum, & Harlow, 1973).

Since the 1950s, primate nursery facilities have developed specific protocols for rearing infants. In general, nursery-reared (NR) infants are removed from their mothers within the first few days of life and are placed in incubators for the next few weeks where they are fed milk formula by human caregivers. Treatment thereafter depends on which rearing protocol the infants have been assigned to. Although, protocols vary slightly from one facility to the next, the fundamental practices are comparatively standard: infants are reared with either peers-only or with an inanimate surrogate mother and limited peer exposure Peer-reared (PR) infants are removed from their incubators and placed in a cage with 3-4 other infants, for either a few hours a day or continuously. The amount of time the PR infants spend in the incubators, as well as the number of peer cagemates, varies across facilities (Wisconsin Regional Primate Research Center: Clarke,

1993; Laboratory of Comparative Ethology, NICHD: Shannon, Champoux, & Suomi, 1998; Yerkes National Primate Research Center: Winslow, Noble, et al., 2003; California National Primate Research Center: Capitanio, Mendoza, et al., 2005). Surrogate-peer reared (SPR) infants, instead of being housed with peers, are housed individually with cloth-covered surrogate mothers. They are given daily contact with other SPR infants for about 30-120 minutes (Laboratory of Comparative Ethology, NICHD: Shannon, Champoux, & Suomi, 1998; University of Washington Primate Center: Sacket, Ruppenthal, & Davis, 2002). Infants typically remain in the nursery until they are approximately 8 months of age. For the purposes of this dissertation, the phrase "nursery reared" will be used to refer collectively to both SPR and PR monkeys.

Researchers have demonstrated distinct behavioral and physiological profiles for mother-reared (MR), PR, and SPR rhesus monkeys. Surrogates make for overly-tolerant mothers because they do not ever reprimand inappropriate behavior, including aggressive acts; peers, on the other hand, make inadequate mothers because they allow the other infant(s) to continuously cling and do not reject them, which perpetuates fearful behavior (Harlow & Harlow, 1962). In adulthood, surrogate-peer-reared monkeys have a reputation for increased levels of aggression, whereas peer-reared monkeys display heightened fear and anxious behavior (Dettmer, Novak, et al., 2012). In addition, PR monkeys are more likely than MR monkeys to develop stereotypic behaviors, such as pacing, digit sucking, rocking, and bouncing (Feng, Wang, et al., 2011); SPR monkeys shown an increased vulnerability to self-injurious behavior compared to monkeys MR and PR monkeys (Lutz, Davis, et al., 2007).

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Nursery-reared monkeys tend to be more behaviorally and emotionally reactive to stressful experiences than MR monkeys. For example, PR monkeys responded to social separation stress with more distress calls and more self-directed behaviors than MR monkeys (Suomi, 1991). Similarly, when PR monkeys were subjected the stress of a novel environment with their peer group, PR monkeys emitted more distress calls and displayed more self-mouthing behaviors than MR and spent more time in contact with their peers (Higley, Hopkins, et al., 1992). In addition, when exposed a loud stimulus sound, PR monkeys demonstrated a heightened startle response, as measure by changes in heart rate, compared to MR monkeys (Parr, Winslow, & Davis, 2002). When exposed to the Human Intruder Test, a mildly stressful experience which introduces the presence of an unfamiliar experimenter, both PR and SPR adults responded with more freezing behavior and less locomotive behavior than MR adults, suggesting that they were behaviorally inhibited (Corcoran, Pierre, et al., 2012). This behavioral reactivity suggests that NR monkeys have a heightened response to stressful experiences.

1.3.1 Nursery Rearing and the HPA Axis

Surrogate and peer reared rhesus monkeys, like other species that have had adverse early experiences, display dysregulated HPA axis activity. Differences between NR and MR monkeys are measurable within the first few months of life. In general, NR infants have lower baseline levels of plasma ATCH and cortisol than MR monkeys (Barr, Newman, et al., 2004; Capitanio, Mendoza, et al., 2005; Clarke, 1993; Davenport, Novak, et al., 2003; Shannon, Champoux, & Suomi, 1998). In addition, NR infants demonstrate attenuated responses to stressful experiences, such as lower cortisol levels following

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social isolation in SPR infants (Shannon, Champoux, & Suomi, 1998) and in PR infants (Barr, Newman, et al., 2004). In addition, PR infants demonstrated an attenuated increase in ACTH following relocation and introduction to social housing compared to MR infants (Clarke, 1993). At six months of age, PR infants had a temporally delayed increases in plasma cortisol concentrations in response to a separation stress (Feng, Wang, et al., 2011).

Pharmacological manipulation of the HPA axis produces similar results; in response to stimulation by administration of exogenous ACTH, PR infants responded with increases in cortisol, yet levels remained comparatively lower than in MR infants. Furthermore, following suppression of the HPA axis by administration of dexamethasone, NR infants had lower plasma cortisol levels and continued to show lower cortisol concentrations than MR infants (Capitanio, Mendoza, et al., 2005). This pattern of activity demonstrated by NR monkeys suggests that nursery rearing experiences confer a lower set point of the HPA axis, which would result in both lower basal activity and response to a stressor or pharmacological manipulation (Capitanio, Mendoza et al., 2005).

It should be noted that not all the results fit the general pattern described above. Some studies report no difference in plasma cortisol concentrations of NR and MR infants (Fahlke, Lorenze, et al., 2002; Winslow, Noble, et al., 2003) or adults (Chen, Novak, et al., 2010) and some report higher levels of plasma cortisol for NR monkeys (Highley, Suomi, & Linnoila, 1992; Barrett, Nobel, et al., 2009). Nursery-reared monkeys sampled within the first month of life had higher baseline plasma cortisol levels

than MR monkeys (Champoux, Coe, et al., 1989). Six month old PR infants had a higher

plasma cortisol response following separation stress than MR infants (Fahlke, Lorenze, et

al., 2000). At 4 years of age, PR monkeys had significantly higher plasma cortisol

responses to venipuncture and an amplified plasma cortisol and ACTH response to social

separation than MR monkeys (Higley, Hasert, et al., 1991). (However, it is important to

note that in the latter study, monkeys were participating in a study of alcohol

consumption, a possible confounding factor).

Variability in the pattern of dysregulation observed in NR monkeys may be due to

a variety of factors. For example, some data suggest that the circadian rhythms of

cortisol secretion in NR monkeys are different from those of MR monkeys. One study

demonstrated that the circadian rhythm of HPA axis activity in SPR monkeys, as

measured by salivary cortisol, appeared to be temporally delayed; the authors described

the circadian rhythm of SPR monkeys, not as *predictive* as is the case under normal

condition, but rather as *reactive* (Boyce, Champoux, et al., 1995). In addition, juvenile

PR monkeys were shown to have higher plasma cortisol concentrations than MR

monkeys at noon; however, this difference was not observed at any other time during the

day. In addition, PR monkeys had disrupted sleep patterns, waking earlier in the day,

sleeping for shorter lengths of time, and sleeping more during the day than MR monkeys

(Barrette, Noble, et al., 2009). Differences in circadian rhythmicity could be one variable

which contributes to the inconsistent reports of HPA axis activity in differently reared

monkeys, especially if samples are not collected at a uniform time of day across studies.

The conflicting reports of HPA axis activity in NR may be associated with the way that cortisol levels are assessed and whether the goal is to obtain information on basal levels or the reaction to a stressor. When measured in plasma or saliva, cortisol concentrations reflect point sample measurements and provide information on HPA activity along the scale of minutes. However, many plasma and saliva sampling procedures involve some form or restraint or sedation, which can inadvertently result in activation of the HPA axis. It is therefore difficult to assess baseline activity through plasma and salivary cortisol sampling, as changes in HPA axis activity can rapidly be detected in these matrices (Novak, Hamel, et al., 2013). Furthermore, point sampling, as was demonstrated above, is subject to alteration by circadian variation (Barrette, Noble, et al., 2009). Assessing baseline HPA axis activity with point samples is very difficult; thus, the development of a technique to measure chronic levels of cortisol is incredibly valuable. Extraction of cortisol from hair has proven to be a powerful technique in accurate assessment of HPA axis activity (Davenport, Tiefenbacher, et al., 2006). As hair grows, cortisol is deposited in the hair shaft; therefore, hair samples provide information on cortisol concentrations over a long period of time. Hair, as a matrix, provides accurate measurements of chronic HPA axis activity (Novak, Hamel, et al., 2013).

Measurement of hair cortisol in differently reared rhesus monkeys gives a chronic and precise assessment of differences in HPA axis activity as a function of rearing experience. Reportedly, PR monkeys had higher hair cortisol concentrations than MR and SPR monkeys at 6 months of age; at 19 months of age, both PR and SPR monkeys had higher hair cortisol concentrations, and no difference was identified at 24 months of

age (Dettmer, Novak, et al., 2012). Conversely, Feng and colleagues report that at 2 and 3.5 years of age, PR monkeys had lower hair cortisol than MR monkeys (Feng, Wang, et al., 2011). Although the pattern of HPA axis activity across these two studies differs, taken together, they may suggest that at a young age, NR monkeys experience high levels of HPA axis activity and that this hypercortisolism may then result in dysregulation of the HPA axis and, eventually, hypocortisolism. However, further research is required to support this hypothesis.

1.3.2 Nursery Rearing and the Oxytocin System

Although there is very little research that assesses oxytocin concentrations in differently reared monkeys, some evidence suggests that the oxytocin system is also modified by nursery rearing. Nursery reared rhesus monkeys between 1 and 3 years of age had lower concentrations of CSF oxytocin than similarly aged MR monkeys. Furthermore, NR monkeys spent less time engaged in affiliative behaviors; CSF oxytocin concentrations, a reflection of central oxytocin levels, were positively correlated with time spent engaged in affiliative behavior (Winslow, Noble, et al., 2003). These data suggest that nursery rearing results in central changes to the oxytocin system and that these modifications may have direct influences on social behavior. However, as this is the only reported comparison, there has yet to be any evaluation of differences in oxytocin system activity between differently reared rhesus monkey infants or adults.

1.3.3 Mechanisms for Modulation of Physiological Systems by Nursery Rearing

Clearly, the early developmental window is a critical and sensitive period. A significant amount of data demonstrate that nursery rearing in non-human primates plays a role in development of the HPA axis, the oxytocin system, and behavioral output. Nursery reared infants are physiologically and behaviorally distinct from MR counterparts. Research on differently reared monkeys has provided some insight into what factors are important for proper development; for example, the presence of a responsive social companion during development is significant. Socially isolated infants develop the most extreme forms of abnormal behavior (Mitchell, 1968). Research has demonstrated that the presence of social companions buffers against the effects of stress, attenuating the HPA response (Heinrichs, Baumgartner, et al., 2003). Feedback from a social companion seems necessary for normal development and it is possible that absence of social buffering early in life is important in development of NR phenotypes. The presence of peers (Chamove, 1973), or even a canine surrogate mother (Capitanio, 1984; 1985), can protect NR monkeys from developing the high levels of behavioral aberrations seen in isolate-reared monkeys. Furthermore, a sentient companion (peer, dog) or an inanimate surrogate mother provide the infant with contact comfort and the development of an attachment bond, which may also be necessary for normal development.

The exact physiological mechanism by which nursery rearing produces distinct phenotypes in macaques is unclear at this time. Furthermore, it is unclear how early experiences either promote or disrupt the normal development of neuroendocrine systems. Research in rodent models indicates potential neurological alterations related to

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early experiences. Prairie voles that experienced repeated handling by an experimenter during the first week of life displayed changes in their central oxytocin system: vole pups that were handled had lower oxytocin receptor binding than non-handled pups (Bales, Boone, et al., 2011). Rats that had been repeatedly separated from their mother during the first week of life had increased levels of hypothalamic CRF mRNA (Plotsky & Meaney, 1993). Differences in expression of neural components of the HPA axis and oxytocin system may result in the functional differences observed between individuals with different rearing experiences.

Understanding, at a neural level, the changes that result from adverse early experiences could inform treatment for individuals with psychopathology related to traumatic events during development. The nursery rearing paradigm represents a good model of adverse early experience and reliably produces lasting alterations to the HPA axis, the oxytocin system, and behavior. Rhesus monkeys are physiologically similar to humans and the nursery provides an extremely well controlled early environment. However, invasive procedures, such as those used to evaluate neurological changes in rodent models, are not routinely performed on non-human primates, making it difficult to draw conclusions about mechanistic causes for changes that result from nursery rearing. Yet, in order for future research to assess important questions about mechanistic changes that occur within nursery reared infants, patterns of dysregulation need to be better understood. The present dissertation evaluates differences in activity of the HPA axis and oxytocin system as well as in behavior of differently rhesus monkeys using both physiological and behavioral methods to further illuminate and explore the effects of

nursery rearing. In addition, this dissertation assesses the interaction between the HPA axis and oxytocin system and the relationship between neuroendocrine activity and behavior.

CHAPTER 2

**METHODOLOGICAL PROCEDURES FOR MEASURING ACTIVITY OF THE
HPA AXIS AND THE OXYTOCIN SYSTEM**

2.1 Biological Sample Collection

Biological samples were obtained on infant, juvenile, and adult rhesus monkeys reared and housed at various primate centers. Data from infant and juvenile monkeys were obtained from the National Institutes of Health, Laboratory of Comparative Ethology (LCE). The LCE conducts research on monkeys reared in different paradigms, and their staff routinely collect biological samples, some of which were then shipped to the University of Massachusetts for analysis in the UMass Assay Core Lab (directed by Jerry Meyer). As a part of this collaboration, the LCE also provided behavioral data. Data on adult monkeys were obtained from the New England Primate Research Center and the UMass Primate Laboratory. These monkeys are part of a large grant to study psychological well-being at 5 national primate research centers.

All biological samples were collected either as a part of a program of research (LCE) or during routine health exams. In order for samples to be collected, rhesus monkeys are customarily sedated to ensure safety for both the monkey and the experimenters. Ketamine HCl (100mg/ml) was administered intramuscularly at 0.10 ml/kg. Once the monkeys were sedated, they were removed from their home environment to an area designated for examination. Hair samples were collected from the nape of the neck using standard hair clippers and stored at 4 C in a labeled tin foil packet. Blood samples were collected from the femoral vein and deposited into EDTA

tubes. The samples were centrifuged at 2,500 rpm for 15 minutes and the plasma portion

was aliquoted into a 2 mL microcentrifuge tube. Saliva was collected in a cotton swab

which was placed into the cheek pouch for approximately 5 minutes. The saturated

cotton was centrifuged at 2,500 rpm for 15 minutes in a Salivette® tube. Saliva samples

were then aliquoted into 2 mL Eppendorf tubes. Cerebrospinal fluid samples were

collected from the cisterna magna of infant monkeys. Following CSF sample collection,

infants were given ketoprofen (100mg/ml) intramuscularly at 0.02 ml/kg to alleviate any

discomfort from collection. Once collected, plasma, saliva, and CSF samples were

placed on ice until they could be stored at -70 C. After the health exam, monkeys were

placed back into their home environment and monitored until they had recovered from

sedation and were fully awake.

All biological samples were shipped to the University of Massachusetts Amherst

on dry ice, except for hair samples which do not require freezing. Upon arrival, the

samples were transferred to a -70°C freezer for storage.

2.2 Measuring Cortisol Concentrations

2.2.1 Plasma and Saliva Sample Analysis

Prior to analysis, plasma samples were diluted 1:99 in deionized water. Both

saliva and plasma samples were analyzed in duplicate using an enzyme immunoassay

(EIA) to identify point sample cortisol concentrations. Manufactured EIA kits specific to

each matrix were used following the manufacturer's recommended procedures (saliva:

Salimetrics, State College, PA, intra-assay CV=1.47%, inter-assay CV = 2.99%; plasma:

Enzo Life Sciences, Farmingdale, NY, intra-assay CV=4.41%, inter-assay CV = 6.75%).

2.2.2 Hair Processing and Analysis

The technique of analyzing hair cortisol to determine long-term concentrations

was pioneered by our lab (Davenport, Tiefenbacher et al., 2006) and is now widely used

by numerous other labs for analysis of hair from many different species. In order to

analyze hair cortisol concentrations, samples first underwent processing for cortisol

extraction. First, approximately 250 mg of each hair sample was washed twice with

isopropyl alcohol to remove any external contaminants. Once the hair had dried, it was

ground to a fine powder in a Retsch MM200 ball mill at 25 Hz for 6 minutes in a 10 mL

stainless steel grinding jar by a 12mm stainless steel grinding ball. To extract cortisol, 1

mL methanol was added to approximately 50 mg of the hair power and the samples were

rotated overnight. The samples were then centrifuged for 1 minute at 10,000 RPM and

600 μL of the supernatant was dried down under a vacuum evaporator. The cortisol was

then reconstituted in 400 μL of EIA assay buffer (Meyer, Novak, et al., 2013). The

reconstituted samples were analyzed in duplicate by an EIA (Salimetrics, State College,

PA) following the protocol recommended by the manufacturer (intra-assay CV=1.92%,

inter-assay CV=7.14%).

2.3 Measuring Oxytocin

2.3.1 Plasma & Saliva Analysis

Before analysis of oxytocin concentrations within plasma or saliva can take place, the samples must be purified. This is because complex proteins that are present within both matrices interfere with analysis by EIA, yielding erroneous results (Fishman, 1992). We have validated a solid-phase extraction procedure for sample purification in our lab by testing recovery of [3H] oxytocin from ten monkey saliva samples (Holt-Lundstad, Birmingham, et al., 2008). Recovery of oxytocin from the sample was approximately 100%.

A strata-X polymeric reverse phase 96-well plate with 60 mg sorbent beds was used to purify plasma and saliva samples (Phenomenex, Torrance, California). The plate was first equilibrated with 1 ml of methanol and then with 1 ml of deionized (DI) water. Once samples were thawed, 0.8 ml of the sample was acidified with 0.4 mL of 1.5% trifluoroacetic acid (TFA). Samples were then centrifuged for 20 minutes at 5°C at 6000 g and then 1.1 mL of each supernatant was applied to a column of the strata-X plate. Next, the columns were washed with 1.5 ml 0.1% TFA and then the oxytocin was eluted from the treated sorbent bed with 1 ml of 80% acetonitrile. The eluants were collected in conical polypropylene tubes, dried down under a continuous stream of nitrogen, and then reconstituted in 600 μl of assay buffer. The purified samples were analyzed in duplicate using a sensitive and specific oxytocin EIA (Enzo Life Sciences, Farmingdale, NY), following the procedure recommended by the manufacturer (intra-assay CV=6.7%, inter-assay CV=13.75%).

2.3.2 Cerebrospinal Fluid Analysis

CSF samples were analyzed without purification as there are fewer and less complex proteins within the matrix that interfere with oxytocin analysis (Fishman, 1992). Samples were analyzed for oxytocin concentrations in duplicate using a sensitive oxytocin-specific EIA (Enzo Life Sciences, Farmingdale, NY) and followed the procedures recommended by the manufacturer (intra-assay CV=7.84%, inter-assay CV=7.55%).

2.3.3 Hair Oxytocin Analysis and Validation

Current techniques for oxytocin measurement do not extend beyond point samples. Therefore, developing a procedure for measurement of chronic oxytocin system activity would greatly enhance methods of assessment. We sought to develop and validate this procedure in our lab by first performing a parallelism test. Twelve unwashed rhesus monkey hair samples were ground to a powder using the procedure described above for hair cortisol extraction (see section 2.2.2 "Hair Processing and Analysis"). In order to extract oxytocin (Kojima, Stewart, et al., 2012), 500 uL of cold PBS solution and 500 uL of 0.1 M acetic acid were added to the powder. The tubes were then incubated in a dri-bath for 10 minutes at 95 C with the caps off and then centrifuged for 10 minutes at 6 C. Six hundred uL of supernatant from each sample was pooled, frozen at -70 C, and then evaporated in a freeze dryer. The oxytocin was reconstituted in 1 mL EIA assay buffer and 5 samples were created with serial dilution: A, B (75% A), C (50% A), D (37.5% A), and E (25% A). The samples were analyzed in duplicate by an EIA (Enzo Life Sciences, Farmingdale, NY) following the protocol

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recommended by the manufacturer. Observed and expected oxytocin concentrations

were plotted linearly and the observed values were significantly different than expected

(linear regression: $F_{(1,2)} = 21.84$, $p = 0.04$) (Fig. 2.1).
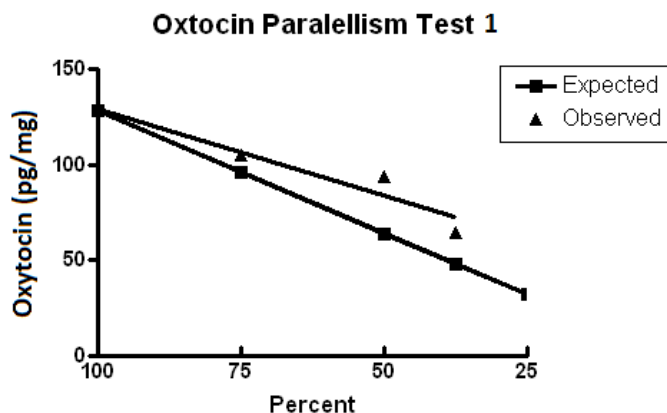
Figure 2.1. Observed and expected hair oxytocin concentrations from first hair oxytocin validation.

Following failure to validate the procedure, a solid phase extraction procedure

was enlisted to purify samples and increase accuracy in hair oxytocin measurement

(Szeto, McCabe, et al., 2011). Following evaporation, extracted oxytocin samples were

reconstituted in 1 mL TFA and stored at -70 C. Ten 3 mL 200 mg c18 columns were

equilibrated with 3 mL 60% acetonitrile and twice with 3 mL 0.1% TFA. Then 1 mL of

the sample was applied to the column and, in order to ensure use of the entire sample, the

sample tube was rinsed with 1 mL 0.1% TFA which was then also applied to the column.

Columns were then washed with 3 mL 0.1% TFA and twice with 3 mL deionized water.

The oxytocin was then eluted from the sorbent bed in 60% acetonitrile and collected in

12 x 75 mm glass tubes. The samples were dried down under a stream of nitrogen and

reconstituted in 250 uL assay buffer and analyzed in duplicated. Resultant oxytocin concentrations were very low (mean: 13.09 pg/mL, range: 8.88 - 25.59 pg/mL, intrassay CV: 2.58%).

In order to validate the solid phase extraction procedure, we tested recovery of [3H] oxytocin diluted 1:49 in 80% ethanol. Recovery of oxytocin following extraction was extremely poor at 0.9%. Therefore, the procedure was repeated and all washes were collected. Very little [3H] oxytocin was detected in each wash and was hypothesized to instead be contained within the sorbent beds of the C18 columns. The columns were then washed with 60% acetonitrile in TFA rather than deionized water and recovery of oxytocin in this solution was approximately 100%. In subsequent variations of the validation procedure, a 100 mg sorbent bed rather than the 200 mg bed, and an additional elution with 60% acetonitrile proved to maximize recovery of the oxytocin.

A second parallelism test was performed using the same procedure as the previous test, but with the 100 mg sorbent bed and an additional elution, on 10 unwashed rhesus monkey hair samples. The expected and observed oxytocin concentrations were plotted linearly and, although the lines were not significantly different from one another (linear regression: $F_{(1,3)} = 0.192$, $p = 0.69$) the observed values were significantly different from expected (Fig. 2.2). In order to preserve the oxytocin, which is heat-labile, the samples underwent extraction without the 10 minute dri-bath and were instead rotated overnight at 6 C. Using this procedure, a third parallelism test was performed and observed concentrations were again significantly different from expected value (linear regression: $F_{(1,3)} = 14.34$, $p = 0.03$) (Fig. 2.3).

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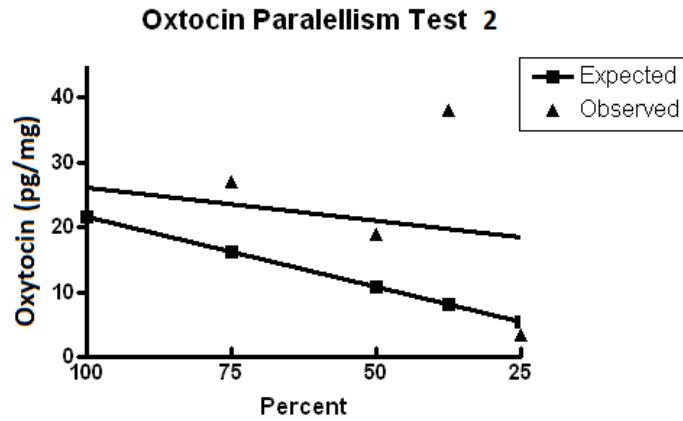
Figure 2.2. Observed and expected hair oxytocin concentrations from second hair oxytocin validation.
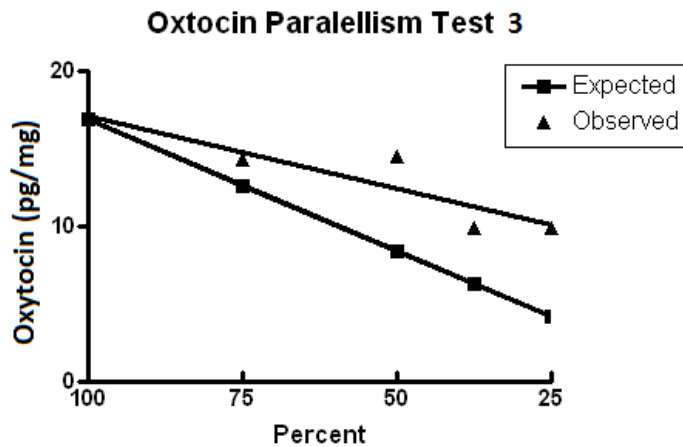
Figure 2.3. Observed and expected hair oxytocin concentrations from third hair oxytocin validation.

These inconsistent results demonstrated that assay of oxytocin concentrations in hair is not a viable procedure for measuring chronic hormone levels. Oxytocin is a peptide hormone and may not be deposited into the growing hair shaft in the same way as cortisol. Little is known about how cortisol is incorporated into the growing hair shaft apart from the fact that, as a steroid hormone, it is lipophilic. Cortisol from surrounding

tissues and local blood supply likely diffuses into the hair shaft during hair growth, where

it is deposited (Meyer & Novak, 2012). The peptide structure of oxytocin, however,

likely prevents diffusion and/or deposition into the hair shaft, preventing hair as a viable

matrix from which to measure oxytocin concentrations.

CHAPTER 3

INTERACTIONS BETWEEN THE HPA AXIS, OXYTOCIN SYSTEM, AND BEHAVIOR IN DIFFERNTLY REARED RHESUS MONKEY INFANTS

3.1 Introduction

Adverse early experiences in rhesus macaques have been shown to have a profound impact on both physiology and behavior. However, conflicting reports of cortisol concentrations in differentially reared infant rhesus monkeys necessitate further examination of these populations in order to fully understand whether nursery rearing is associated with hypo- or hyperfunctionality of the HPA axis. In addition, there is no account in the literature of effects of nursery rearing on central or peripheral domains of oxytocin system in infant monkeys. Therefore, the goal of the experiment described in this chapter was to assess the extent to which early rearing experience modulates the developing HPA axis and oxytocin system. Furthermore, although HPA axis activity has been shown to be modulated by the oxytocin system, little is known about this interaction in differentially reared infants. The present experiment examined whether measures of HPA axis and oxytocin system activity are related and additionally, how they relate to measures of temperament and behavior during the first six months of life.

Nursery rearing was posited to convey dysregulation of the HPA axis. Specifically, NR monkeys were predicted to have lower levels of plasma and hair cortisol, as a majority of the literature demonstrates this pattern. Similarly, nursery rearing was expected to be associated with dysregulation of the oxytocin system, as a result of the relationship with the HPA axis. Nursery reared infants were predicted to

display lower oxytocin concentrations in CSF and plasma, following the pattern previously demonstrated (rhesus monkeys: Winslow, Nobel, et al., 2003; humans: Heim, Young, et al., 2009). Nursery reared infants were also expected to exhibit a more reactive temperament and behavioral responses during early life assessments, consistent with previous reports of higher emotionality in NR infants (Gottlieb & Capitanio, 2013).

Assessments of HPA axis and oxytocin system activity were predicted to be correlated, given the posited relationship between the two systems. Oxytocin concentrations measured in central and peripheral matrices were not expected to be correlated because of their separation by the blood-brain barrier. Finally, measures of HPA axis and oxytocin system activity were predicted to be correlated with temperament and behavior assessed during early developmental evaluations.

3.2 Subjects

Differently reared infant rhesus monkeys (*Macaca mulatta*) born at the Laboratory of Comparative Ethology (LCE) of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) at the National Institutes of Health Animal Center in Poolesville, Maryland participated in this study. Approximately, 50 infants are born at the LCE each year and are randomly assigned to either MR or NR conditions. Samples collected from MR and NR infant monkeys born during the 2007, 2012, and 2013 birth seasons contributed to this data set (see Table 3.1 for demographic information).

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Table 3.1. Demographic information on subjects, including sex and rearing history

| | | Rearing Experience | | |
	Sex	Mother-Peer Reared	Surrogate-Peer Reared	Peer-only Reared
Males	84	35	19	30
Females	65	31	16	18
Totals	149	66	35	48

3.2.1 Rearing

Infants are born at the LCE each year and are randomly assigned to one of three rearing conditions as a part of their research program. For this dissertation, all three rearing conditions were examined: mother-peer-reared (MPR), surrogate-peer-reared (SPR), and peer-reared (PR).

3.2.1.1 Mother-Peer Rearing

At the LCE, MPR infants were raised in social groups with their biological mothers. The social group included 2 adult males, 6-8 adult females, and their respective offspring. The enclosure consisted of indoor (2.44 x 3.05 x 2.21 m) and outdoor components (2.44 x 3.0 x 2.44 m) connected by a guillotine door. The enclosure walls were made of galvanized steel mesh and the floors were covered with wood chips. Within the indoor enclosure, the lights were maintained on a 12:12 cycle (0700-1900). The adults' diet consisted of Purina Monkey Chow (#5038), fruit supplemented three times a week, and nuts which were provided daily as forage. Water was available *ad libitum*. For the first six to eight months of life, infants remained in this social group

until they were relocated to a large, mixed-rearing social group with other similar-aged infants.

3.2.1.2 Surrogate-Peer Rearing and Peer-Only Rearing

At birth, infants designated for nursery rearing were assigned to either the surrogate-peer-reared (SPR) or peer-reared (PR) condition. However, for the first 37 days of life, both SPR and PR infants received the same treatment. Infants assigned to either nursery-rearing condition were separated from their mothers between 1-3 days of age. The infants were then transferred to the nursery where they were individually housed in incubators (51 x 38 x 43 cm) which were maintained at approximately 27 C. Infants had auditory, visual, and olfactory, but not tactile, contact with other infants. Incubators were furnished with inanimate, cloth-covered "surrogate mothers" which were enclosed in a heating pad and fleece fabric to provide warmth as well as contact comfort for the infants.

At 15 days of age, nursery reared infants were moved, with their surrogates, to a larger housing room. Infants were individually housed in wire mesh cages (64 x 61 x 76 cm) where they continued to be able to see, hear, and smell other infants, but not physically interact with them. The environment was maintained at 22-26 C and 50-55% humidity, with a light cycle of 14:10 (0700-2100). All nursery-reared infants were assigned to social groups, whether they had been designated for SPR or PR. When the youngest infant of each assigned group reached 37 days of age, the social groups were formed. Infants assigned to PR social groups were moved into larger cages (71 x 81 x

152 cm) with 3 other similar-aged infants with whom they were housed 24 hours a day. Infants assigned to the SPR condition remained in individual cages with their surrogates. For 2 hours a day, the SPR infants were moved to a larger play cage (71 x 81 x 12 cm), furnished with one surrogate, along with 3 other similarly-aged SPR infants that comprised their social group. During this 2 hour period, infants typically engaged in social play behavior.

All nursery-reared infants were hand fed a mixture of Similac (Ross Laboratories, Columbus, OH) and Primilac (Bio-Serv, Frenchtown, NJ) formulas. When the infants were capable of feeding independently, formula was provided *ad libitum*. The infants' diet was supplemented with Purina monkey chow (#5038) and water *ad libitum* from 1 month of age on. At 4 months of age, the infants were placed on a ration of 300 mL of formula a day and at 5 months of age, the ration was decreased to 200 mL a day. Infants were weaned entirely off of formula at 6 months of age. Infants remained in the nursery until they were 8 months of age, when they were relocated to mixed-rearing social housing with other same-aged infants from all 3 rearing conditions: MPR, SPR, and PR.

3.3 Biological Sample Collection

Collection of hair, plasma, and CSF samples coincided with LCE mandated health exams which take place at 14 and 30 days and 6 months of life. All samples were shipped to the University of Massachusetts Amherst on dry ice, except for hair samples, which do not require freezing. Samples were assayed for cortisol and oxytocin

concentrations (as described in Sections 2.2 "Measuring Cortisol Concentrations" and Section 2.3 "Measuring Oxytocin Concentrations").

3.4 Brazelton Neonatal Behavioral Assessment

The Brazelton Neonatal Assessment Scale (BNAS) is a standardized 20-minute battery of tests administered to infants in order to systematically assess emotional, social, and motor responses that develop within the first month of life. Originally designed to assess development in humans (Brazelton, 1973), the BNAS was modified for use in rhesus macaques (Schneider & Suomi, 1992). These variables fall into three umbrella categories: temperament, interactive, and neuromotor. For the purpose of this dissertation, only a subset of variables were analyzed, those that involved assessments of temperament and emotional behavior. The BNAS was administered to infants at 7, 14, 20, and 30 days of age.

3.4.1 Temperament Assessment

During the 20-minute test battery, the goal was to keep the infant quiet and alert; therefore, temperament was continuously monitored throughout. The first set of assessments focused on attention, reflexes, and responses to different physical orientations. Following these exercises, the experimenter rated the infant's temperament by evaluating various behavioral and emotional categories. For the purposes of this analysis, temperament categories of interest included: soothability (needing experimenter intervention for soothing), contact resistance (resistance to maintaining close physical contact with the experimenter), and tremulousness (amount of trembling). Each domain

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was scored by a trained observer on a five point scale; scores ranged from 0 to 2 and included half scores (ie, 0.5 or 1.5). Higher scores for soothability and tremulousness indicated higher levels of distress while higher scores on contact resistance indicated a higher resistance to experimenter-initiated cuddling.

The next step in the test was a five minute isolation test in which the infant was placed alone in an incubator for five minutes. Nursery-reared infants were given a toy (Tweety Bird stuffed animal) and MR infants were given a blanket in addition to the toy. The number of times the infant vocalized was tallied for the first minute of the test and will be referred to as the "1 minute vocalization" score. Throughout the 5 minute test, the experimenter recorded the duration or frequency of target behaviors on a palm pilot. Behavioral categories that were selected as relevant to the dissertation included environmental exploration (any examination, exploration, or manipulation of the environment), locomotion (any self-induced change in location), and sleep (nonactive and eyes closed). At the end of the test, experimenters rated the infant's overall response to the novel environment. Infant's level of distress was scored on a five point scale between 0 and 2 ("isolation distress") with higher scores indicating higher distress or greater difficulty self-soothing.

Following the isolation test, experimenters rated the infant's overall temperament across the entire test. Categories selected for analysis included: irritability (amount of protest or distress), consolability (ease of calming by experimenter), and fearfulness (timidity or apprehension). Again, higher scores indicated higher distress. Following testing on 14 and 30, the infants were anesthetized for collection of biological samples

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(see section 2.3 "Biological Sample Collection") while after testing on days 7 and 20 infants were directly returned to their home environment.

3.5 Data Analysis

3.5.1. Examination of Cortisol and Oxytocin Concentrations

Each biological measurement first underwent a Kolmogorov-Smirnov test for normality and any measurements that were not normally distributed ($p<0.05$) were log transformed: hair cortisol (day 14), hair cortisol (day 180), CSF oxytocin (day 14), CSF oxytocin (day 30), plasma cortisol (day 14), plasma oxytocin (day 14), and plasma oxytocin (day 30). Plasma cortisol (day 30) was also log transformed in order for comparisons to be made with other log transformed measures. The log transformed values were used in all subsequent analyses.

For the purposes of comparing across rearing conditions, initial analyses combined SPR and PR conditions, as these two groups were treated the same throughout the first month of life, into a nursery rearing (NR) category. An initial one-way ANOVA demonstrated that there was no significant effect of cohort (between subjects variable) on biological measures or response to the BNAS (within subject variables). Therefore, subjects were collapsed across cohort for all subsequent analyses.

A mixed design ANOVA was applied to each biological sample type: (1) hair cortisol (day 14 and day 180), (2) CSF oxytocin (day 14 and day 30), (3) plasma cortisol (day 14 and day 30), and (4) plasma oxytocin (day 14 and day 30). For each analysis, the within subject variables were the biological sample measure collected at the two different

points during development, and the between subjects variables were rearing condition and sex. Because NR infants were assigned to different rearing conditions between the day 14 and day 180 hair sample, the hair cortisol analysis included all three rearing conditions, whereas the other 3 analyses combined the two nursery rearing conditions. Significant effects were examined using post hoc t-tests. Independent samples t-tests compared biological measures (within subject variable) across either rearing condition or sex (between subjects variables) while paired samples t-tests compared biological samples (within subject variable) across sampling date (between subjects variable). A post hoc ANOVA with Bonferroni corrections was applied to hair cortisol concentrations to examine differences between the 3 rearing conditions.

In order to assess the relationship between the HPA axis and oxytocin system, Pearson correlations examined the relationship between cortisol and oxytocin concentrations within sampling matrices: (1) plasma cortisol by plasma oxytocin (day 14) and (2) plasma cortisol by plasma oxytocin (day 30). Similarly, to determine the relationship between central and peripheral oxytocin system activity, Pearson correlations were applied across sampling matrices (yet within sampling period): (1) CSF oxytocin by plasma oxytocin (day 14), and (2) CSF oxytocin by plasma oxytocin (day 30).

3.5.2 Assessment of Temperament through the Brazelton Neonatal Assessment

Target measures scored on the Brazelton Neonatal Assessment (BNAS) included 7 temperament measures and 4 behavioral measures. Within each of the measures, scores across the 4 test days (days 7, 14, 20, and 30) were averaged to create an overall mean

score. Measures of temperament included: soothability, contact resistance, tremulousness, irritability, consolability, fearfulness, and isolation distress. A composite temperament category, "DISTRESS", was created to reflect overall infant distress during the test and calculated by averaging the mean scores for soothability, consolability, and irritability. The category, isolation distress, was purposefully excluded from this category as it specifically reflects infants' response to the isolation test. In addition, a composite "FEAR" category was created by averaging the temperament measures tremulousness and fearfulness in order to reflect levels of infant fear during the BNAS. Overall, there were 4 temperamental categories, including the two composite scores and contact resistance and isolation distress. Measures of behavioral response to the isolation test included environmental exploration, locomotion, sleep, and the 1 minute vocalization score, for a total of 4 behavioral categories.

In order to determine the contribution of rearing condition (MR vs NR) and sex on measures of temperament, a multivariate analysis of variance was performed (between subjects factors: rearing condition and sex; within subject factors: the average scores on DISTRESS, FEAR, isolation distress, and contact resistance). Similarly, a multivariate analysis was performed to determine the effects of the same factors on behavioral measures collected during the isolation test (between subjects factors: rearing condition and sex; within subject factors: environmental exploration, locomotion, sleep, and 1 minute vocalization score).

3.5.3 Determining the Relationship between Temperament or Behavior and Measures of Cortisol and Oxytocin Concentrations

In order to determine whether prenatal exposure to maternal glucocorticoids predicted temperament or behavior, Pearson correlations were performed between Day 14 hair cortisol measures and each of the 8 temperament and behavior measures of interest. As hair cortisol was expected to be the most stable measure of HPA axis activity, Pearson correlations were performed between the Day 180 hair cortisol measure and each of the 8 average temperament and behavior measures of interest. Because the central and peripheral oxytocin systems are believed to be distinct, two rounds of correlations were performed to assess the relationship between temperament and both arms of the oxytocin system: (1) between the 8 average temperament and behavior scores and the Day 30 CSF (central) oxytocin measure, and (2) between the 8 average temperament and behavior scores and the Day 30 plasma (peripheral) oxytocin measure.

3.6 Results

3.6.1 The Effects of Rearing Condition and Sex on Cortisol and Oxytocin Concentrations

Mixed design ANOVAs revealed significant effects of rearing condition and sex across the different developmental time points (Table 3.2). For hair cortisol, there was a significant main effect of developmental age ($F_{(1,55)}$=220.622, p<0.001) such that day 14 concentrations were higher than day 180 concentrations (Fig. 3.1a), and an interaction between rearing condition and age ($F_{(2,53)}$=7.352, p=0.002) (Fig. 3.1b). As predicted MPR infants had higher hair cortisol concentrations than both SPR (p=0.038) and PR (p=0.008) infants on day 180 (post hoc ANOVA with Bonferroni correction; Fig. 3.1b).

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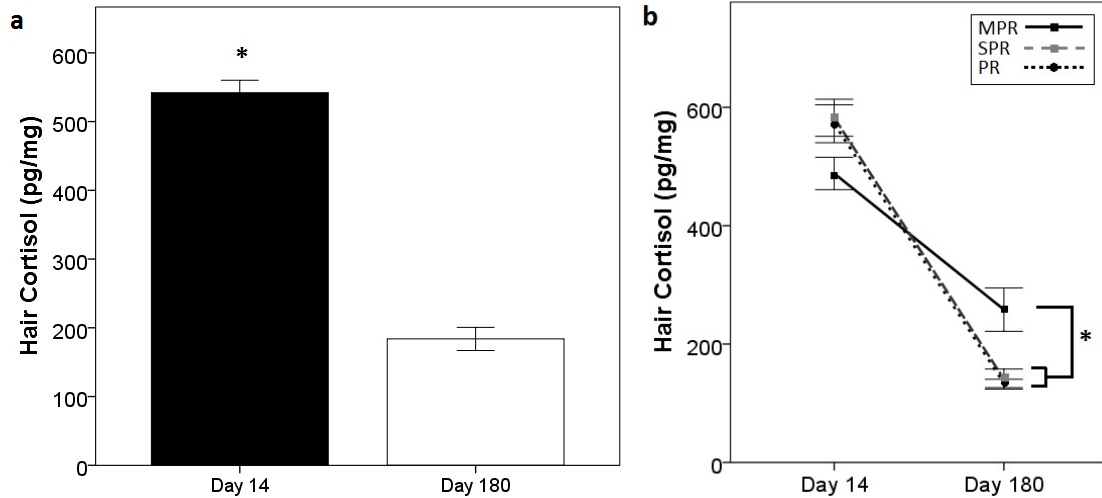
Figure 3.1. The main effect of (a) sampling date and (b) the interaction between sample date and rearing condition on hair cortisol, *$p<0.05$. Error bars represent ±1 SE.

As predicted, there was a main effect of rearing condition on plasma cortisol concentrations ($F_{(1,48)}=32.718$, $p<0.001$; Fig. 3.2a). As with hair cortisol concentrations, MPR infants showed higher plasma cortisol levels than NR infants. In addition, there was a main effect of developmental age on plasma cortisol concentrations ($F_{(1,48)}=5.089$, $p=0.029$); Day 30 plasma cortisol concentrations were higher than those measured on Day 14 (Fig. 3.2b). There was also a nearly significant interaction between sex and sampling date ($F_{(1,48)}=3.950$, $p=0.053$) on plasma cortisol concentrations. A post hoc paired t-test demonstrated that females had higher plasma cortisol on day 30 than day 14 ($t_{(24)}=-14.026$, $p<0.001$) whereas a post hoc independent samples t-test demonstrated that females had higher plasma cortisol than males on day 30 ($t_{(54)}=-3.282$, $p=0.002$) (Fig. 3.2c).
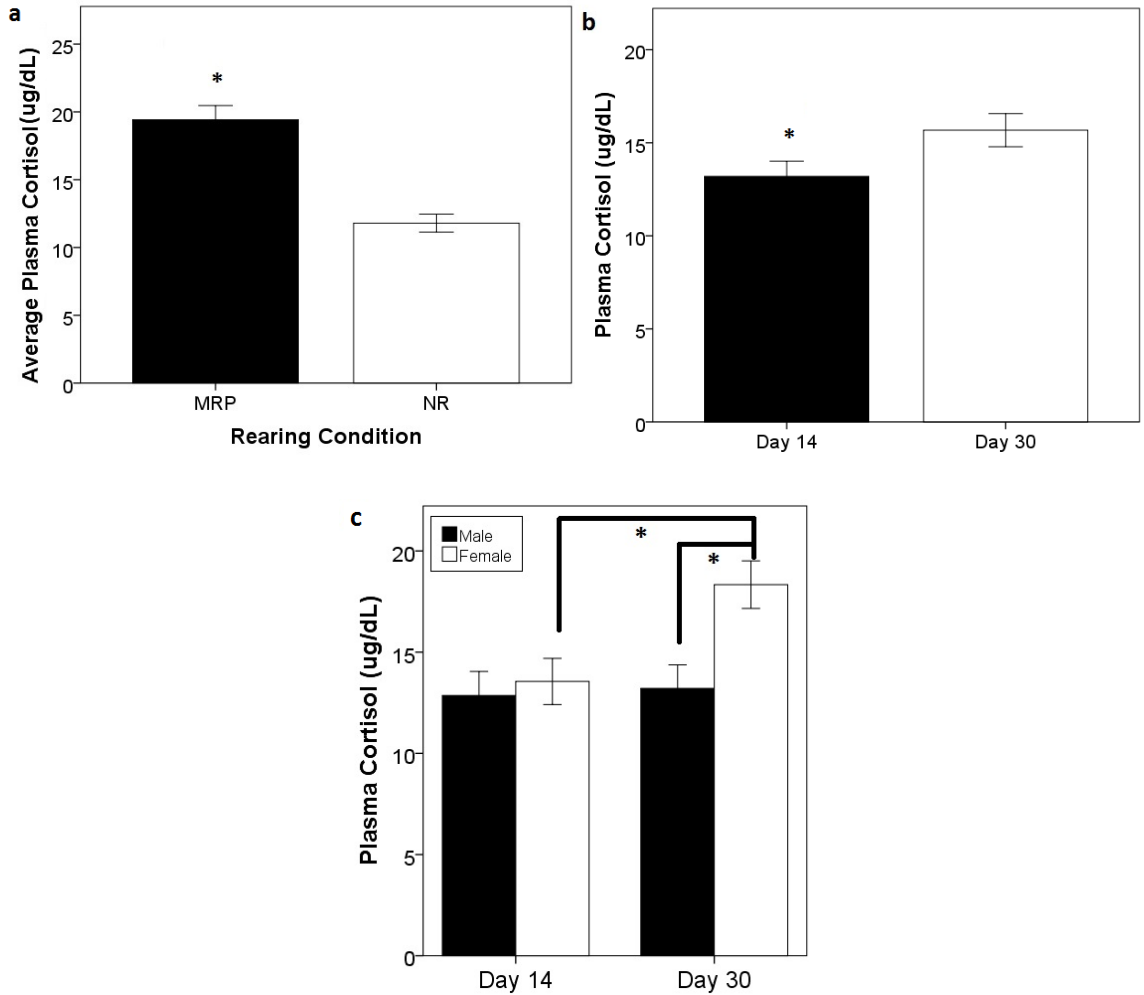
Figure 3.2. The (a) main effects of rearing condition and (b) age, and (c) the interaction between sex and age, on plasma cortisol concentrations, *p<0.05. Error bars represent ±1 SE.

With respect to the oxytocin system, a mixed design ANOVA revealed a significant main effect of rearing condition ($F_{(1,84)}$=5.755, p=0.019). Contrary to both predictions and to the plasma cortisol findings, NR infants had higher CSF oxytocin concentrations overall than MPR infants (Fig. 3.3a). In addition, there was a significant main effect of developmental age on CSF oxytocin ($F_{(1,84)}$=5.205, p=0.025), such that concentrations were higher overall on day 14 than day 30 (Fig. 3.3b).

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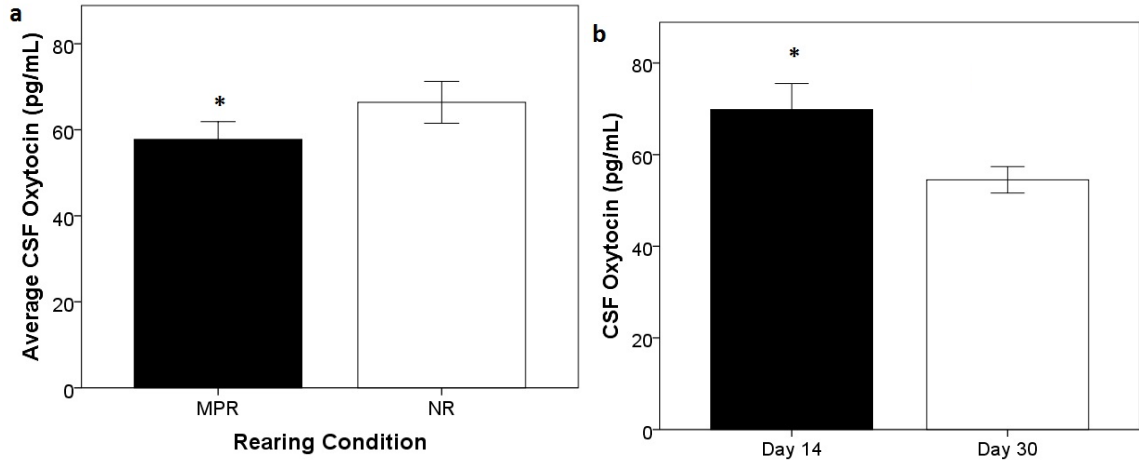
Figure 3.3. The main effects of (a) rearing condition and (b) sampling day on CSF oxytocin concentrations, $p < 0.05$. Error bars represent ±1 SE.

Table 3.2. The effects of rearing, age, and sex on hair cortisol, plasma cortisol, plasma oxytocin, and CSF oxytocin (p-values)

	Hair Cortisol	Plasma Cortisol	Plasma Oxytocin	CSF Oxytocin
Main Effect of Rearing	0.075	**<0.001**	0.138	**0.019**
Main Effect of Sex	0.595	0.093	0.898	0.899
Main Effect of Age	**<0.001**	**0.029**	0.710	**0.025**
Rearing x Age Interaction	**0.002**	0.417	0.552	0.378
Sex x Age Interaction	0.435	0.053	0.861	0.528

Note: Bolded p-values <0.05.

3.6.2 Cortisol and Oxytocin Correlations Across and Within Sampling Matrices

The prediction of a correlation between HPA axis and oxytocin system activity was not met; there was no significant correlation between plasma cortisol and plasma

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oxytocin concentrations (day 14: $p=0.151$; day 30: $p=0.851$). Contrary to predictions, there was no correlation between central and peripheral oxytocin system activity measured in CSF and plasma (day 14: $p=0.108$; day 30: $p=0.322$).

3.6.3 Effects of Rearing Condition and Sex on Temperament and Behavioral Reactivity

A multivariate ANOVA identified significant effects of rearing, but not sex, on temperament measures (Table 3.3). There was a significant effect of rearing condition on DISTRESS ($F_{(1,116)}=310.921$, $p<0.001$), FEAR ($F_{(1,116)}=6.717$, $p=0.011$), isolation distress ($F_{(1,116)}=171.499$, $p<0.001$) and contact resistance ($F_{(1,116)}=24.264$, $p<0.001$). Mother-peer reared infants had higher composite DISTRESS (Fig. 3.4a) and FEAR scores (Fig. 3.4b) as well as higher isolation distress (Fig. 3.4c) and contact resistance scores (Fig. 3.4d) than NR infants.
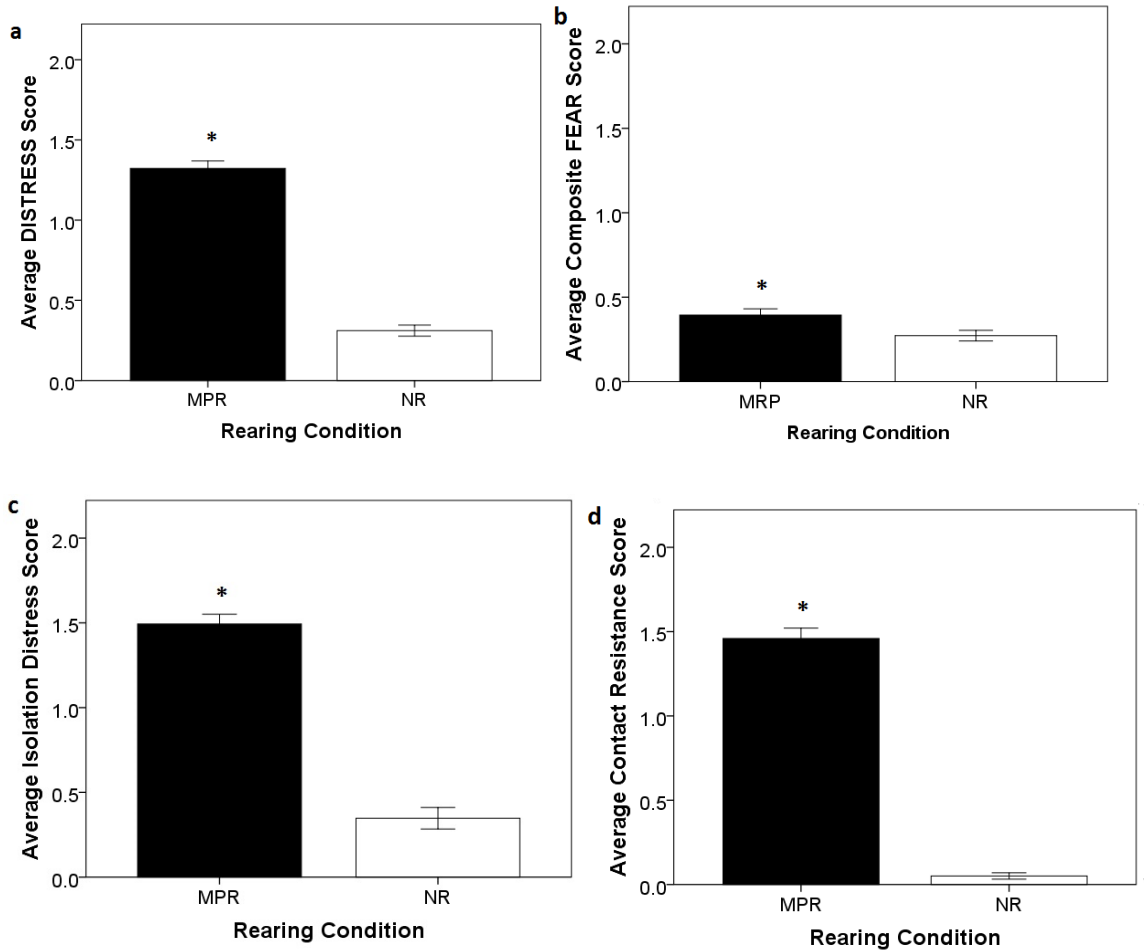
Figure 3.4. The effect of rearing on composite temperament scores (a) DISTRESS and (b) FEAR and on (c) isolation distress and (d) contact resistance, * *p*<0.01. Bars represent ±1 SE.

A multivariate ANOVA demonstrated that rearing significantly affected behavioral response to the novel environment test, whereas sex did not (Table 3.3). Both types of active behavior, environmental exploration ($F_{(1,116)}$=56.622, *p*<0.001) and locomotion ($F_{(1,116)}$=33.379, *p*<0.001) were modified by rearing condition, although in different directions. Mother-peer reared infants spent less in time environmental exploration (Fig. 3.5a) and more time in locomotion (Fig. 3.5b) than NR infants. The number of vocalizations made during the first minute of the isolation test

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($F_{(1,116)}=112.245$, $p<0.001$) and amount of time spent sleeping ($F_{(1,108)}=6.078$, $p=0.020$) were also significantly modified by rearing condition. In the isolation test, MPR monkeys vocalized more during the first minute of the isolation test (Fig. 3.5c) and slept significantly less (Fig. 3.5d) than NR monkeys.
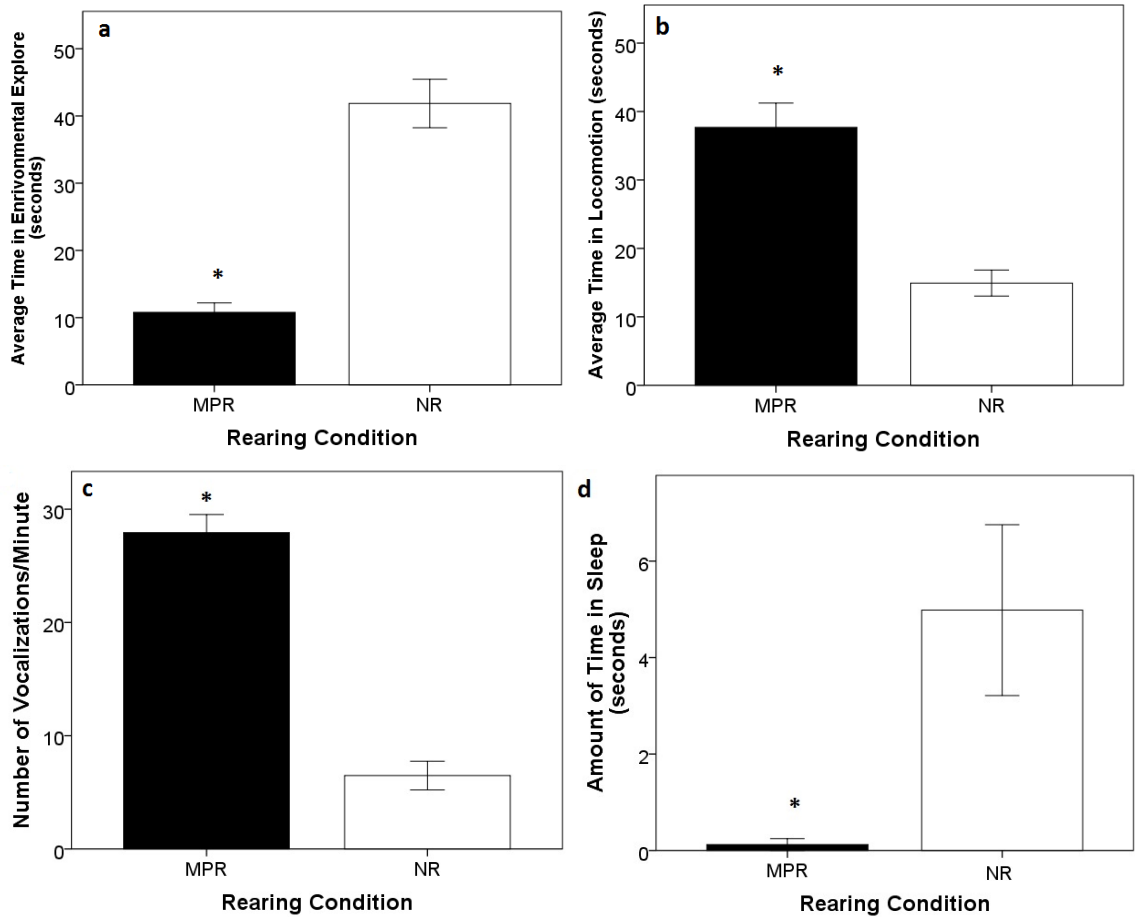
Figure 3.5. The effect of rearing on time spent in (a) environmental exploration and (b) locomotion, (c) number of vocalizations during the first minute, and (d) amount of time spent in sleep during the isolation test, $p<0.02$. Bars represent ±1 SE.

3.6.4 Relationships between Measures of Cortisol and Oxytocin Concentrations and Temperament

Significant Pearson correlations were identified between Day 14 hair cortisol concentrations and average temperament scores of DISTRESS ($r_{(57)}=0.499$, $p=0.001$; Fig.

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3.6a) and contact resistance ($r_{(46)}$=0.337, p=0.031; Fig. 3.6b). In terms of behavioral measures, Day 14 hair cortisol was correlated with amount of time in locomotion ($r_{(57)}$=0.324, p=0.036; Fig 3.7) (Table 3.3).
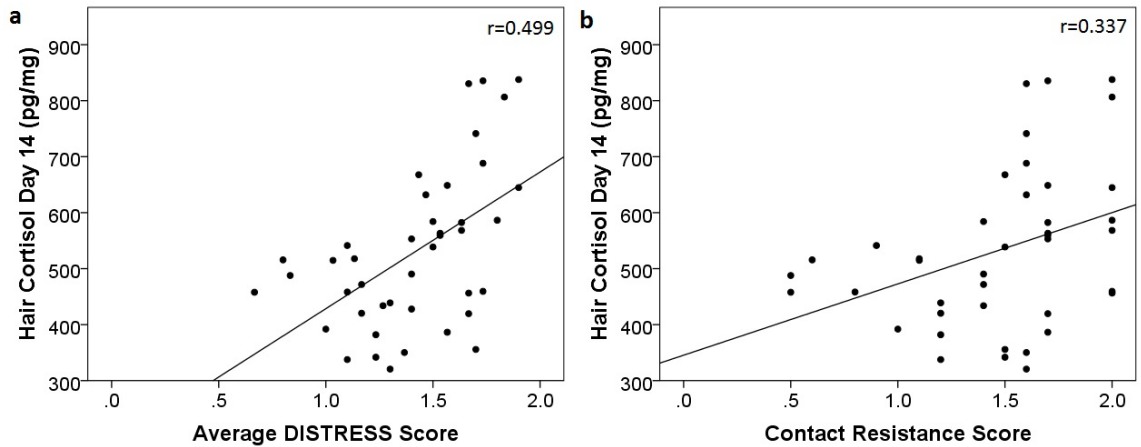
Figure 3.6. The relationship between Day 14 hair cortisol and (a) DISTRESS (p=0.001) and (b) contact resistance (p=0.031).
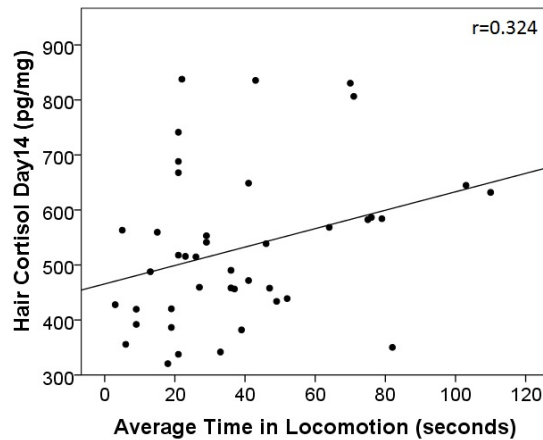
Figure 3.7. The relationship between Day 14 hair cortisol and locomotion (p=0.036).

Pearson correlations identified significant positive correlations between Day 180 hair cortisol concentrations and average temperament scores of DISTRESS ($r_{(97)}$=-0.345,

$p=0.001$; Fig. 3.8a), isolation distress ($r_{(97)}=0.286$, $p=0.005$; Fig. 3.8b), and contact resistance ($r_{(97)}= 0.506$, $p<0.001$; Fig. 3.8c) (Table 3.3).
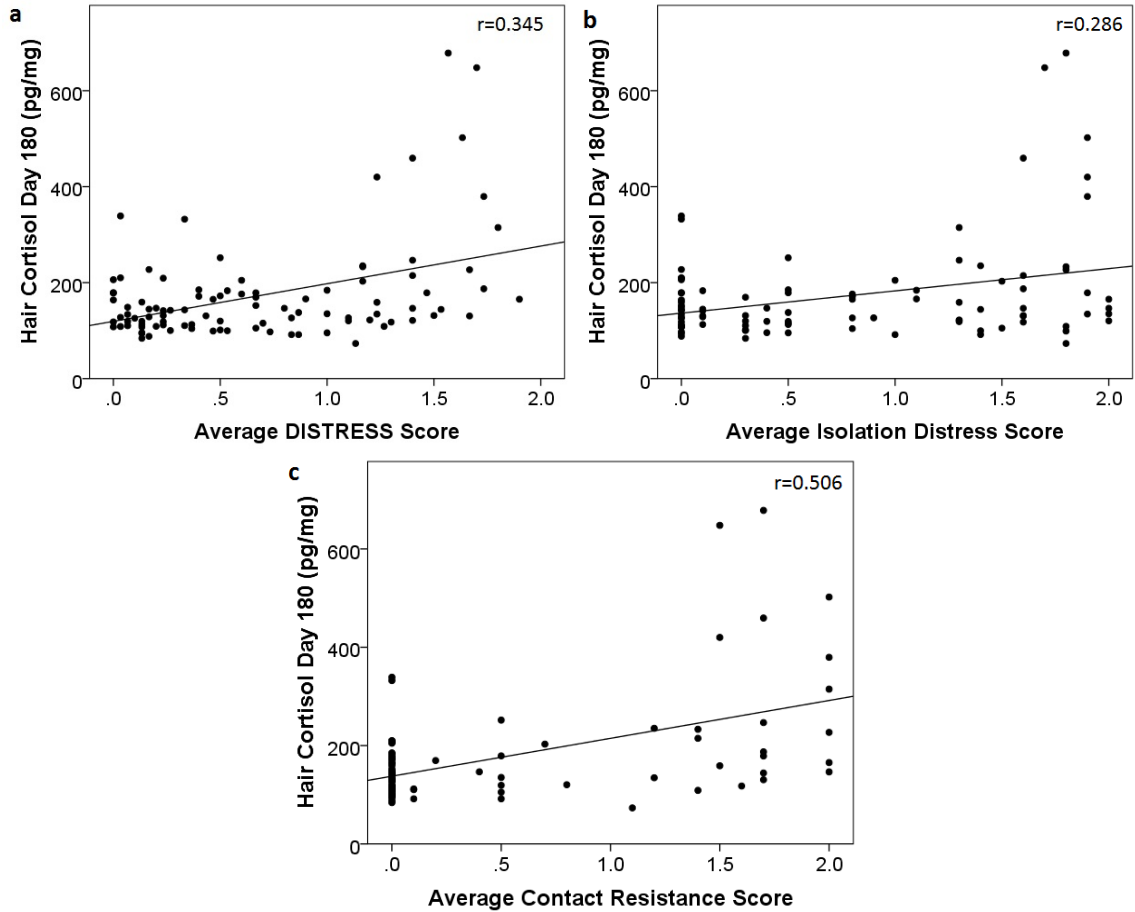
Figure 3.8. The relationship between Day 180 hair cortisol and (a) DISTRESS ($p<0.001$), (b) isolation distress ($p=0.005$), and (c) contact resistance ($p<0.001$).

There was also a significant positive correlation between Day 180 hair cortisol concentrations and vocalizations per minute ($r_{(97)}=0.284$, $p=0.005$; Fig. 3.9a) and a significant negative correlation with environmental exploration during the isolation test ($r_{(97)}=-0.234$, $p=0.021$; Fig. 3.9b) (Table 3.3).
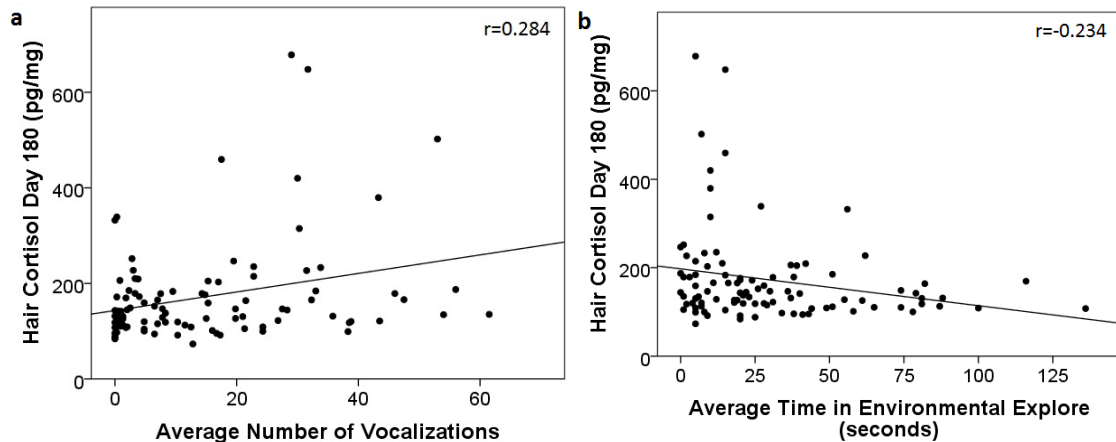
Figure 3.9. The relationship between Day 180 hair cortisol and (a) average number of vocalizations (*p*=0.005), and (b) amount of time spent in environmental exploration (*p*=0.021).

In terms of the oxytocin system, there were no significant correlations between Day 30 CSF oxytocin concentrations and any of the temperament or behavioral scores. However, peripheral oxytocin system activity, as measured in plasma, was significantly related to measures of temperament. In contrast to positive correlations with hair cortisol, significant negative correlations were identified between Day 30 plasma oxytocin concentrations and DISTRESS ($r_{(45)}$=0.361, *p*=0.015; Fig. 3.10a), isolation distress ($r_{(45)}$=-0.429, *p*=0.003; Fig. 3.10b) and contact resistance ($r_{(56)}$=0.314, *p*=0.038; Fig. 3.10c) There were no significant correlations between Day 30 plasma oxytocin concentrations and measures of behavior during the isolation test (Table 3.3).
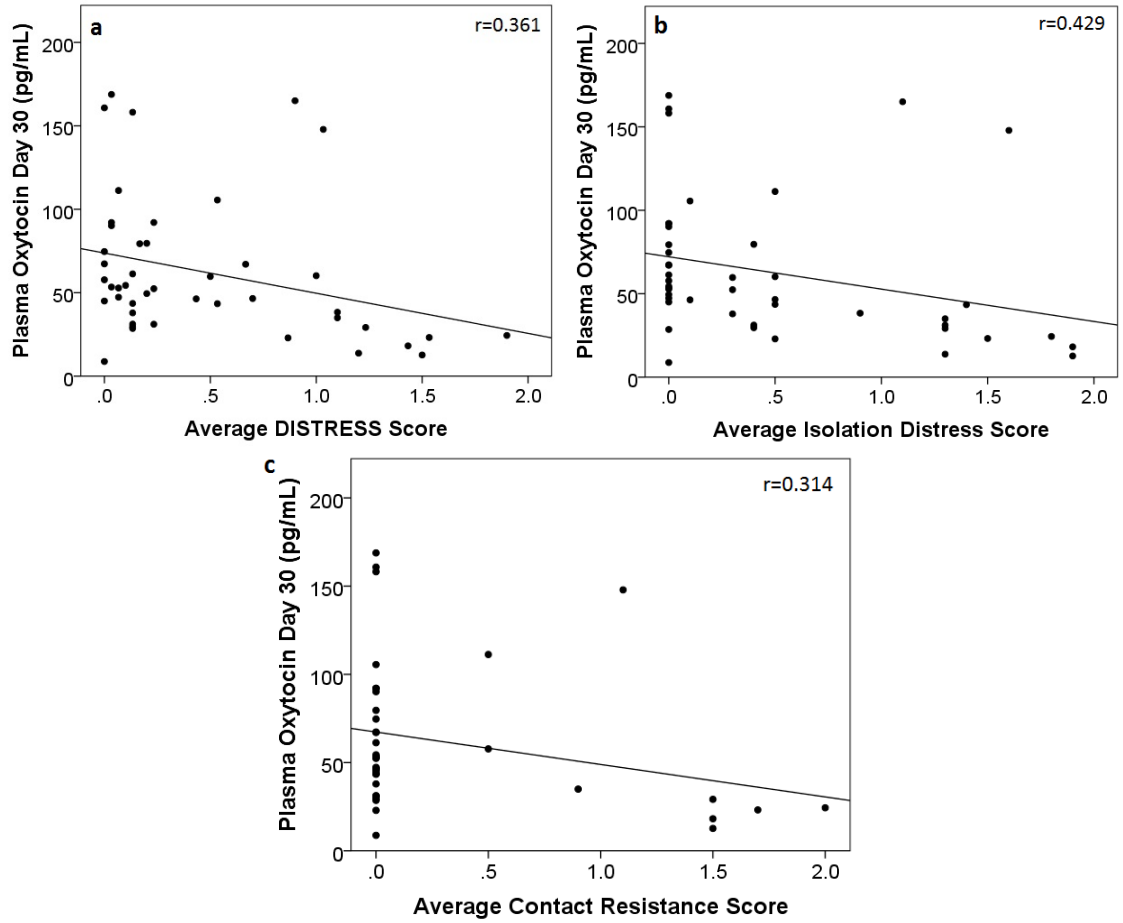
Figure 3.10. The relationship between Day 30 plasma oxytocin concentrations and (a) DISTRESS ($p=0.015$), (b) isolation distress ($p=0.003$), and (c) contact resistance ($p=0.046$).

Table 3.3. The effect of rearing on the temperament and behavioral scores as well as their correlations with day 180 hair cortisol, day 30 CSF oxytocin, and day 30 plasma oxytocin (*p*-values)

		Temperament Scores				Behavioral Scores			
		DISTRESS	FEAR	Isolation Distress	Contact Resistance	Environmental Exploration	Locomotion	1 Min. Voc. Count	Sleep
	Main Effect of Rearing	**<0.001**	**0.011**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.015**	**<0.001**
Correlations	**Hair Cortisol Day 14**	**0.001**	0.110	0.369	**0.031**	0.951	**0.036**	0.301	0.412
	Hair Cortisol Day 180	**0.001**	0.059	**0.005**	**<0.001**	**0.021**	0.543	**0.005**	0.359
	CSF Oxytocin Day 30	0.253	0.505	0.784	0.952	0.110	0.093	0.093	0.571
	Plasma Oxytocin Day 30	**0.015**	0.103	**0.003**	**0.038**	0.213	0.153	0.075	0.177

Note: Bolded *p*-values <0.05, voc=vocalization

3.7 Discussion

3.7.1 The Effect of Rearing Condition and Sex on HPA Axis and Oxytocin System Activity

As predicted, NR infants demonstrated lower plasma and hair cortisol concentrations than MPR infants. The significant effect of rearing on plasma cortisol corresponds with previous research from MPR and NR infants reared at the NICHD (Davenport, Novak, et al, 2003; Shannon, Champoux, & Suomi, 1998), in which NR

infants showed lower plasma cortisol concentrations within the first month of life, yet differs from reports of higher cortisol in NR infants (Champoux, Coe, et al., 1989) and of similar cortisol levels in differently reared infants (Clarke, 1993). Importantly, at the NICHD, plasma samples were collected after the BNAS and therefore represent the infants' acute responses to removal from the home environment, being handled by an experimenter, and the 20-minute evaluation. Decreased levels of plasma cortisol in NR monkeys following the BNAS could be interpreted in multiple ways: (1) that NR monkeys have lower baseline HPA axis activity, (2) that response of the HPA axis to stress is blunted in NR monkeys, or (3) that NR infants are less reactive to the separation and handling during the BNAS.

A previous report by Capitanio and colleauges similarly demonstrated lower levels of plasma cortisol in NR monkey infants (Capitanio, Mendoza, et al. (2005). More importantly, however, is that although both MR and NR monkeys responded similarly to pharmacological manipulation of the HPA axis, NR monkeys continued to have lower cortisol levels both after the dexamethasone suppression test, in which HPA axis activity was pharmacologically dampened, and in response to activation of the HPA axis by the ACTH stimulation test. These findings led Capitanio and colleagues to conclude that NR monkeys have an overall lower HPA axis set point (2005), which corresponds to the first hypothesis.

As predicted, both SPR and PR infants had lower hair cortisol concentrations than MPR infants on day 180. As these data reflect chronic HPA axis activity over a period of months and are not susceptible to modulation by the stress of sample collection, they

refute the hypothesis that NR infants are simply less reactive to handling and sampling and support that NR monkeys have a lower HPA axis set point. Although our data corroborate lower levels of hair cortisol in PR monkeys at 2 and 3.5 years (Feng, Wang, et al., 2011) previous experiments of infants reared at the NICHD have demonstrated conflicting results, in which MPR and NR infants were reported to have similar levels of hair cortisol at day 180 (Dettmer, Novak, et al., 2009). These variable effects of rearing on hair cortisol, particularly from one facility, are intriguing and warrant future investigation. One possible explanation could be genetic variation, as breeding males are cycled in and out of commission across breeding seasons.

Although NR monkeys were predicted to have blunted oxytocin system activity, oxytocin concentrations, measured in CSF, but not plasma, were higher in NR infants. These data are the first to demonstrate rearing effects on oxytocin system activity in infant monkeys. The single previous account of oxytocin concentrations in differently reared monkeys reported that NR monkeys at 18, 24, and 36 months of age had *lower* CSF oxytocin than MR monkeys (Winslow, Noble, et al., 2003). It should be noted, however, that (1) the monkeys in the present experiment were significantly younger and (2) that samples were collected following the BNAS. As with HPA axis activation, CSF oxytocin concentrations may reflect response to the stress of testing. Previous research with rodents has shown elevated central oxytocin following the Shaker Stress Test (Niskioa, Anselmo-Franci, et al., 1998). Therefore, these data may indicate that NR infants demonstrate elevated oxytocin system activity following a stressor. Furthermore, it is possible that increased oxytocin system activity may be related to the lower set point

hypothesized for HPA axis activity in NR infants. In addition, it is of note that these data correspond with previous patterns of decreased HPA axis activity associated with increased oxytocin system activity following a stressor in rodents (Windle, Shanks, et al., 1997) and following intranasal oxytocin administration in rhesus monkeys (Simpson, Sclafani, et al., 2014).

There was no significant effect of rearing experience on plasma oxytocin concentrations. This lack of effect may be related to the separation of central and peripheral oxytocin systems by the blood-brain barrier (Amico, Challinor, et al., 1990). Therefore, as rearing experience is associated with differences in central levels of oxytocin, it may have specific effects on parvocellular neurosecretory neurons, which have extra-hypothalamic projections that target central brain areas, rather than magnocellular neurons which project to the pituitary gland and cause release of peripheral oxytocin. Further research is required to determine the validity of this hypothesis.

3.7.2 The Effect of Rearing Condition on Temperament and Behavior

Infants' responses to the BNAS varied by rearing condition; however, contrary to predictions, MPR infants demonstrated higher levels of reactivity than NR infants. Overall, MPR infants displayed higher composite DISTRESS and FEAR scores and higher contact resistance. During the isolation test MPR infants displayed higher distress, more vocalizations and locomotion, and less time asleep or in environmental exploration. This pattern of results is consistent with a pattern of heightened distress and arousal in

MPR infants and likely reflects a protest response to the brief maternal separation required for developmental assessment in this group of monkeys. Although NR monkeys have been shown to develop attachment bonds with surrogate mothers (Harlow, 1962) and demonstrate negative reactions to separation (Meyer, Novak, et al., 1975), attachment bonds may not be as secure as those formed between a mother and a MPR infant. Alternatively, these data may suggest that NR infants are better able to calm themselves during the BNAS, perhaps as a result of more experience self-soothing. Furthermore, NR infants may be less reactive to testing as they more familiar with the testing environment as well as the close proximity of human experimenters; NR infants showed less contact resistance, which supports increased familiarity with experimenters. Finally, elevated concentrations of oxytocin in NR infants may attenuate HPA axis response to the acute stress of testing, and thus their behavioral reactivity. These various hypotheses are not mutually exclusive and may each contribute to responses of MPR and NR infants to the BNAS.

3.7.3 Relationships between HPA Axis and Oxytocin System Activity and Results of BNAS

Hair cortisol concentrations, which represent chronic HPA axis activity, were related to measures of temperament and behavioral reactivity. Hair cortisol collected on Day 14 was positively correlated with measures of reactivity, including DISTRESS, contact resistance, and locomotion, during the BNAS in the first month of life. Early postnatal hair samples likely reflect prenatal exposure to maternal glucocorticoids (Duthie & Renolds, 2013). These data suggest that infants with greater exposure to

glucocorticoids prenatally are more behaviorally reactive to acute postnatal stress. Furthermore, Day 14 hair cortisol was negatively correlated with Day 180 hair cortisol (see Appendix A). These data may indicate that early exposure to high levels of maternal glucocorticoids result in a lower set point of the infant HPA axis.

Hair cortisol from Day 180 hair samples was positively correlated with the infants' earlier responses to the BNAS. Higher hair cortisol was associated with higher composite DISTRESS score, isolation distress, contact resistance, 1 minute vocalization score, and locomotion. These measures may indicate that infants with higher overall HPA axis activity were more emotionally reactive during the BNAS. Alternatively, these data may instead reflect the relationships of rearing condition with HPA axis activity and behavioral response to the test. For example, MPR infants both demonstrated higher hair cortisol concentrations and greater behavioral reactivity during the assessment. Regardless, there is a clear relationship between temperament and chronic HPA axis activity.

Although no chronic measure of oxytocin system activity is available, infants' responses to the BNAS were strongly correlated with plasma oxytocin concentrations. In contrast to plasma cortisol measures, plasma oxytocin was *negatively* correlated with the composite DISTRESS score, isolation distress, and contact resistance. One possible interpretation is that emotional response to the BNAS may have been diminished by high levels of oxytocin, which have been demonstrated to reduce the response of HPA axis to a stressor (Agren, Lundeberg, et al., 1995; Light, Grewen, & Amico, 2005). There may be a relationship between physical contact, increases in oxytocin concentration, and

decreased HPA axis response to the BNAS. Contact with humans is both familiar and comforting to NR monkeys and may be associated with increased oxytocin system activity (Agren, Lundeberg, et al., 1995; Light, Grewen, & Amico, 2005). Specifically, NR infants demonstrated less contact resistance, lower DISTRESS scores, and lower plasma cortisol concentrations and therefore may have experienced a stress-ameliorating benefit of physical contact with the experimenter. Of course, there is no way to determine from the present data whether there is in fact an interaction between these measures. However, these data may provide further support that the oxytocin system moderates response of the HPA axis.

Interestingly, there was no correlation between central levels of oxytocin and temperament or behavior. As central and peripheral oxytocin systems are separated by the blood-brain barrier, it is possible that magnocellular projections from the hypothalamus that are associated with release of peripheral oxytocin from the pituitary gland may be specifically associated with behavioral and emotional reactivity. Even more intriguing, is that differences in oxytocin concentrations across rearing conditions were only observed in CSF and not plasma, suggesting that changes to the oxytocin system as a result of nursery rearing involve projections which connect other, central, regions and not those that project to the pituitary. These data suggest that different sections of the oxytocin system may be have specific and separate functions.

3.7.4 Relationship between the HPA Axis and Oxytocin System and between Central and Peripheral Oxytocin Activity

Given the established relationship between the HPA axis and oxytocin system, cortisol and oxytocin concentrations were predicted to be correlated. However, there was no significant correlation between plasma oxytocin and plasma cortisol. Previous accounts similarly reported a lack of correlation between plasma cortisol and plasma oxytocin in infant rhesus monkeys (Parker, Hoffman, et al., 2010) and between plasma cortisol and CSF oxytocin in juvenile monkeys (Winslow, Noble, et al., 2003). However, as discussed above, HPA axis and oxytocin system activity were both related to behavioral measures. In addition, as predicted, there were no correlations between oxytocin concentrations measured in central and peripheral matrices. This result is consistent with the view that central and peripheral oxytocin systems are separated by the blood-brain barrier (Amico, Challinor, et al., 1990) and supports the hypothesis that they may have different functions.

3.7.5 Conclusions

Overall, rearing experience did have significant effects on HPA axis and oxytocin system activity as well as on behavior. The data suggest that HPA axis activity in NR infants may be dysregulated, resulting in a lower overall set point than in MPR infant counterparts. In addition, NR monkeys displayed higher central oxytocin concentrations following developmental assessment, which may reflect oxytocin system modulation of HPA axis activity during stress. Mother-reared infants demonstrated more behavioral reactivity to maternal separation and administration of the assessment, whereas NR

infants demonstrated less distress. These data may indicate a relationship between higher

oxytocin concentrations, lower HPA axis activity, and behavioral reactivity during

separation and testing and may ultimately provide some support for a relationship

between HPA axis and oxytocin system activity. Overall, these data demonstrate that

early rearing experiences can exert rapid and measureable changes on HPA axis and

oxytocin system activity in infant rhesus monkeys.

CHAPTER 4

**RESPONSE OF THE OXYTOCIN AND HPA AXIS TO THE STRESS OF
RELOCATION IN DIFFERENTLY REARED RHESUS MONKEYS**

4.1 Introduction

One method of assessing functional differences in HPA axis activity is to examine

response to relocation, which is a well-known stressor for both human and nonhuman

primates. At the NICHD, infants of all rearing conditions are "weaned" at 8 months of

age, a process which mimics the weaning experience as it would occur in a natural

environment. To accomplish the weaning, MPR infants are removed from their mothers,

whereas SPR and PR infants are removed from the nursery. All infants from that birth

cohort are then placed together in one group and relocated to a large indoor-outdoor pen

environment. At the NICHD, the natural weaning process is compounded by relocation

and the introduction of unfamiliar conspecifics, both of which have been previously

demonstrated to be stressful experiences (Davenport, Lutz, et al., 2008; Mendoza, Coe, et

al., 1978). This standard laboratory practice affords the opportunity to assess the

response of differently reared infants to a major life stress event. The goal of this

experiment was to identify possible dysregulation of the HPA axis and the oxytocin

system in nursery reared monkeys by assessing their response to weaning, relocation, and

the formation of a new social group.

All subjects, regardless of rearing condition, were expected to demonstrate

increases in hair and plasma cortisol in response to the stress of relocation. In addition,

plasma oxytocin levels were also predicted to be higher post-relocation, as HPA axis

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activation has been associated with activation of the oxytocin system. Nursery rearing was expected to be associated with dysregulation of the HPA axis and oxytocin system; baselines levels of cortisol were predicted to be lower in NR infants. Conversely, oxytocin system activity was predicted to be higher in NR infants, as was demonstrated in the previous experiment. In response to the stress of relocation, NR infants were expected to demonstrate attenuated HPA axis and oxytocin system responses. All monkeys were expected to demonstrate positive correlations between pre- and post-relocation cortisol and oxytocin concentrations. Additionally, oxytocin and cortisol concentrations were expected to be correlated given the demonstrated relationship between HPA axis and oxytocin system activity. Response to the stressor was predicted to be associated with early measures of HPA axis and oxytocin system activity as well as measures of temperament. Monkeys with heightened reactions to weaning and relocation were expected to have demonstrated higher hair cortisol levels at 180 days and higher levels of distress during the BNAS. Finally, plasma oxytocin concentrations post-relocation were predicted to be related to response of the HPA axis to the stressor.

4.2 Subjects

Differently reared infant rhesus monkeys (*Macaca mulatta*) born at the LCE during the 2013 birth season participated in this study. Samples collected from 47 (28 male, 19 female) infant monkeys contributed to this data set (Table 4.1).

Table 4.1. Demographic information on subjects including sex and rearing history

| | | Rearing Condition | | |
	Subjects	Mother-Peer Reared	Surrogate-Peer Reared	Peer-Only Reared
Male	28	15	4	9
Female	19	12	3	4
Totals	47	27	7	13

4.3 Relocation to Mixed-Rearing Social Housing

At the NICHD, when infants reach 8 months of age, all MPR and NR infants born in one birthing season are relocated to form one large mixed-rearing social group made up of approximately 60 infants. The infants are socially housed in an environment where they have access to both indoor and outdoor areas. The indoor enclosure (7.3 x 3.4 x 3.7 m) contains perches and swings with wood shavings on the floor. The outdoor enclosure is composed of a circular corncrib (5.03 x 5.49 m). The infants are able to move freely between the two enclosure except during husbandry or inclement weather (<4 C).

Relocation of infants into mixed-rearing social housing occurred in stages. In December 2013, 13 MPR infants were moved and the remaining infants (12 MR and 20 NR) were moved in January 2014. The infants were fed Purina monkey chow (#5038) and water was available *ad libitum*. Their diet was supplemented with fruit three times a week and nuts were provided daily as forage.

4.4 Biological Sample Collection

Hair and plasma samples were collected both prior to and after relocation to mixed-rearing social housing. Samples were shipped to the University of Massachusetts Amherst for cortisol and oxytocin analysis as previously described (see Chapter 2).

4.5 Data Analysis

Each biological measurement first underwent a Kolmogorov-Smirnov test for normality and measures which were not normally distributed ($p<0.05$) were log transformed: post-move plasma cortisol, day 14 hair, day 180 hair, and day 14 CSF oxytocin. In order to make comparisons, pre-move plasma cortisol and day 30 CSF oxytocin were also log transformed. The log transformed values were used in all subsequent analyses.

4.5.1 The Effect of Relocation, Rearing Condition, and Sex on HPA Axis and Oxytocin System Activity

In order to determine the effect of relocation on HPA axis and oxytocin system activity in differently reared monkeys, three mixed design ANOVAs were performed with rearing condition and sex as the between subjects variables, pre- and post- relocation as the repeated measure, and with hair cortisol, plasma cortisol, and plasma oxytocin as the dependent measures. In order to quantify changes in HPA axis and oxytocin system activity associated with relocation stress, change scores were calculated for each measure (Post-Move concentration - Pre-Move concentration). A multivariate ANOVA was performed using the change scores for hair cortisol, plasma cortisol, and plasma oxytocin as within subject variables and rearing condition and sex as between subject variables.

Significant effects were further analyzed using post hoc univariate ANOVAs with Bonferroni corrections (between subjects variable: rearing, within subject variable: biological measure).

4.5.2 Relationships between Pre-Relocation and Post-Relocation Hormone Concentrations

Pearson correlations compared pre- and post-relocation concentrations for each measure (hair cortisol, plasma cortisol, and plasma oxytocin). To determine whether cortisol measures were correlated across matrix (yet within sampling period) hair cortisol was compared with plasma cortisol for both pre- and post-relocation samples. Finally, correlations compared plasma cortisol and plasma oxytocin for both pre- and post-relocation samples to determine whether HPA axis and oxytocin system activity were related within matrix (and sampling period).

4.5.3 The Relationship between Early Measures of HPA Axis Activity or Behavior and Response to Relocation

Data from hair, CSF, and plasma samples collected during the first 6 months of life, and composite DISTRESS scores from the BNAS (see Chapter 3), were included in the data set to examine the extent to which early measures predict later response to relocation stress. In order to determine if early measures of HPA axis activity predicted response of the HPA axis and oxytocin system to relocation stress, Pearson correlations compared hair cortisol sampled on day 180 and change scores calculated for hair cortisol, plasma cortisol, and plasma oxytocin. Pearson correlations compared the DISTRESS score from the BNAS with the change scores calculated for hair cortisol, plasma cortisol,

and plasma to determine whether early behavioral reactivity predicted later HPA axis response to relocation-stress.

4.5.4 The Relationship between Oxytocin System Activity and Response of the HPA Axis to Relocation

Pearson correlations compared post-move plasma oxytocin concentrations with change scores calculated for both hair cortisol and plasma cortisol in order to assess whether activity of the oxytocin system was related to the response of the HPA axis to the relocation stressor.

4.6 Results

4.6.1 The Effect of Rearing Condition and Relocation on HPA Axis and Oxytocin System Activity

Mixed design ANOVAs identified significant effects of rearing condition and relocation stress on hair cortisol, plasma cortisol, and plasma oxytocin concentrations (Table 4.2) As predicted, there was a main effect of relocation on hair cortisol concentrations ($F_{(1,35)}$=6.204, p=0.018) with levels higher following relocation (Fig. 4.1a). In addition, there was a main effect of rearing condition on hair cortisol ($F_{(1,35)}$=4.798, p=0.014). A post hoc univariate ANOVA with Bonferroni corrections confirmed our predictions and revealed that MPR infants had higher hair cortisol concentrations than SPR (p=0.011) but not PR (p>0.1) infants (Fig. 4.1b). The interaction of relocation with hair cortisol as a function of rearing is depicted even though only the main effect was significant. There was no effect of sex on hair cortisol.

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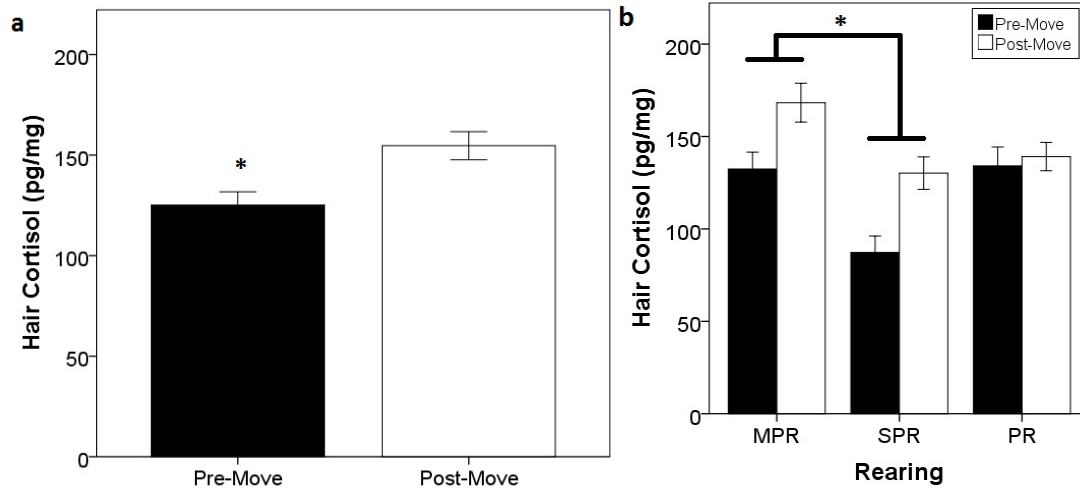
Figure 4.1. The main effect of (a) relocation and (b) rearing condition on hair cortisol concentrations, * $p<0.05$. Error bars represent ±1 SE.

There was a main effect of relocation ($F_{(1, 31)}=37.529$, $p<0.001$), but not rearing condition or sex, on plasma cortisol concentrations. As predicted, plasma cortisol levels were higher in the post-relocation phase (Fig.4.2).
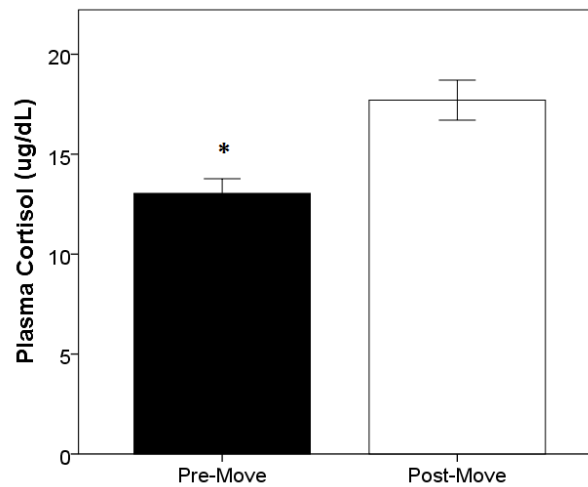
Figure 4.2. The main effect of relocation on plasma cortisol concentrations, * $p<0.001$. Error bars represent ±1 SE.

There was a significant main effect of relocation on plasma oxytocin ($F_{(1,19)}$=7.035, p=0.016). As predicted, plasma oxytocin levels were higher following relocation (Fig. 4.3). There was no significant effect of rearing condition or sex on plasma oxytocin concentrations.
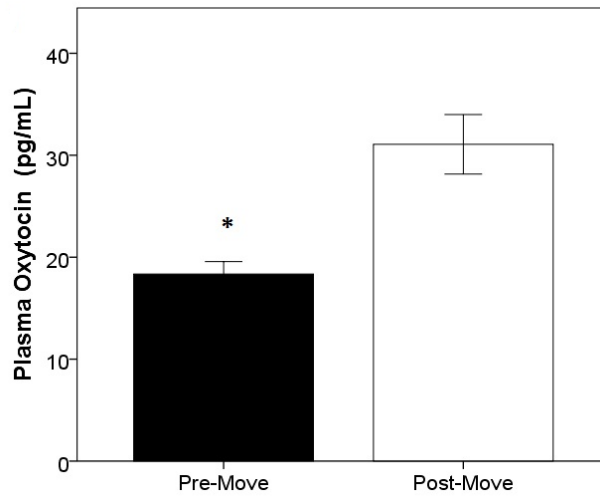
Figure 4.3. The main effect of relocation on plasma oxytocin concentrations, * p=0.016. Error bars represent ±1 SE.

There was no effect of rearing or sex on the change scores calculated for hair cortisol, plasma cortisol, or plasma oxytocin.

Table 4.2. The effect of rearing and relocation on hair cortisol, plasm cortisol, and plasma oxytocin mean scores and change scores (p-values)

	Hair Cortisol	Plasma Cortisol	Plasma Oxytocin
Main Effect of Rearing	**0.014**	0.161	0.853
Main Effect of Relocation	**0.018**	**<0.001**	**0.016**
Rearing x Change Score Interaction	0.386	0.082	0.084

Note: Bolded p-values <0.05.

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4.6.2 The Relationship between Pre-Relocation and Post-Relocation Hormone Concentrations

Pearson correlations between hair cortisol and plasma cortisol measures were statistically significant. As predicted hair and plasma cortisol were positively correlated in both the pre- ($r_{(47)}$=0.511, p<0.001; Fig. 4.4a) and post- relocation ($r_{(35)}$=0.419, p=0.017; Fig. 4.4b) samples. No predicted correlations were found between plasma cortisol and plasma oxytocin concentrations before or after relocation.
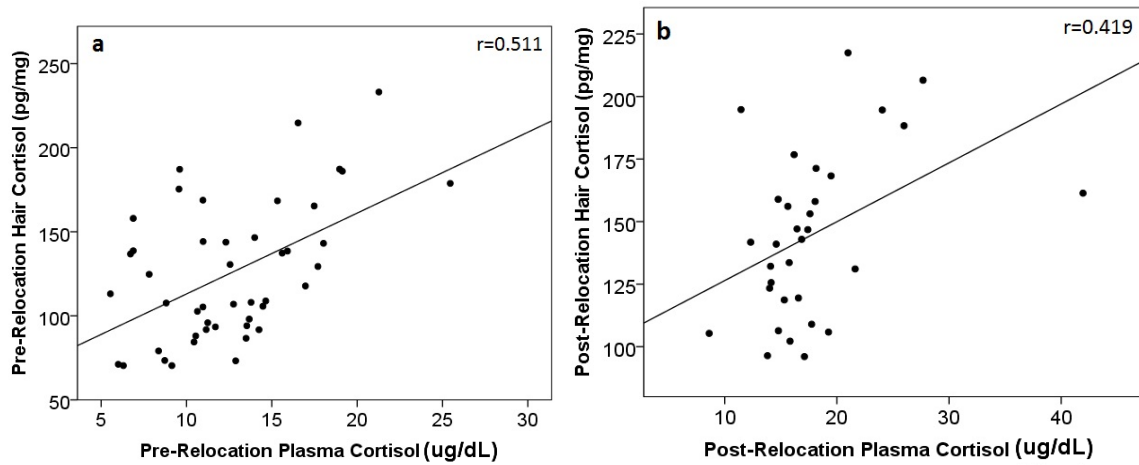
Figure 4.4. The correlation between (a) pre-location (p<0.001) and (b) post-relocation (p=0.017) hair cortisol and plasma cortisol concentrations.

As expected, there was a significant correlation between pre- and post-relocation concentrations for plasma cortisol ($r_{(35)}$=0.551, p=0.001; Fig. 4.5), but not for hair cortisol or plasma oxytocin.

69
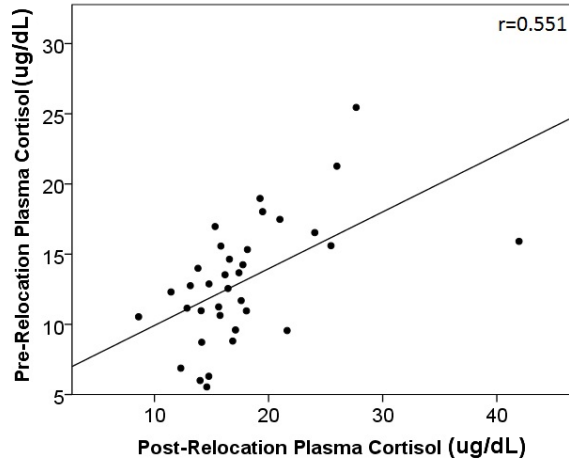
Figure 4.5. The correlation between pre- and post-relocation plasma cortisol concentrations (*p*=0.001).

4.6.3 The Relationship between Early Measures of HPA Axis Activity and Response to Relocation

As predicted, early measures of HPA activity were correlated with response to the stress of relocation, but only with change in hair cortisol concentration (Table 4.2). Pearson correlations identified a significant correlation between day 180 hair cortisol concentration and hair cortisol change score (the difference between pre- and post-relocation hair cortisol concentrations) ($r_{(41)}$=0.376, *p*=0.020; Fig. 4.6), but not with plasma cortisol or plasma oxytocin change scores.

70
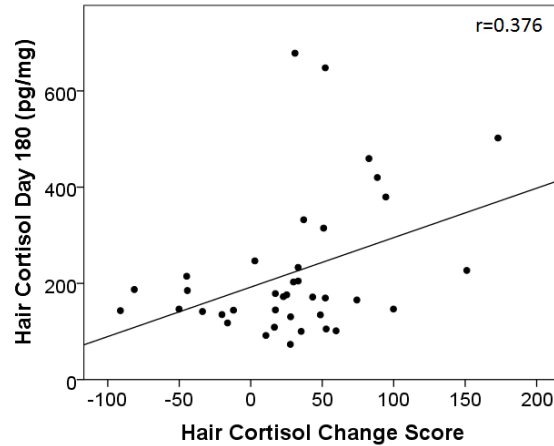
Figure 4.6. The correlation between hair cortisol sampled on day 180 and the difference between pre- and post- relocation hair cortisol concentrations (*p*=0.020).

4.6.4 The Relationship between Early Measures of Behavioral Reactivity and Response to Relocation

As predicted, early measures of behavioral reactivity predicted later response to relocation stress, but only the change in plasma cortisol concentration (Table 4.3). There was a significant correlation between BNAS composite DISTRESS score and plasma cortisol change score (the difference between pre- and post- relocation plasma cortisol concentration) ($r_{(35)}$=0.432, *p*=0.009; Fig. 4.7), but not with change scores for hair cortisol or plasma oxytocin.

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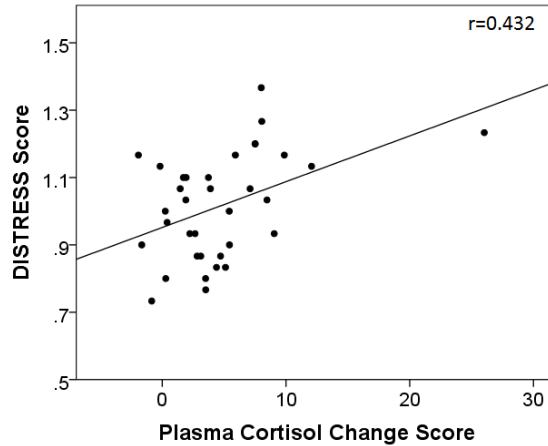
Figure 4.7. The correlation between composite DISTRESS score on the BNAS and plasma cortisol change score (*p*=0.009).

Table 4.3. The correlations between early measures of hair cortisol or DISTRESS during the BNAS and hair cortisol, plasma cortisol, and plasma oxytocin change scores after relocation (*p*-values)

	Change Scores		
	Hair Cortisol	**Plasma Cortisol**	**Plasma Oxytocin**
Day 180 Hair Cortisol	**0.020**	0.699	0.304
Composite BNAS DISTRESS Score	0.070	**0.009**	0.261

Note: Bolded *p*-values <0.05.

4.6.5 The Relationship between Activity of the Oxytocin System and Response of the HPA Axis to Relocation

There was a significant correlation between post-move plasma oxytocin concentrations and the plasma cortisol change score ($r_{(5)}$=0.492, *p*=0.014; Fig. 4.8) but not the hair cortisol change score ($r_{(41)}$=0.275, *p*=0.215).

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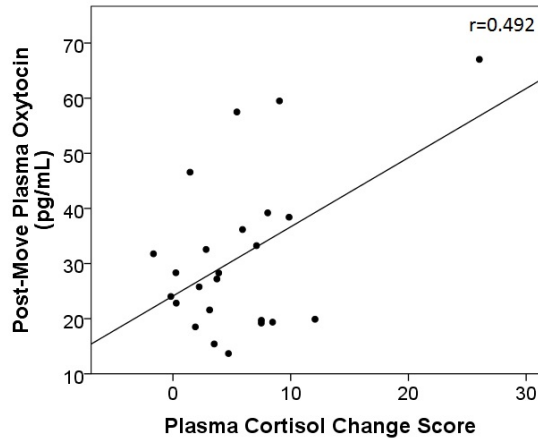
Figure 4.8. The correlation between post-move plasma oxytocin concentrations and plasma cortisol change score (*p*=0.014).

4.7 Discussion

4.7.1 The Effect of Rearing Condition and Relocation on HPA Axis and Oxytocin System Activity

As predicted, relocation of infants into mixed-rearing social housing elicited HPA axis and oxytocin responses, although response did not vary by rearing condition. Both chronic (hair) and acute (plasma) measures of cortisol were significantly higher post-relocation. These data are consistent with the view that relocation into mixed-rearing social housing was a stressful event. Specifically, increases in plasma cortisol suggest an increase in response of the HPA axis to the acute stress of sample collection following relocation. In terms of the oxytocin system, this study is the first to demonstrate an effect of stress on oxytocin system activity in infant rhesus monkeys. As previous research with rats has demonstrated increased central levels of oxytocin in response to a stressor (Niskioka, Anselmo-Franci, et al., 1998), higher oxytocin levels in post-relocation infants may reflect greater activation of the oxytocin system as a result of increased HPA axis

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activation. Furthermore, as higher post-relocation plasma cortisol concentrations likely reflect greater reactivity of the HPA axis to the acute stress of sampling, greater oxytocin concentrations may likewise reflect higher reactivity of the oxytocin system to acute stress post-relocation. Alternatively, plasma oxytocin levels could be higher in post-relocation samples as a result of the change in social environment, as relocation represents a large increase in the number of conspecifics within the living environment for all infants. There is no evidence to suggest that social interactions are associated with increases in oxytocin system activity, although physical contact has (Stock & Unväs-Moberg, 1988). Thus increases in baseline levels of oxytocin may reflect increases in social contact and interaction, although further research is necessary.

Hair cortisol concentrations, but not plasma cortisol or oxytocin concentrations, differed by rearing condition; as predicted, MPR monkeys had significantly higher hair cortisol than SPR monkeys, while PR monkeys had intermediate levels. The effect of rearing on hair cortisol concentrations replicates previously reported findings of lower concentrations in NR monkeys (Davenport, Novak, et al., 2003; Feng, Wang, et al., 2011) and supports the hypothesis that dysregulation of the HPA axis in NR monkeys results in a lower set point (Capitanio, Mendoza, et la., 2005). Furthermore, these data follow the same pattern of HPA axis activity by rearing condition as was demonstrated in plasma cortisol concentrations from the same subjects at earlier time points (see Chapter 3). In terms of changes in oxytocin system, there was no effect of rearing condition, although concentrations of oxytocin were higher in NR infants at an earlier time point (see Chapter

3). This may suggest that early effects of nursery rearing on oxytocin system activity may be acute rather than chronic.

Finally, there was no effect of rearing condition on the difference between pre- and post-relocation cortisol or oxytocin concentrations; infants responded to the stress of relocation in a uniform pattern. However, in a previous report, SPR infants relocated at 8 months of age at the NICHD demonstrated a greater increase in hair cortisol concentrations following relocation than MPR or PR infants (Dettmer, Novak, et al., 2012). This report also specifies that prior to relocation, PR infants had higher hair cortisol concentrations than both MPR and SPR infants, whereas in the present experiment, MPR infants had the highest levels of hair cortisol prior to relocation (see Chapter 3). The inability to replicate the effect of rearing on response to relocation may therefore be the result of differences in baseline rearing effects, which may be related to differences in genetic variation across birth seasons by introduction of different breeding males.

4.7.2 The Relationship between Pre- and Post- Relocation Concentrations of Cortisol and Oxytocin

Measurement of HPA axis activity in different matrices revealed significant positive correlations between hair and plasma cortisol levels both before and after relocation. Correlations between hair samples, which represent chronic HPA axis activity, and plasma samples, which likely represent acute response to sample collection, suggest a relationship between chronic HPA axis activity and reactivity to acute stressors. Infants with higher baseline HPA activity were likely to respond to the stress of sample

collection with comparably high levels of cortisol secretion. In addition, pre-relocation levels of plasma cortisol were positively correlated with post-relocation levels. These results indicate that infants reactive to the acute stress of sample collection were similarly reactive to sampling following relocation. As with cortisol concentrations, pre- and post-relocation oxytocin concentrations were expected to be correlated; however, the data did not support this prediction. This negative result may support the previous hypothesis that changes to the oxytocin system following relocation may not reflect changes in HPA axis activity and may instead be related to changes in the social environment.

4.7.3 The Relationship between Early Measures of HPA Axis Activity or Behavioral Reactivity and the Response to Relocation

Early measures of HPA axis activity and behavior were correlated with infants' response to relocation stress. Hair cortisol concentrations collected on day 180 and composite BNAS DISTRESS scores were both positively correlated with the change score calculated for plasma cortisol. Increases in plasma cortisol following relocation likely represent an increase in reactivity to the acute stress of sampling. Therefore, correlations with early measures may indicate that infants with high overall HPA axis activity or behavioral reactivity to acute stress may be similarly more reactive to acute stress following relocation. Alternatively, MPR had the highest levels of hair cortisol at day 180 as well as higher DISTRESS scores (see Chapter 3), and therefore may demonstrate the highest increases in responsivity to sampling. In other words, MPR infants may have a greater response to relocation and maternal separation. The lack of a correlation between early measures and changes in hair cortisol concentration in response

to relocation may be a result of the stable nature of the measure. These data may indicate that changes in hair cortisol concentration were somewhat uniform across infants. Finally, as early measures were not correlated with changes in oxytocin system activity, increases in oxytocin concentrations may not reflect a response to relocation, but may alternatively reflect response of the oxytocin system to a changing social environment.

4.7.4 The Relationship between Oxytocin System Activity and Response of the HPA Axis to Relocation

There was a positive correlation between oxytocin concentrations measured in the post-relocation sample and the change score calculated for plasma cortisol. Monkeys that responded with greater HPA axis activity following the relocation stress also had higher oxytocin concentrations. This data may indicate increased oxytocin system activity in monkeys with a greater HPA axis response following relocation and supports a relationship between the two neuroendocrine systems.

4.7.5 Conclusions

Infants exposed to the stress of relocation demonstrated increases in HPA axis activity, although rearing experience did not modulate this effect. However, MPR infants had overall higher hair cortisol concentrations than SPR infants, which supports that NR confers a lower HPA axis set point. Oxytocin system activity was higher in infants following the relocation and may reflect changes to infants' social environment and interactions, rather than HPA axis response to relocation. However, earlier observations of higher oxytocin system activity in NR infants were not replicated. Early measures of HPA axis activity and behavioral reactivity predicted later increases in plasma cortisol

following relocation. Overall, infants responded to relocation with increased HPA axis and oxytocin system activity, the extent of which was associated with early markers of physiological and behavioral reactivity, but not with early rearing experience.

CHAPTER 5

THE HPA AXIS, OXYTOCIN SYSTEM, AND BEHAVIOR IN DIFFERENTLY REARED ADULT RHESUS MONKEYS

5.1 Introduction

Previous comparisons between MR and NR monkeys have almost primarily examined infants or adolescents; therefore, little is known about whether there are prolonged effects of early rearing experience on activity of the adult HPA axis. Furthermore, there is no known account in the literature about oxytocin system activity in differently adults. Adverse early experiences were hypothesized to produce dysregulation of the HPA axis and oxytocin system that persists into adulthood. Specifically, nursery reared monkeys were predicted to demonstrate lower baseline cortisol concentrations and higher oxytocin concentrations compared to mother reared counterparts. In addition, cortisol concentrations were predicted to be correlated across plasma and saliva samples, as were oxytocin concentrations, as both are peripheral measures. Given the purported relationship between the oxytocin system and HPA axis, cortisol and oxytocin concentrations were also predicted to be correlated within sampling matrices (ie, plasma cortisol and plasma oxytocin).

Differential rearing experiences have previously been associated with observable differences in behavior, although little is known about this effect in adults. In order to assess behavioral responses of differently reared adult monkeys to a mildly stressful situation, monkeys were administered the Human Intruder Test (HIT), which is widely used to assess anxiety and behavioral reactivity in monkeys (Coleman & Pierre, 2014).

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Nursery reared monkeys were predicted to respond to the HIT with greater levels of reactivity. Response to the HIT was also predicted to correlate with biological measures, specifically, that monkeys with higher chronic cortisol concentrations, as measured in hair, would demonstrate higher levels of reactive behavior.

5.2 Subjects

A total of 36 adult male monkeys were included in this experiment. Subjects housed at the New England Primate Research Center (NEPRC) in Southborough, MA consisted of 23 male rhesus monkeys that ranged in age from 5 to 30 years (mean age of 13.2 years). Subjects housed at the University of Massachusetts Amherst Primate Lab in Amherst, MA consisted of 13 rhesus monkeys, who ranged in age from 6 to 19 years (mean age of 11.45 years).

5.2.1 Rearing

An unforeseen challenge was the closing of the NEPRC, which limited the number of subjects available for study. The majority of the available subjects from the NEPRC were MR, two were PR, and none of the subjects were SPR whereas, at UMass, monkeys were either MR or SPR (Table 5.1). Therefore, determining effects of early rearing experience on biological and behavioral measures across the two primate labs was not possible as the data were confounded by facility. Finally, as the majority of available subjects were males, the few available female monkeys were excluded from analysis to limit variability. Therefore, sex differences could not be assessed on any of the measures of interest and the findings are all specific to males.

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Table 5.1. Demographic information on subjects including facility and rearing history

		Rearing			
	Subjects	Mother Reared	Surrogate-Peer Reared	Peer-only Reared	Unknown
NEPRC	23	20	-	2	1
UMass	13	3	8	-	2
Totals	36	23	8	2	3

5.2.2 Facility of Origin

Monkeys housed at the NEPRC and UMass primate facilities were born at a variety of different primate and research centers including the NIH Animal Center (SPR, MR), the Caribbean Primate Research Center (MR), the NEPRC (MR and PR), MERCK (MR and PR), and the UMass primate facility (MR) (Table 5.2). The rearing histories and facility of origin for 3 of the monkeys was unknown, although the subjects were included in the data set to provide more data on how biological samples relate to one another, regardless of rearing history.

Table 5.2. Rearing information on subjects including facility of origin and rearing history

	Subjects	Rearing			
		Mother Reared	Surrogate-Peer Reared	Peer-only Reared	Unknown
NIH	11	3	8	-	-
CPRC	13	13	-	-	-
NEPRC	3	1	-	2	-
MERCK	4	4	-	-	-
UMass	2	2	-	-	-
Other	3	-	-	-	3
Totals	36	23	8	2	3

5.2.3 Housing

Monkeys housed at the NEPRC were housed individually in auditory, visual, and olfactory contact with approximately 5-6 other conspecifics. The monkeys were fed Harlan Tekland commercial monkey chow, were supplemented with fruit and forage (a mixture of nuts and dried fruit), and had access to water *ad libitum*. The environment was kept at a 12:12 (0630-1830) light cycle with temperature set at 22 C and humidity between 30-70%.

Monkeys located at the UMass Amherst Primate lab were housed in a variety of enclosures, including Allentown cages and floor-to-ceiling pens equipped with shelves and hanging enrichment devices. Some were housed socially and others individually, but all monkeys were housed in colony rooms with between 2-5 other conspecifics. The monkeys were given Purina monkey chow (#5038) twice daily and were supplemented

with a variety of fruits, nuts and grains. They had access to water *ad libitum*. The light cycle in the colony rooms was 13:11 (0700-2000); the environment was maintained at 23 C and between 35-50% humidity. The monkeys were maintained on a 5 day/week environmental enrichment schedule which rotated various activities and treats including television, music, ice cube treats, and forage. In addition, the monkeys participated in a number of cognitive studies in which they were rewarded, including studies of social cognition, social learning, and spatial memory.

5.3 Biological Sample Collection & Analysis

Hair, plasma, and saliva samples were collected during routine health exams. Two rounds of sample collection were performed at the NEPRC: Round 1 occurred during January and February 2013 and Round 2 occurred between March and May 2013. All samples were transported to the University of Massachusetts Amherst for analysis of cortisol and oxytocin concentrations as described in Chapter 2. The samples collected at the NEPRC included the following 10 variables: hair cortisol 1 and 2, plasma cortisol 1 and 2, salivary cortisol 1 and 2, plasma oxytocin 1 and 2, and salivary oxytocin 1 and 2.

Sample collection at the University of Massachusetts Amherst took place in January 2013. The small quantity of the plasma samples obtained only allowed for analysis of a single biological factor and it was decided that oxytocin would be analyzed rather than cortisol, given that oxytocin was also only measureable in saliva whereas cortisol is measureable in both saliva and hair. The samples collected at the University of Massachusetts Amherst consisted of the following 4 measures: hair cortisol, plasma

oxytocin, saliva cortisol, and saliva oxytocin, which were combined with data from the first NEPRC sampling (ie, hair cortisol 1, plasma oxytocin 1, saliva cortisol 1, and saliva oxytocin 1).

During health exams, in order to determine whether the order in which the subjects in each colony room were sampled had any effect on HPA axis activity, time at which the veterinary technician first entered the colony room to anesthetize the first animal was noted as "Time Vet Tech Entered" and the time at which the blood sample was taken for each subject was noted as "Time Sample Collected". The variable "Latency to Sample" was calculated by (Time Sample Collected - Time Veterinarian Entered). As there was no significant correlation between Latency to Sample and plasma cortisol concentrations (Pearson correlation: r=0.088, n=23, p=0.691), this measure was excluded from further analyses.

5.4 The Human Intruder Test

The Human Intruder Test (HIT) presents subjects with the mildly stressful presence of an unfamiliar experimenter, including both threatening and non-threatening social situations, while the monkeys' responses are video recorded. The HIT is composed of 4 consecutive 2 minute phases. Before the test begins, a video camera is placed within 2 m of the monkey's home enclosure. Ten minutes are allowed to pass for the subject to acclimate to the presence of the camera; the final 2 minutes of this phase are designated as the Baseline period. The human intruder then enters the colony room and positions herself 1 m from the subject, standing quietly with her profile oriented

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towards the monkey for 2 minutes (Profile Phase). The intruder then turns 90   to face

the monkey, making direct eye contact for 2 minutes (Stare Phase). Finally, the intruder

turns 180   to orient her back towards the subject for two minutes (Back Phase). At the

end of the final phase, the intruder leaves the room and the test is over.

5.4.1 Human Intruder Test Videoscoring

Videos captured during the HIT were sent to the University of Massachusetts for

videoscoring. Eight behaviors of interest were identified and scored for duration: (1)

back of cage (positioning oneself with at least 3 limbs in the back half of the cage), (2)

lipsmack (rapidly opening and closing the lips), (3) fear grimace (a large grin-like facial

expression showing the teeth), (4) aggress (shaking the cage or threatening [open-mouth

stare, lunging or swiping at] the intruder), (5) scratch, and (7) yawn. Videos were scored

in a frame-by-frame analysis using Mpeg Streamclip with an interobserver reliability of >

90%. Start and stop times for each behavior were noted and duration of each behavioral

bout was calculated as Stop Time - Start Time. For each behavior, individual bout

durations were totaled within each phase to create a behavioral score for each of the 4

phases (Baseline, Profile, Stare, and Back) and were also totaled across phases to create a

global score. Finally, behavioral categories thought to be associated with anxiety (back

of cage, scratch, yawn) were combined first within the 4 phases, to create individual

phase scores, and across phases to create a global Anxiety Score. These data were scored

by trained observers in the UMass Primate Lab and provided for this dissertation.

5.5 Data Analysis

5.5.1 Examination of Cortisol and Oxytocin Concentrations

Each biological measurement underwent a Kolmogorov-Smirnov test for normality and any measurements that were not normally distributed ($p<0.05$) were log transformed: salivary cortisol 1, hair cortisol 2, plasma oxytocin 1 and 2, and salivary oxytocin 1 and 2. For accurate comparisons to be made, both salivary cortisol 2 and hair cortisol 1 were also log transformed. The log transformed values were used in all subsequent analyses.

Due to the confounded nature of rearing condition and facility, to determine the effects of rearing experiences on the HPA axis and oxytocin system, an ANOVA was applied to the biological variables that were collected at the UMass facility (dependent variables: hair cortisol 1, plasma cortisol 1, salivary cortisol 1, and salivary oxytocin 1; independent variable: rearing). To determine the effect of facility, an analysis of variance was used to determine differences in biological variables sampled from MR monkeys across facilities (dependent variables: hair cortisol 1, plasma cortisol 1, salivary cortisol 1, and salivary oxytocin 1; independent variable: facility).

Considering the wide range of ages across subjects, age was also included as a variable of interest. Subjects were categorized by age into two groups using a median split; monkeys older than the median age of 16 were considered "old" (n=15) while monkeys younger than, or equal to, the median age were considered "young" (n=21). Repeated measures analysis of variance were performed to determine if oxytocin and

cortisol concentrations differed by time across the two sample collections (dependent variables: (1) hair cortisol 1 and 2, (2) plasma cortisol 1 and 2, (3) salivary cortisol 1 and 2, (4) plasma oxytocin 1 and 2, and (5) salivary oxytocin 1 and 2; independent variable: age group).

Finally, Pearson correlations were applied to cortisol and oxytocin concentrations to examine the relationship across sampling matrices, yet within sampling periods (hair cortisol 1 by both salivary cortisol 1 and plasma cortisol 1 separately, salivary cortisol 1 by plasma cortisol 1, hair cortisol 2 by both salivary cortisol 2 and plasma cortisol 2 separately, salivary cortisol 2 by plasma cortisol 2, salivary oxytocin 1 by plasma oxytocin 1, and salivary oxytocin 2 by plasma oxytocin 2). The correlation of cortisol and oxytocin concentrations within sampling matrices and round of sampling was also performed (salivary cortisol 1 by salivary oxytocin 2, salivary cortisol 2 by salivary oxytocin 2, plasma cortisol 1 by plasma oxytocin 1, and plasma cortisol 2 by plasma oxytocin 2) to assess the relationship between HPA axis activity and oxytocin system activity.

5.5.2 Assessment of Behavior during the Human Intruder Test

To determine differences in response to the Human Intruder Test across rearing conditions at UMass, a multivariate ANOVA examined the 7 behavioral categories (within subjects variables: back of cage, lipsmack, fear grimace, aggress, yawn, scratch, and anxiety score) across rearing condition (between subject variable). A similar multivariate ANOVA was performed with facility as the between subjects variable to

assess differences in behavior across MR monkeys at UMass and the NEPRC (within

subject variables: 7 behavioral categories). Finally, a multivariate ANOVA compared

response of differently aged monkeys to the HIT (within subject variables: 7 behavioral

categories; between subjects variable: age group).

5.5.3 The Relationship between Behavioral Response to the Human Intruder Test and Cortisol and Oxytocin Concentrations

In order to assess the relationship between cortisol concentrations and behavior

during the HIT, Pearson correlations were performed using the hair cortisol data, as hair

cortisol provides the most stable measure of HPA axis activity. Separate correlations

between hair cortisol 1 and each of the 7 behaviors measured during the HIT were

performed. Since oxytocin cannot be measured in hair, we used the measure of plasma

oxytocin to assess the relationship between oxytocin concentrations and behavior.

Furthermore, plasma had been collected from more subjects than saliva and presented a

better opportunity to examine how the oxytocin system may relate to behavioral reaction

to the HIT. Again, separate Pearson correlations between plasma oxytocin 1 and the 7

target behaviors were performed.

5.6 Results

5.6.1 The Effect of Rearing, Facility, and Age on Cortisol and Oxytocin Concentrations

Analysis of MR and SPR monkeys at UMass did not identify predicted

differences in cortisol or oxytocin concentrations. In terms of facility, no significant

differences between cortisol or oxytocin concentrations in MR monkeys at NEPRC and at

UMass were identified. Finally, a repeated measures analysis of variance demonstrated

that age group had a significant main effect on plasma cortisol concentrations

($F_{(1,20)}$=6.062, p=0.023), with cortisol concentrations higher in older monkeys (Fig. 5.1).
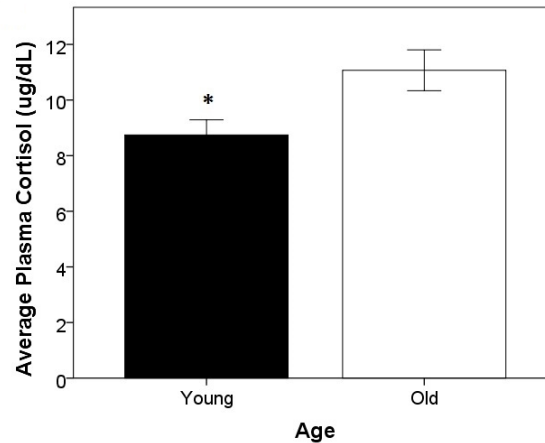
Figure 5.1. The main effect of age group on plasma cortisol, * p=0.023. Error bars represent ±1.

5.6.2 Correlations of Cortisol and Oxytocin across Sampling Matrices

As predicted, cortisol concentrations were correlated across sampling matrices.

Hair cortisol concentrations were correlated with salivary cortisol within the first

sampling period ($r_{(24)}$=0.434, p=0.039) (Fig. 5.2a) whereas the correlation between hair

cortisol and plasma cortisol within the first sampling period was nearly significant

($r_{(22)}$=0.421, p=0.051) (Fig. 5.2b). Similarly, hair and plasma cortisol concentrations

measured in the second sampling period were correlated ($r_{(21)}$=0.580, p=0.006) (Fig.

5.2c). Despite predictions, oxytocin concentrations were not significantly correlated

across plasma and salivary matrices.
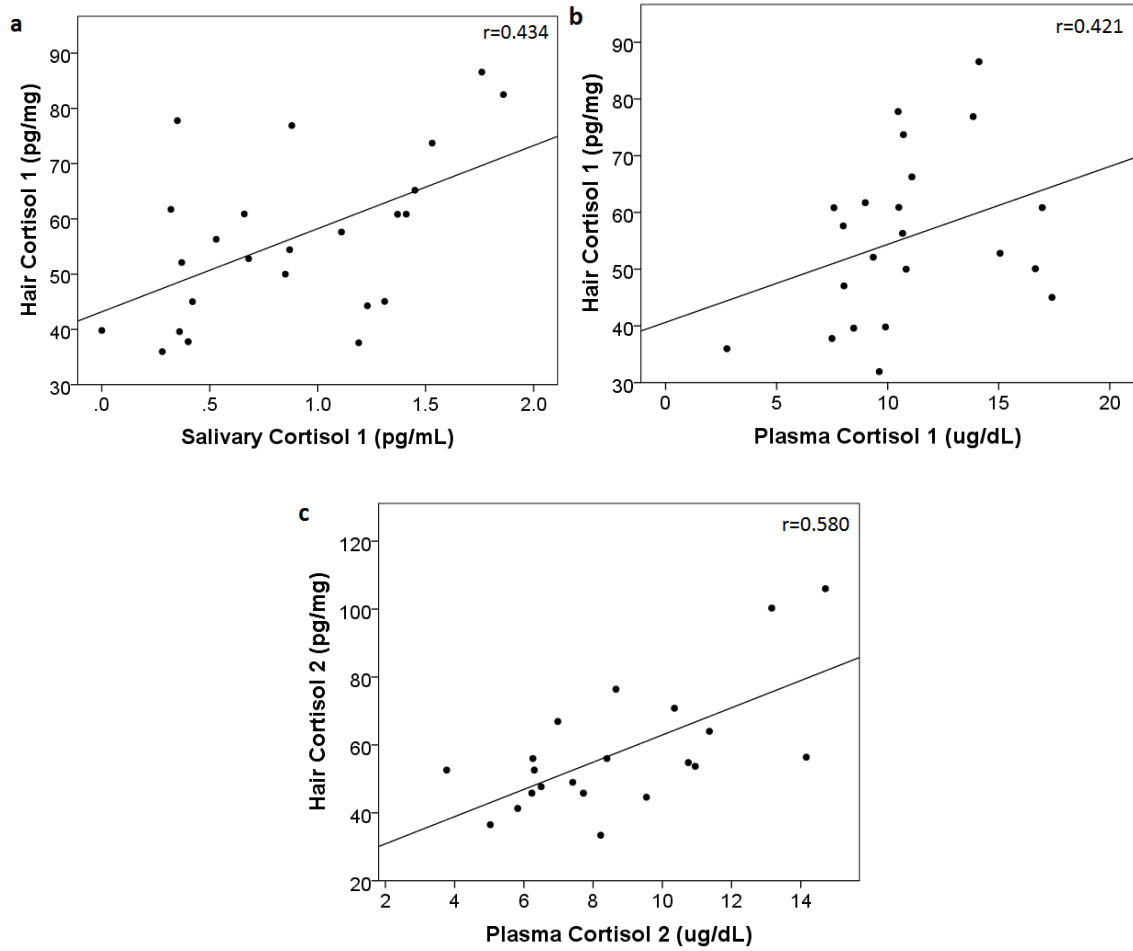
Figure 5.2. Cortisol concentrations correlated across (a) hair and saliva 1, (*p*=0.039), (b) hair and plasma 1 (*p*=0.051) and (c) hair and plasma 2, (*p*=0.006).

5.6.3 Correlations within Sampling Matrices

Although HPA axis and oxytocin system activity were expected to be related within each sampling matrix, correlations between cortisol and oxytocin in either plasma or saliva did not reach significance.

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5.6.4 Assessment of Behavior during the Human Intruder Test

Contrary to predictions, there was no significant effect of rearing condition on response of UMass monkeys to the HIT. In addition, MR monkeys at UMass and the NEPRC reacted similarly to the HIT. Finally, there was no significant effect of age on response of the monkeys to the HIT.

5.6.5 The Relationship between Behavioral Response to the Human Intruder Test and Cortisol and Oxytocin Concentrations

Hair cortisol was significantly positively correlated with only one variable, amount of time spent in aggression ($r_{(30)}$=0.404, p=0.027) (Fig. 5.3). No significant correlations between behavior and plasma oxytocin concentration were identified (Table 5.4).
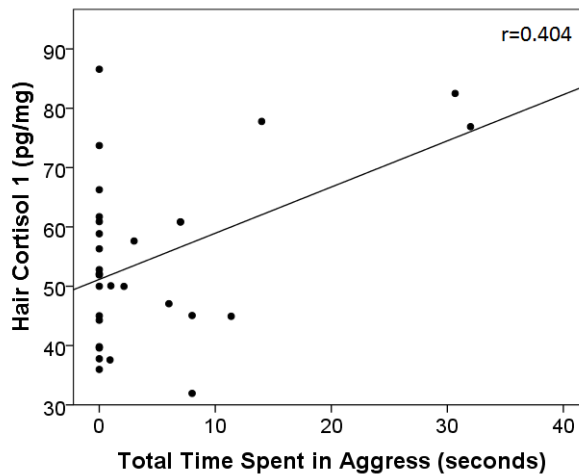
Figure 5.3. The correlations between hair cortisol concentration and total amount of time spent engaged in aggress during the Human Intruder Test

Table 5.3. The correlations between hair cortisol or plasma oxytocin and behavior during the HIT (*p*-values)

	BOC	LS	FG	Aggress	Yawn	S	Anxiety Score
Hair Cortisol	0.888	0.107	0.503	**0.027**	0.382	0.662	0.827
Plasma Oxytocin	0.120	0.127	0.263	0.804	0.145	0.319	0.071

Note: Bolded *p*-values <0.05; BOC=back of cage, LS=lipsmack, FG=fear grimace, S=scratch.

5.7 Discussion

5.7.1 Effects of Rearing Condition, Facility, and Age on HPA Axis and Oxytocin System Activity

Although rearing experience was predicted to result in different HPA axis and oxytocin system activity, as well as behavior in adult monkeys, no effects of rearing were observed. Similarly, facility was not a significant predictor of either neuroendocrine activity or behavior. Unfortunately and unexpectedly, the distribution of rearing conditions across the two facilities was uneven, such that SPR monkeys could only be found at the UMass facility whereas PR monkeys could only be found at the NEPRC. Given that there was a small number of available subjects to begin with, the confounded nature of the rearing condition and facility variables resulted in an even smaller number of subjects. Therefore, the experiment was likely underpowered, making it difficult to find any significant effects of rearing on the biological and behavioral measures.

The absence of an effect of rearing condition on HPA axis activity in adult monkeys corresponds to previous null results in hair cortisol concentrations of 24 month old monkeys (Dettmer, Novak, et al., 2012), although differences in HPA axis activity in

differently reared monkeys has been reported in plasma of 1-3 year old monkeys (Davenport, Novak et al., 2003) and hair in monkeys as old as 3.5 years (Feng, Wang, et al., 2011). However, as the previously reported data represent monkeys of sub-adult age, the disappearance of an effect of early rearing experience on HPA axis activity in adult monkeys may be related to changes in HPA axis activity with age. For example, cortisol has been shown to increase with increasing age in female rhesus macaques (Maestripieri, Hoffman, et al., 2008) and humans (Van Cauter, Leproult, & Kupfer, 1996). Furthermore, in the present experiment, plasma cortisol was higher in older monkeys. Increased HPA axis activity in older monkeys may mask any rearing condition effects. If so, that may suggest that the effects of early rearing experiences are transitory in nature and diminish over time. However, there was no effect of age on chronic HPA axis activity, as measured in hair. The effect of age on an acute measure of HPA axis activity, but not a chronic measure, may suggest that HPA axis response to the acute stressor of sedation and sample collection, rather than chronic activity, is altered with age.

Similarly, oxytocin system activity did not differ in adult monkeys with different rearing histories, despite previous reports of both higher (see Chapter 3) and lower (Winslow, Noble, et al., 2003) oxytocin in sub-adult nursery reared monkeys. As oxytocin system activity has been shown to increase with age (Parker, Hoffman, et al., 2010), effects of rearing may diminish as system activity increases over time, as suggested above with HPA axis activity. However, age was not a significant predictor of oxytocin system activity in the present experiment, which may be a result of insufficient statistical power. Regardless, the data do not support that early rearing experiences have prolonged effects of oxytocin system activity in adult monkeys.

5.7.2 Effects of Rearing Condition, Facility, and Age on Behavioral Response to the HIT

There was no effect of rearing condition, facility, or age on monkeys' responses to the HIT. However, the one previous report of differently reared monkey adults administered the HIT describes that NR monkeys demonstrated less locomotion and environmental exploration and more freeze during the HIT (Corcoran, Pierre, et al., 2011). The authors concluded that NR monkeys were more behaviorally inhibited than MR monkeys. Similarly, Capitanio and colleagues reported lower activity in NR infants during the HIT (2006). The data presented here are not in disagreement with these findings, as behaviors of interest included measures of anxiety and communicative facial expressions rather than measures of activity. Therefore, NR may be associated with behavioral inhibition in adults, but not with anxious behavior or reactive expressions, as demonstrated by the present experiment.

5.7.3 Relationships between HPA Axis and Oxytocin System Activity and across Matrices

Hair cortisol, a stable measure of chronic HPA axis activity, was predicted to correspond with measures of reactivity during the HIT in adult monkeys. However, the only behavior to correlate with hair cortisol concentration was aggression. Rhesus monkeys are a highly aggressive species and, therefore, this response to the HIT is not surprising. Our lab has recently demonstrated that rhesus monkey adults with characteristically high hair cortisol levels are more likely than monkeys with low cortisol levels to respond to the HIT with aggression (Hamel, Lutz, submitted). Hair cortisol concentrations, which are highly stable measures, are likely to map onto temperament, or

trait-like behavioral characteristics, such as aggressive reactivity. Furthermore, since aggression is a common species-typical behavior, it is not surprising that it would be reflected by a stable measure of physiological reactivity, such as hair cortisol.

Plasma oxytocin concentrations did not demonstrate predicted correlations with behavior during the HIT. This finding is in contrast to previous evidence that plasma oxytocin concentrations are correlated with behavioral reactivity in infants during the acute stress of developmental assessment (see Chapter 3). However, it should be noted that sampling in the previous study occurred directly after assessment whereas sampling in this study occurred within a month of the HIT. This suggests that point sample measuring of oxytocin concentration does not necessarily reflect temperament or behavioral reactivity.

Cortisol, but not oxytocin, concentrations were correlated across sampling matrices. Hair and plasma cortisol concentrations were correlated, both within the first and second sample collections, whereas hair and saliva concentrations were correlated, but only within the first sample collection. As hair cortisol provides a stable measure of activity, it is unsurprising to find that it was correlated with cortisol in plasma and saliva. Therefore, these correlations suggest that individuals with chronically high HPA axis activity respond to the acute stress of sampling with a correspondingly high level of reactivity. Finally, given the relationship between HPA axis and oxytocin system activation, cortisol and oxytocin concentrations were expected to be correlated within a sampling matrix. However, this prediction was not supported. Previous assessments of oxytocin and cortisol concentrations were similarly unable to demonstrate significant

correlations (Parker, Hoffman, et al., 2010; Winslow, Noble, et al., 2003; and see Chapters 3 and 4).

5.7.4 Limitations

There are a number of limitations to this experiment that could have interfered with the ability to find significant effects of early rearing experience in adults. One unforeseen challenge was the closing of the NEPRC, which contributed to the low number of subjects available for study and left the data set underpowered. In addition, determining rearing history of older monkeys proved to be a challenge, especially for subjects that had transferred from facility to facility a number of times. Records for these animals were difficult to track down and any available records neglected to specific rearing history. Finally, as so few females were available for study, only males were included in the analysis. Therefore, sex differences on the variables measured could not be assessed.

5.7.5 Conclusions

The results of the present experiment are difficult to interpret, given the confounded nature of rearing condition and facility. Regardless, there was no support for the prediction that early rearing experience would result in prolonged changes to HPA axis and oxytocin system activity or behavior. Effects of early rearing experiences on HPA axis and oxytocin system activity may diminish as neuroendocrine activity increases throughout the aging process. Finally, hair cortisol provided a stable assessment of HPA axis activity that was both correlated with level of aggressive reaction

to the HIT as well as acute measures of HPA axis activity, whereas point samples of oxytocin system activity do not appear to predict behavior in general.

CHAPTER 6

GENERAL DISCUSSION

The goals of research outlined in this dissertation were to examine the effects of rearing experience on activity of the HPA axis and oxytocin system, as well as on behavior, and to assess the relationships between neuroendocrine activity and behavioral output. Determining the effects of rearing condition is important for understanding whether adverse early experiences produce detrimental, and possibly prolonged, changes to important neuroendocrine systems.

6.1 The Effect of Rearing Experience on the HPA Axis, Oxytocin System, and Behavior

Rearing experience had more consistent effects on HPA axis activity than on the oxytocin system. Dysregulation of the HPA axis in NR monkeys was observed in multiple samples and across multiple matrices throughout the first year of life whereas a significant difference in oxytocin concentrations was observed only in CSF measured during the first month. These data indicate that rearing experience may exert more influence on the HPA axis than on the oxytocin system, and particularly during infancy, as the effects of rearing on the HPA axis and oxytocin system were only observed in monkeys within the first year of life. Nursery rearing was associated with a blunting of HPA axis activity, as measured in both hair and plasma, but by one year of age, differences in cortisol concentrations across rearing conditions were absent. CSF oxytocin levels were higher in NR infants less than 1 month of age; however, differences in oxytocin activity were not observed beyond that time point.

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Rearing effects were particularly pronounced in response to the mild, acute stress of developmental assessment (BNAS). Mother-peer reared infants demonstrated higher levels of distress and arousal than NR monkeys whereas NR monkeys engaged in more exploration, sleep, and contact with the experimenter. Conversely, differently reared rhesus monkey infants of weaning age responded uniformly to the stress of relocation and group formation with comparatively similar increases in cortisol and oxytocin concentrations. As adults, differently reared monkeys responded to the mildly stressful presence of an unfamiliar experimenter with similar levels of reactivity. These data suggest that, whereas infant rhesus monkeys may be susceptible to the effects of rearing experience, adult rhesus monkeys do not demonstrate robust effects of early experience.

6.1.1 The Interaction between Genetic Variation and Rearing Experience on HPA Axis Activity and Behavior

Although the nursery rearing paradigm employs a controlled manipulation of early life experiences in rhesus monkeys, there may be other factors that contribute to the effects of nursery rearing on neuroendocrine activity and behavior. Various genetic factors have been identified that modulate the adverse effects of early rearing experiences, such that infants with certain genetic polymorphisms may be more susceptible to the effects of nursery rearing experiences. Genetic modulators that have been identified include polymorphisms in genes that code for the serotonin transporter, tryptophan hydroxylase 2, and monoamine oxidase A.

Variation in the regulatory region of the serotonin transporter gene has been associated with adverse effects in NR, but not MR monkeys (Suomi, 2006). During

behavioral testing, NR infants carrying the short allele displayed higher levels of distress than NR infants homozygous for the long allele and all MR infants (Champoux, Bennett, et al., 2002). Variation in the gene that encodes tryptophan hydroxylase 2 (rhTPH2), the enzyme that synthesizes central serotonin, has been associated with higher cortisol levels and more aggressive threat displays, but only in NR monkeys (Chen, Novak, et al., 2010). A third genetic polymorphism, located in the promoter region of the monoamine oxidase A gene (MAOA-LPR) has similarly been demonstrated to modulate the effects of nursery rearing in rhesus monkeys. Monkeys with the low activity allele demonstrated more anxious behavior during the HIT, but only if they had been reared alone with their mother versus a large social group (Karere, Kinally, et al., 2009).

Two different hypotheses have been proposed to explain these gene by environment interactions: 1.) that possession of good genes can protect against the effects of adverse early experiences, and 2.) that a good environment during development can protect against the effects of bad genes (Suomi, 2006). These hypotheses are not mutually exclusive and may simultaneously explain the interactive effects of rearing experience and genes. Regardless, these data demonstrate that the effects of rearing experience are modulated by genetic factors and that rearing experience does not exert a simple cause and effect influence on HPA axis activity and behavior. Therefore, genetic variation may account for some of the variability in how rearing experience modulates neuroendocrine activity and behavior.

6.2 The Relationship between Measures of HPA Axis and Oxytocin System Activity and Relationships with Behavioral Correlates

Measures of HPA axis activity were more consistently associated with other neuroendocrine and behavioral assessments than measures of the oxytocin system. Within a single sampling time point, hair cortisol was shown to correlate with saliva and plasma concentrations whereas oxytocin concentrations were not demonstrated to correlate across matrix, even across peripheral measures. While both cortisol and oxytocin were demonstrated to correlate with measures of behavior and temperament on the BNAS, early measures of reactivity only correlated with infants' subsequent HPA axis response to the relocation stressor. Finally, only cortisol concentrations correlated with adult behavior during the HIT. These data support that measures of HPA axis activity may be more consistently represented across different matrices than measures of oxytocin system activity and that HPA axis activity may exert more influence over temperament and behavior than activity of the oxytocin system.

Previous research has supported that hair cortisol, a chronic and stable measure of HPA axis activity, relates to temperament in non-human primates. Vervet monkeys with high levels of hair cortisol were less likely to explore a novel object placed outside of the cage (Laudenslager, Jorgensen, et al., 2011). This response was consistent across session and therefore likely representative of a more reactive temperament. With the help of collaborators, I have demonstrated that rhesus monkeys with high hair cortisol are more reactive during the HIT (Hamel, Lutz, submitted). Monkeys with high levels of hair cortisol were consistently more reactive to the presence of the intruder, demonstrating more aggressive and anxious behaviors, across two testing sessions. These data provide

support for the relationship between HPA axis activity and behavioral reactivity or temperament.

6.3 The Relationship between the HPA Axis and the Oxytocin System

Rearing experience did not seem to have any bearing on the relationship between the HPA axis and the oxytocin system. Furthermore, there was limited data to support the purported relationship between these two neuroendocrine systems, specifically that oxytocin concentrations following a major stressor were correlated with the response of the HPA axis to the stress. Although cortisol and oxytocin concentrations were not directly associated with one another, they were, however, correlated with similar temperamental measures. Both cortisol and oxytocin concentrations were correlated (in opposite directions) with measures of infant distress during the BNAS. Furthermore, the pattern of activity in NR infants is consistent with previous research in rodents (Windle, Shanks, et al., 1997), monkeys (Parker, Buckmaster, et al., 2005), and humans (Heinrichs, Meinlschmidt, et al., 2001) which demonstrates that higher oxytocin concentrations during an acute stressor are associated with lower HPA axis activity. In addition, both neuroendocrine systems demonstrated increased activity directly following a stressful life event, which supports the idea that increased oxytocin activity during stress functions to modulate the HPA axis response. The relationship between these neuroendocrine systems may be more complex than can be demonstrated by simple correlations between measures and may only be possible when sampling is associated with a stressor.

6.4 Conclusions

Overall, this dissertation has demonstrated that rearing experience is associated with dysregulation of the HPA axis in infant, but not adult, rhesus monkeys, whereas rearing experience was only associated with changes to the oxytocin system within the first month of life. Behavior and temperament were also modified by rearing experience in infant rhesus monkeys, but not in adults. Therefore, although rearing experience is associated with early differences in neuroendocrine function and behavior, it may not exert prolonged effects. In addition, the effects of rearing experience may be complicated by genetic interactions. Finally, there was only some behavioral evidence to support a relationship between HPA axis and oxytocin system activity, although it was not modified by rearing experience. Influence of the oxytocin system on HPA axis activity may be most evident directly following a stressor, rather under normal conditions.

APPENDIX

TEMPORAL CORRELATIONS OF CORTISOL AND OXYTOCIN ACROSS SAMPLING TIME POINTS

In order to determine the stability of the various oxytocin and cortisol measures,

Pearson correlations were performed across samples collected at different ages or dates.

Infant Monkeys (14 - 180 days)

Temporal correlations compared (1) hair cortisol concentrations sampled on day

14 and day 180, (2) CSF oxytocin, (3) plasma cortisol, and (4) plasma oxytocin

concentrations sampled on day 14 to day 30. Pearson correlations identified a significant

correlation for both hair cortisol ($r_{(59)}$=-0.327, p=0.011; Fig. A.1a) and plasma cortisol

($r_{(52)}$=0.476, $p<0.001$; Fig. A.1b) sampled across day 14 and day 30. Temporal

correlations for plasma and CSF oxytocin were not significant.
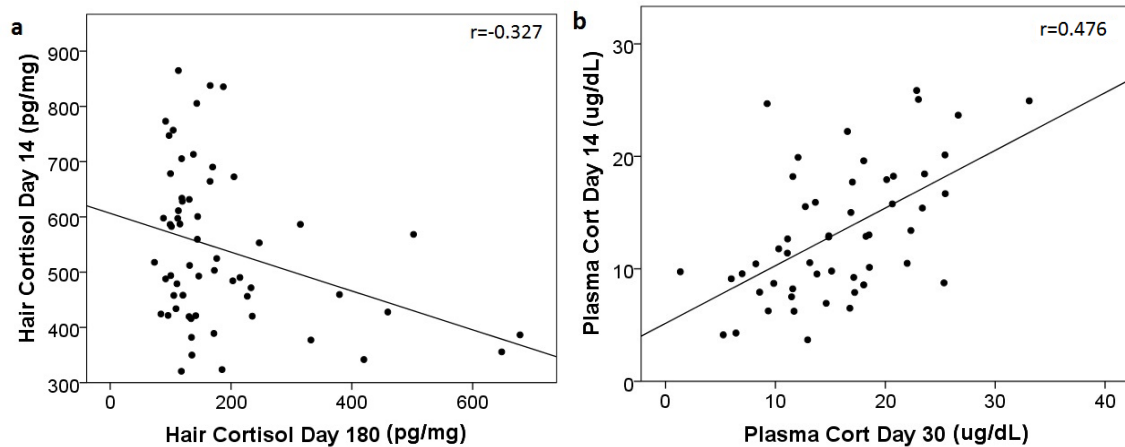
Figure A.1. The correlation between (a) hair cortisol measured on day 14 and day 180 ($p<0.001$) and (b) plasma cortisol measured on day 14 and day 30 ($p=0.011$).

Adult Monkeys

Temporal correlations compared (1) hair cortisol, (2) saliva cortisol, (4) plasma oxytocin, and (4) saliva oxytocin concentrations sampled on two separate occasions with an inter-sample interval of approximately 3 months. Hair cortisol ($r_{(21)}$=0.862, $p<0.001$) (Fig. A.2a), salivary cortisol ($r_{(17)}$=0.642, p=0.024) (Fig. A.2b) and salivary oxytocin ($r_{(19)}$=0.456, p=0.05) (Fig. A2c) were significantly correlated across the two sample dates.
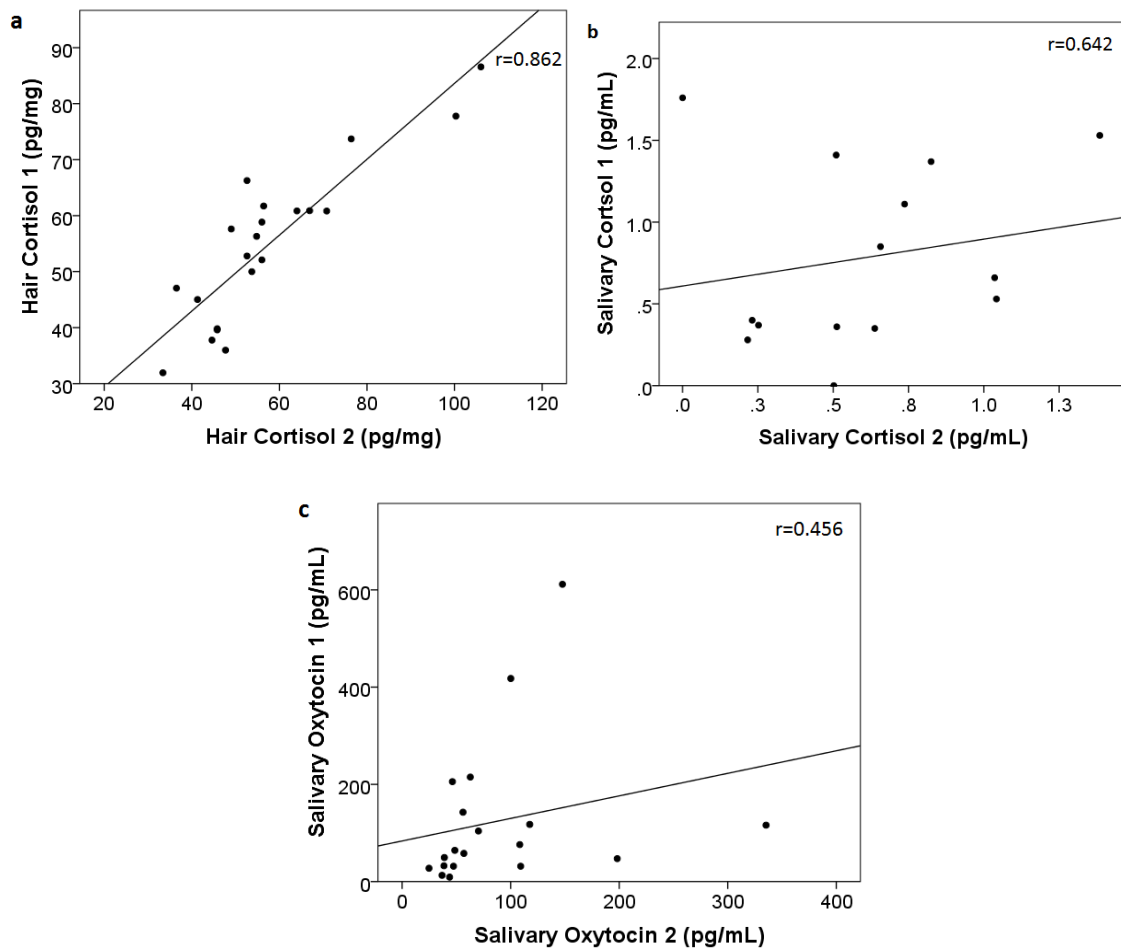
Figure A.2. Temporal correlations between first and second sampling periods for (a) hair cortisol ($p<0.001$), (b) salivary cortisol (p=0.024) and (c) salivary oxytocin (p=0.05).

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